Applied bacteriology : an introductory handbook .. / by T.H. Pearmain and C.G. Moor.

APPLIED BACTERIOLOGY

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AN INTRODUCTORY HANDBOOK FOR THE USE OF STUDENTS, MEDICAL OFFICERS OF HEALTH, ANALYSTS, AND OTHERS.

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PREFACE.

This work is intended to be an introductory handbook for the use of students, medical men, and others who require a practical acquaintance with Bacteriology without having at command the necessary time for a comprehensive study of the mass of work which it comprises.

Our purpose is therefore to give a concise account of the principal facts which may fairly be considered to have practical applications, and of the methods of examination by which they can be investigated. To persons familiar with the science it is needless to say that it has been impossible in the space at our disposal to give more than a small fraction of the material which would be available for a treatise having the same object. We regret this circumstance the less because persons engaged on other subjects of study or of professional occupation, especially those who have little or no previous knowledge of the subject, could not assimilate anything like the whole mass of conflicting observations which have been published, of which, indeed, a large number must ultimately be superseded. We have endeavoured, however, to include those results which may be considered as definitely established, or appear most likely to be definitely developed in the near future.

We have pleasure in acknowledging our indebtedness to the following periodicals and standard works on Bacteriology:

The Local Government Board Reports.
The British Medical Journal.
The Lancet.
The Journal of State Medicine.
The Medical Press and Circular.
The Pharmaceutical Journal.
The Journal of Pathology and Bacteriology.
Crookshank, 'Manual of Bacteriology.'
Frankland, 'Micro-organisms in Water.'
Hansen, 'Practical Studies in Fermentation.'
Jorgensen, 'Micro-organisms and Fermentations.'
Kanthack and Drysdale, 'Practical Bacteriology.'
Migula, 'Introduction to Practical Bacteriology.'
Prudden, 'Story of the Bacteria.'
Schenk, 'Elements of Bacteriology.'
Sternberg, 'Manual of Bacteriology.'
Woodhead, 'Bacteria and their Products.'
Würtz, 'Précis de Bactériologie Clinique.'

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APPLIED BACTERIOLOGY.

CHAPTER I.

INTRODUCTION.

Bacteria: their history and place in Nature—The abiogenesis and biogenesis theories—Structure of micro-organisms—Types and size of organisms—Method of reproduction—Rate of growth—Movements of bacteria — Classification of micro-organisms — Conditions and products of growth—Resistance of bacteria to physical influences: light, heat, etc.—Sterilisation—The antagonism of micro-organisms.

Far down in the scale of life is a large group of organisms which are spoken of in a general way as micro-organisms, bacteria, microbes, germs, etc. The bacteria are so small and simple in their structure, that it has been no easy task for the biologist to decide whether they belong to the animal or vegetable kingdom. It is now definitely settled, however, that they are plants, and are closely related to the algæ.

Bacteria are distributed everywhere in Nature; they cling to the surface of every substance, and are to be found in greater or lesser numbers in air, water, dust, etc. We only perceive their presence under ordinary circumstances, however, when the conditions are favourable to their growth and development. Sometimes they give rise to a putrefactive smell, or impart a colour to the body on which

they grow, or acquire a colour of their own. If some slices of boiled vegetables, such as carrots or potatoes, be exposed to the air for a few minutes, and then covered up and allowed to remain for a few days in a warm place, bacterial colonies will be seen to have developed, spreading over the surface of the media, giving rise to characteristic appearances, such as various white and coloured patches in the form of little droplets, or more or less slimy masses with irregular outlines. While the slices of vegetables were exposed to the air, various bacterial germs fell upon them, and then developed at the spots where they fell into colonies, which remain isolated on the solid media. Occasionally, however, it may happen that two or more germs fall on the same spot, in which case the resulting colony is impure from the first.

The history of the science of bacteriology may be said to commence with the observations of Anton Leuwenhoeck, of Delft, Holland, who in 1675 constructed a microscope of sufficient power to demonstrate minute organisms in water, putrefying fluids, saliva, etc., of a kind which up to that time were quite unknown.

A century later, namely, in 1775, the Danish investigator Muller named and described some three hundred organisms occurring in the waters about Copenhagen. The first experiments in connection with the sterilisation of apparatus by heat were made by the Abbé Spallanzani, about the year 1775.

Scarcely any advance was made in our knowledge of the bacteria until Ehrenberg, the naturalist, in 1830 studied them with the aid of improved instruments. The lack of culture-methods, however, prevented him from recognising the true nature of these organisms.

Cohn a few years later shed fresh light upon the subject by showing that the bacteria are plant-cells, with which

they agree in way of growth as well as in structure. In 1837 the important discovery was made by Schwann that the phenomenon of alcoholic fermentation was connected with the presence and life of the yeast-plant, and that putrefaction was due 'to something in the air which heat was able to destroy.'

Messrs. Schröder and Dusch in 1854 introduced the use of cotton-wool for filtering air to free it from micro-organisms, and for plugging apparatus. But it remained for Pasteur to make the greatest advance in this department, by making pure cultures of various organisms, thus rendering an accurate study of them for the first time possible.

Pasteur's first experiments were devoted to the study of the yeasts, and the part they play in the phenomena of fermentation. As Pasteur's classical experiments laid the foundations of the modern study of bacteriology, it may be well to describe the general lines on which he worked. He first carefully observed the nature of the organic material in which certain fermentations took place, studying both synthetically and analytically the best medium for the purpose, and then by careful microscopical study determined what organisms developed most rapidly during the fermentation process. After making a solution of the substance to be fermented, he added a small quantity of albuminous material and a trace of the ash of the yeast under examination, so that there should be a sufficient quantity of the necessary mineral constituents present. The medium was then carefully sterilised by being boiled in flasks to which only filtered air had access. To the germ-free solution he added a small trace of the special yeast which he wished to examine. By this means, after growing the organism through two or three generations, he obtained pure cultures. Pasteur also employed the 'dilution,' or 'fractional,' method of cultivation. A drop of the

liquid containing the organism that is desired to be grown is largely diluted with sterile nutrient fluid favourable to its growth. Drops of this diluted culture are then inoculated into separate test-tubes containing nutrient fluid. By the extension of this process pure cultures are eventually obtained. It is of interest to note that Pasteur's first experiments, which have led to such far-reaching results, were made to disprove the spontaneous generation theory.

The great controversy which started during the latter part of the last century in connection with this subject was briefly as follows: Those on one side regarded bacteria as produced from organic matter by the process of putrefaction, while those on the other hand believed they were derived from living germs already present. The first theory is that of 'abiogenesis,' or 'spontaneous generation'; the second that of 'biogenesis,' or 'life from life.' The supporters of the former theory made the mistake of supposing that all forms of life were destroyed by simple boiling; but, on the other hand, the Abbé Spallanzani, as early as 1777, showed that once boiling was not sufficient to destroy all living germs, but that repeated and prolonged boiling, care being taken to keep out aerial germs, will entirely prevent meat-broth, etc., from undergoing putrefactive changes. In spite of this, however, the discussion was continued for many years, until Pasteur, Tyndall, and others, demonstrated that all putrefaction is due to the action of bacteria, and that meat-infusion, milk, wine, and other putrescible bodies, will keep indefinitely, if due care be taken to protect them from germs after proper sterilisation.

If the 'abiogenesis' theory were correct, it would be useless to fight against harmful bacteria, as these would again and again be generated afresh. Fortunately, however, the truth is found in the contrary view, that bacteria only

appear where their germs are already present, and it is sufficient to exclude these germs if their intrusion is to be prevented.

The great discovery was made by Davaine, in 1863, of the bacillus of anthrax in the blood of animals suffering from splenic fever. This year will be ever memorable in the annals of medicine, on account of the fact that this is the first notice of a specific organism in connection with disease. This opened the way to the many brilliant discoveries which from then have taken place almost year by year, and have thrown so much light upon the cause and prevention of disease. The mysterious veil which for many centuries has hung over some of the most widespread and terrible of the diseases which afflict the human race is being gradually drawn aside.

In 1873 Obermeier described the spiral organism which bears his name, in the blood of patients suffering from relapsing fever. Hansen in 1879 described the bacillus of leprosy. In 1880 Eberth discovered the typhoid bacillus, which was artificially cultivated by Gaffky in the following year. Koch in 1881 devised his beautiful method of using solid culture media, which is now so universally used. He thickened nutrient meat-broth with gelatine, whereby the organisms inoculated into the liquid are fixed *in situ* when it cools and sets, thus rendering it easy to obtain pure cultures of any micro-organism by picking out a fragment of a colony and planting it on a fresh surface. Löffler in 1882 discovered the organism of glanders. In 1883 Nicolaier described and investigated the bacillus of tetanus, and Koch the bacillus of tubercle. Koch, again, in 1884 published his discovery of the spirillum of Asiatic cholera (Koch's comma baciilus), while, perhaps, the latest discovery is that of the bacillus of influenza in 1892 by Pfeiffer.

Twenty years ago it would have seemed chimerical to have said that we could cultivate at will, in the laboratory, the very living essence and cause of such diseases as cholera, diphtheria, typhoid, tuberculosis, and others, as a gardener does his plants, and from the knowledge thus gained plan new and efficient methods for combating and preventing disease. This new field of study opened up during the last twenty-five years by medical men such as Davaine, Koch, Virchow, Klein, Sanderson, Lister, Löffler, and by chemists like Pasteur, Kitasato, Frankland, and others, has not yet been by any means perfectly explored. The results so far obtained, however, are so important that it has become an absolute necessity for those concerned in the study and treatment of disease to have some knowledge of this branch of science. But enough has been said to pave the way for the better appreciation of the marvellous manner in which these investigations have led and are leading to the most important and far-reaching results in medicine.

Structure of Micro-organisms.—The bacteria appear under the microscope as pale, translucent bodies; they consist of unicellular organisms composed of protoplasm surrounded with a membrane, or skin, of a body allied to cellulose. This outer skin swells up in some cases to form a jelly-like casing, by which the internal protoplasm is covered.

The cells sometimes contain a nucleus which is readily stained with the usual staining reagents.

Types of Organisms.—The organisms vary very much in shape and size. Many are globular or spherical in shape, and are generally known as micrococci; others, on the other hand, are rod-like bodies, hence are termed bacilli; whilst others, having a spiral or corkscrew shape, are known as spirilla. Some spirilla sometimes appear in a much shorter form, and resemble the shape of a comma. Very

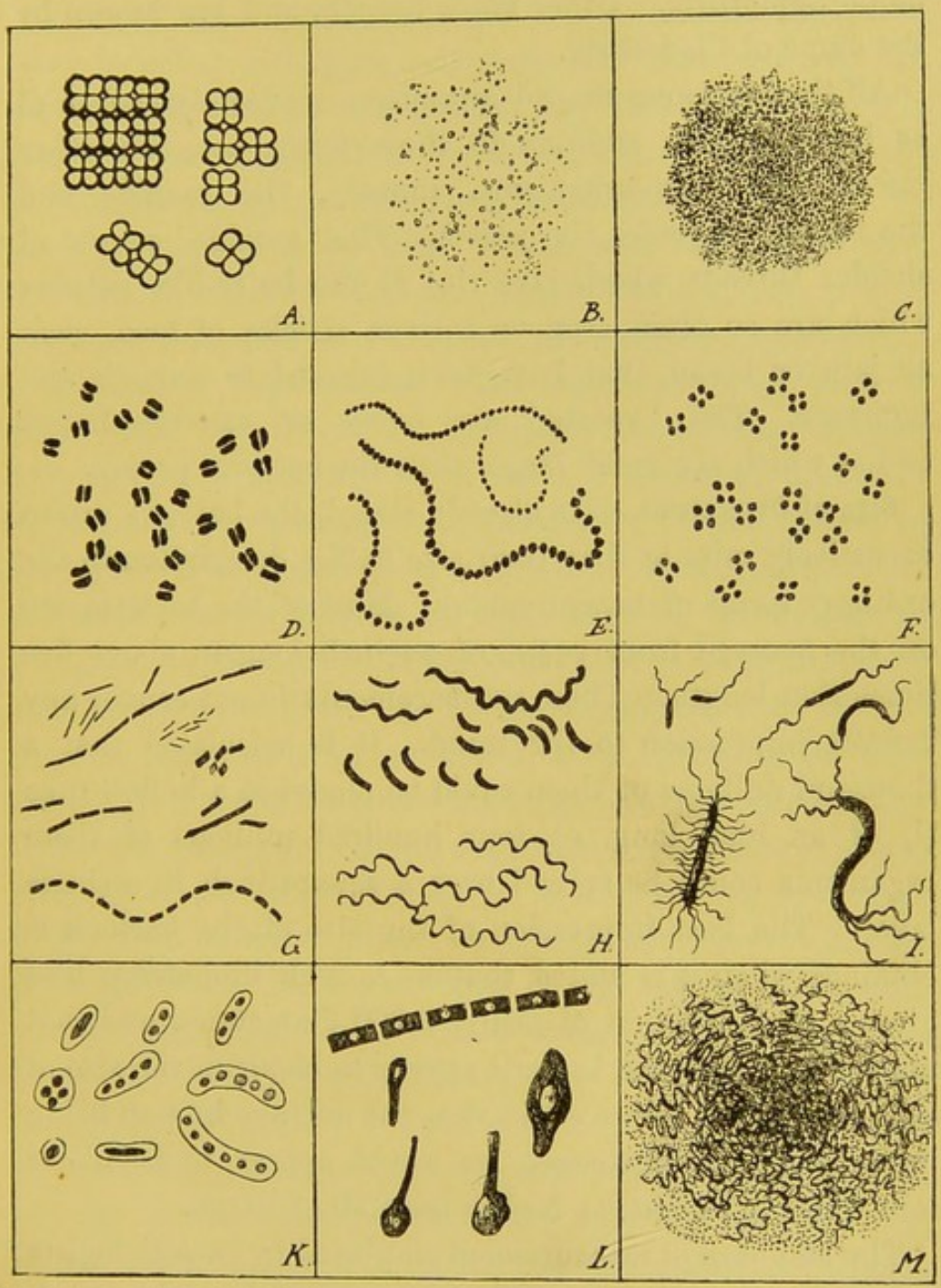


FIG. 1.—TYPES OF ORGANISMS.

A, sarcina; *B*, micrococci; *C*, staphylococci; *D*, diplococci; *E*, streptococci; *F*, tetracocci; *G*, bacilli; *H*, spirilla; *I*, bacteria with flagella; *K*, bacteria with capsules; *L*, bacteria with spores; *M*, zoogloea.

short rodlets are often known as bacteria—if fairly long, as bacilli; very long filaments are included under the

name Leptothrix. Other kinds branch, and are known by the name of Cladothrix.

All these various shaped organisms are loosely spoken of as bacteria. In addition to these forms are two other classes of micro-organisms, namely, the moulds and the saccharomycetes, or yeasts. The 'moulds' consist of slender threads which give rise to the hairy-like patches which are so often seen on various articles of food, such as jam or bread, that have been exposed to warmth and moisture. The 'yeasts' are ovoid or sausage-shaped bodies which are much larger than the bacteria proper.

Size of Organisms.—As already stated, the bacteria are so excessively minute that their size baffles description in the ordinary terms of measurement. Most of the bacteria are on the average from $\frac{1}{25000}$ of an inch long to about five times that length. These measurements do not convey any definite impression to the mind. It is calculated that a thousand millions of them could be placed in a hollow tube $\frac{1}{25}$ of an inch long, or four hundred millions of these organisms could be spread over a square inch in a single layer. The best impression of the size of the bacteria is obtained when it is stated that a $\frac{1}{25}$ inch immersion lens gives a magnification of nearly 2,200 diameters; and that under this power the bacteria appear to be about the size of ordinary print. If we could view the average human being under such circumstances, he would appear to be about four miles in height, or higher than Mont Blanc.

The standard of measurement employed by bacteriologists is the micro-millimetre; this is represented by the Greek letter μ. One μ (micro-millimetre) is equal to about $\frac{1}{25000}$ of an English inch.

The number of cocci in a milligramme of a culture of *Staphylococcus pyogenes aureus* has been estimated by Bujwid, by counting, at eight thousand millions. Not only do various

species differ in dimensions, but considerable differences may be noted in a pure culture of the same species. On the other hand, there are numerous species which so closely resemble each other in size and shape that they cannot be differentiated by microscopic examination alone, and we have to depend upon other characters, such as colour, growth in various culture media, pathogenic power, chemical products, etc., to decide the question of identity.

Methods of Reproduction.—The reproduction of bacteria takes place by 'fission' or by 'spore' formation. Fission is a process of splitting, or division, whereby an organism divides into two parts, each of which lives on and divides in its turn. If an organism is watched under the microscope, the coccus or bacillus, as the case may be, will be seen to elongate somewhat, and at the same time becomes narrower and narrower, until its two halves become free, the two individual organisms so produced again dividing in their turn. If, however, the new organisms do not break away from each other, but remain connected in groups or clusters, they are known as staphylococci; if they remain connected in the form of chains, like a string of beads, they are known as streptococci. If the division in the case of cocci takes place in one plane, diplococci are formed. If division takes place in two directions, tetracocci or tablet-cocci are formed. Again, if the division is in three directions, sarcina or packet-cocci are formed. On account of this multiplication by fission, the generic name of schizomycetes, or 'fission-fungi,' has been given to the bacteria. Some species, such as the various Cladothrices, do not divide, but grow in length, and give rise to branched threads.

The second method by which the bacteria propagate is by the development of spores. These are distinguished by their remarkable power of resistance to the influence of temperature and the action of chemical agents and other

unfavourable conditions. Spore formation may take place in two ways: firstly, by 'endogenous spores' (internal spores); secondly, by 'arthrospores.'

(*a*) *Endogenous Spores.*—When the formation of the spores takes place in the mother-cell, the protoplasm is seen to contract, giving rise to one or more highly refracting bodies, which are the spores. The enclosing membrane of the organism then breaks away, leaving the spores free.

(*b*) *Arthrospores.*—When the spore is not formed in the parent bacillus, but when entire cells (owing to lack of favourable conditions of growth) become converted into spores, the formation is known as 'arthrogenous,' the single individuals being called 'arthrospores.'

When the conditions are again favourable, these spores germinate, giving rise to new bacilli. The germinating spore becomes elongated, and loses its bright appearance; the outer membrane becomes ruptured, and the young bacillus is set free. Certain conditions, such as the presence of oxygen in the case of the anthrax bacillus, give rise to the formation of spores; while various kinds of bacteria secure their existence by developing spores when there is lack of proper food material.

With reference to the incredible rapidity with which the bacteria multiply under conditions favourable to their growth and development, Cohn writes as follows: 'Let us assume that a microbe divides into two within an hour, then again into eight in the third hour, and so on. The number of microbes thus produced in twenty-four hours would exceed sixteen and a half millions; in two days they would increase to forty-seven trillions; and in a week the number expressing them would be made up of fifty-one figures. At the end of twenty-four hours the microbes descended from a single individual would occupy $\frac{1}{10}$ of a hollow cube, with edges $\frac{1}{25}$ of an inch long, but at the end

of the following day would fill a space of 27 cubic inches, and in less than five days their volume would equal that of the entire ocean.'

Again, Cohn estimated that a single bacillus weighs about 0·000,000,000,024,243,672 of a grain; forty thousand millions, 1 grain; 289 billions, 1 pound. After twenty-four hours the descendants from a single bacillus would weigh $\frac{1}{2000}$ of a grain; after two days, over a pound; after three days, sixteen and a half million pounds, or 7,366 tons. *It is quite unnecessary to state that these figures are purely theoretical, and could only be realised if there were no impediment to such rapid increase.*

Fortunately for us, however, various checks, such as lack of food and unfavourable physical conditions, prevent unmanageable multiplication of this description.

These figures show, however, what a tremendous vital activity micro-organisms do possess, and it will be seen later at what great speed they increase in water, milk, broth, and other suitable nutrient media.

Movement of Micro-organisms.—Many of the bacteria are motile, especially the rod-like and spiral forms. This movement, in some of the bacteria, at least, is induced by one or more little hair-like or whip-like processes attached to the ends or body of the organism. These little projections, or cilia, are known as 'flagella.' By means of these minute threads of protoplasm, which perform lashing movements, the bacteria go though a most elaborate and astonishing series of movements, sometimes very rapid, at other times very slow and sinuous. They roll over, dart about, bang against one another, rest awhile, and so on through various phases of movement. Other micro-organisms, particularly in the case of the cocci, are quite motionless. This motility of the bacteria must not be confounded with oscillatory movements, or with such motions

as occur when solid particles are suspended in a fluid medium, which is due to electrical disturbance, and generally called the 'Brownian movement.'

The following bacilli, amongst others, have numerous flagella distributed over the whole of the organism: The typhoid bacillus, the bacillus of blue milk (*Bacillus cyanogenus*), the potato bacillus (*Bacillus mesentericus vulgatus*), the bacillus of malignant œdema, the hay bacillus (*Bacillus subtilis*), *Proteus vulgaris*, etc.

The following have only one or two flagella at the poles: The *Bacillus pyocyaneus*, the *Spirillum Finkleri*, the *Spirillum choleræ Asiaticæ*, the *Spirillum Metschnikovi*, etc.

The following have numerous polar flagella: The *Spirillum undula*, *Spirillum rubrum*, *Spirillum concentricum*, etc.

The *Micrococcus agilis* has also several flagella, which possibly arise from one point.

Classification of Bacteria.—The task of classifying bacteria is one of great difficulty, since they are so little known, and new kinds are constantly being discovered; also, on account of the polymorphic characters of many of the forms, it is only possible to arrange them in a few leading groups according to their shape and general characters, such as spore formation, mode of growth, etc. A great many methods of classification have been put forward from time to time, but the only one we will give here is a modification of that proposed by Hueppe, which is the simplest and most practical. The classification of micro-organisms may be divided into four main divisions:

Coccaceæ, Bacteriaceæ, Leptotricheæ, Cladotricheæ.

These groups may be again divided into groups, as follows:

Coccaceæ—(1) *Micrococcus, or Staphylococcus.*—When the cocci occur in masses like bunches of grapes, they are called *Staphylococci*. Cocci often occur singly, sometimes

in pairs, when they are known as *Diplococci*. Some cocci are not always round, but somewhat oval; when in process of division they are necessarily more or less elongated.

(2) *Streptococcus, or Chain Cocci.*—Division in one direction. The cocci are arranged in chains or bead-like formation.

(3) *Merismopedia, or Pediococcus.*—Division in two directions, forming groups of four, which remain associated in a single plane, giving rise to tablet-like layers of cells.

(4) *Sarcina, or Packet-cocci.*—Division in three directions, forming packets of eight or more elements, which remain associated in more or less cubical masses.

(5) *Ascococcus.*—Cocci in groups, surrounded by a gelatinous intercellular substance. This form is often known as a Zooglœa.

(6) *Leuconostoc.*—Cocci singly or in chains, surrounded by a gelatinous envelope, forming a Zooglœa.

Bacteriaceæ—(1) *Bacillus.*—The straight, rod-like bacteria; reproduction by binary division, or by resting spores; are, as a rule, motile. When, owing to spore formation in the end of the rod, it gives rise to peculiar enlargement resembling a bottle, the bacillus is known as a Clostridium.

(2) *Bacterium.*—Generally shorter than the bacilli, and form no spores and possess no power of locomotion. It is difficult to draw a sharp line between these two classes. It frequently happens that these characteristics are not all present in one species. Some are motionless, and yet form spores. Continually species are being taken out of this class, to be included in the bacilli.

(3) *Spiro-bacteria, or Spirilla.*—These form curved or spiral filaments, rigid or flexible; reproduction by binary division and by spore formation; movements rotatory in the direction of the long axis of the filaments, or they may be motionless.

Spirilla are subdivided into 'comma' bacilli or vibrios, spirilla in the more restricted sense, and spirochætæ. The vibrios usually form strings of cells which strongly resemble spirilla; the spirochætæ are distinguished for their flexibility.

Leptotricheæ.—These form rodlets and longish threads, which show a distinction between the base and apex of the filaments, growing out from a thinner base to a broader apex.

The three most important classes are: (1) Beggiatoa; (2) Crenothrix; (3) Leptothrix.

(1) *Beggiatoa.*—These form long motile threads, consisting of colourless cells, and are distinguished by the presence of strongly refracting granules of sulphur. They occur in sulphur springs and in dirty water.

(2) *Crenothrix.*—These form simple threads, the separate cells of which surround themselves with a distinct sheath, and then change themselves by segmentation at their ends into roundish spores. The threads are motionless, and, especially in their younger stages, group themselves into little patches.

(3) *Leptothrix.* — Threads with or without sheaths. Division not very numerous or well marked. The cells are devoid of sulphur.

Cladotricheæ.—Forms consisting of threads which possess pseudo-branches; the separate cells are provided with sheaths. Spore formation not yet demonstrated. This class has only one division, namely Cladothrix. They are found in dirty water.

Conditions of Growth of the Bacteria.—The bacteria, like the higher organisms, cannot live and multiply unless they have proper nourishment and conditions of growth. As the bacteria do not contain chlorophyll, they are not able to avail themselves of the carbon existing in the air as carbon dioxide (carbonic acid gas), but are dependent for their

nourishment upon the more complex compounds of carbon, the sugars, for instance, and the nitrogenous compounds in the shape of the albuminoids. Some of the bacteria, however, obtain their nitrogen from inorganic materials, such as compounds of ammonia and nitrates.

The bacteria derive their oxygen either from the air or from compounds containing oxygen. In the former case they are termed aerobic, in the latter anaerobic. Pasteur, in 1861, first pointed out the fact that certain species of micro-organisms not only grow in the entire absence of oxygen, but that for some no growth can occur in the presence of this gas. The cultivation of 'strict anaerobics' calls for methods by which oxygen is excluded.

The 'facultative anaerobics' grow either in the presence or absence of oxygen. There are various gradations in this regard, from the strictly aerobic species which require an abundance of oxygen, and will not grow in its absence, to the strictly anaerobic, which will not grow if there is a trace of oxygen in the media in which it is proposed to grow them. According to this relation to oxygen they are classed as 'facultative' and 'obligate' aerobic or anaerobic bacteria, as the case may be. Among the most interesting pathogenic bacteria which are 'obligate' anaerobics are the bacillus of tetanus, malignant œdema, and symptomatic anthrax.

On the other hand, bacteria such as anthrax, for instance, are aerobic, but facultatively so, since they can live for a long time out of contact with oxygen. Again, bacteria cannot live and reproduce unless they have a proper temperature. This varies very much with the different organisms, but in most cases is not less than 12° C. (=54° F.), nor more than 40° C. (=104° F.). There are, however, bacteria which can grow at 0° C. (=32° F.), and others which can do so at from 60° to 70° C. (=140° to

158° F. These are the 'thermophylic' organisms, which have been recently studied by Miquel, McFadyean, and others.

With regard to the conditions of life of the bacteria, they may be divided broadly into two classes. When the organisms draw their nourishment from some living body or 'host,' they are known as 'parasites.' These are further termed 'obligate' parasites if they can only live on this 'host.' If the bacteria draw their nourishment from dead organic matter, they are called 'saprophytes.' These are also divided into 'obligate' and 'facultative' saprophytes. Thus, it will be seen that a parasite under certain circumstances may readily become a saprophyte.

Some of the more important saprophytes are those organisms which play such an important and useful part in our everyday life, such as, for instance, in the phenomena of fermentation, and also as the putrefaction agents which transform dead and decomposing organic matter into their simpler elements, thus completing the great life cycle, and rendering the dead and effete matter again ready for the vital processes.

Amongst other life manifestations of the bacteria may be mentioned those which have the property of generating colouring matter, though not chlorophyll. The bacteria themselves are colourless and transparent, and the pigment is merely formed as a product of their metabolism, especially under the influence of light. Many of the bacteria give rise to various gases and odours, particularly the anaerobic organisms which give rise to very foul putrefactive gases, (ammonia, sulphuretted hydrogen, etc.). The *Bacillus prodigiosus* gives rise to a smell resembling that of trimethylamine.

Micro-organisms have the property of producing various changes in the medium on which they are grown. In many

cases albuminous bodies are peptonized and gelatine is liquefied. Many bacteria have the faculty of resolving organic bodies into their simplest elements; others, again, have the property of converting ammonia into nitric and nitrous acid. Certain microbes have the power of becoming phosphorescent in the dark. These phosphorescent bacteria are often seen on decaying plants and wood; sometimes in tropical climates the sea becomes luminous owing to the presence of countless numbers of these organisms. Again, they are frequently seen on the surface of dead fish, particularly mackerel, which often become so bright as to strongly illuminate the cupboard in which they lie.

The particular class of parasites which produce disease in man and the higher animals are termed 'pathogenic' bacteria. These pathogenic organisms may exert their pernicious power in several ways. They may be injurious on account of their abstracting nourishment from the blood or tissues, or for the purely mechanical reason of their stopping up the minute capillaries and bloodvessels by their excessive multiplication. But the poisonous action of most of the pathogenic bacteria is due to the chemical products secreted by the organisms, and it is to the circulation and absorption within the body of these poisons that must be traced the disturbances of the animal system which characterise disease.

The various products of the metabolism of the bacteria are known as 'ptomaines,' 'toxalbumoses,' 'ferments,' or 'enzymes.' Many of these bodies may be elaborated by micro-organisms when growing on artificial media or articles of food. When meat or albuminous bodies undergo decomposition, *i.e.*, when the organisms of putrefaction alight and develop on them, the result may be the production of these intensely poisonous bodies, which are the cause of the cases of which we frequently hear of indi-

viduals, or even families, being poisoned by partaking of some particular meat, fish, or other food, that has had the opportunity of undergoing partial decomposition. These cases are invariably due to the fact of the food in question being in an unsound condition, whereby it contained organisms which generated the poison; and even though the bacteria may have been destroyed during the process of cooking, the toxic substance remains in the food, to produce the most disastrous effects on its being eaten.

These pathogenic organisms, which under ordinary circumstances cause disease and death, can by proper methods be so modified in their properties that they can be made to serve as antidotes to the very diseases they cause. This discovery, which was due to the genius of Pasteur, is the greatest romance of modern science; it has opened a new epoch in the annals of medicine, and has revolutionized the treatment of disease.

Taking only one case for example: The bacillus of anthrax, if cultivated at a temperature rather higher than blood-heat, becomes no longer fatal when inoculated into animals, but produces only a slight constitutional disturbance, after which treatment the animals are found to be 'immune,' or protected against the virulent form of anthrax. This great principle of an 'attenuated' virus conferring immunity is the basis of many systems of protective treatments which are becoming of ever-increasing importance in the conflict with infectious disease. A full account of the theory and practice of these 'antitoxin' treatments will be found later.

Resistance of the Bacteria to External Influences.

Bacteria, like other living organisms, are exposed to many outside influences. These we will consider under six heads, namely, Light, Heat, Cold, Desiccation, Electricity, Chemical

Agents. The resistance of micro-organisms to these influences is very high, especially in the spore stage.

(1) **Light.**—Messrs. Downes and Blount, in a communication to the Royal Society in 1881, first called attention to the fact that light had an injurious effect upon bacteria, and that cultures may be destroyed by exposure to sunlight. About 1885, Duclaux and others took up this subject, and with various pure cultures of micro-organisms it was found that by exposure to sunlight the spores of various bacteria lose their capacity to germinate. It was also found that cultures lost their power of reproduction in diffused light, and that they also became 'attenuated' in their pathogenic power. In his address before the International Medical Congress at Berlin, in 1890, Koch stated that the tubercle bacillus was killed by the action of sunlight in a time varying from a few minutes to several hours, depending upon the thickness of the layer exposed. Diffused daylight had the same effect, although a considerably longer time was required; when placed close to the window, about a week was required. Similar results have been obtained by Janowski (1891) on the typhoid bacillus.

In the experiments of Momont (1892), dry anthrax spores were found to resist the action of light for a long time, but moist spores freely exposed to the air failed to grow after forty-four hours' exposure to sunlight. In the absence of spores, anthrax bacilli in a moist condition freely exposed to the air failed to grow after from half an hour to two hours' exposure to sunlight; but in the absence of air these same bacilli were not destroyed at the end of fifty hours of exposure. Buchner found that broth cultures of typhoid, *Bacillus coli communis*, *Bacillus pyocyaneus*, and the *Vibrio choleræ Asiaticæ* yielded no growth after one hour's exposure to direct sunlight.

The most recent and conclusive experiments of all, how-

ever, are those of Professor Marshall Ward, with the very resistant and virulent spores of anthrax. Having found that repeated exposure to sunlight destroyed the spores in a few cubic centimetres of Thames water containing a very large number, while a few weeks of bright daylight greatly lessened them, he proceeded to make a series of accurate experiments as follows: Agar plates of anthrax were made in Petri dishes, using for this purpose the virulent and resistant spores obtained by transferring some of the material from an old culture into some sterile distilled water, and keeping at a temperature of 56° C. for twenty-four hours. The plates were then covered with a metal stencil plate in which letters were cut, the dishes stood on a black background, and then exposed to sunlight for from two to six hours, after which the plates were put into an incubator at 20° C. for forty-eight hours. The agar was then found to be gray and cloudy, owing to the development of an immense number of colonies, but the space exposed to the light remained quite clear, showing the form of the letters in the stencil plate. The same results were obtained with other bacteria as well as with fungi. Similar though less marked results were obtained with an electric arc light, so much so that Professor Ward thinks that this form of light may prove to be an effective disinfecting agent. As with sunlight, however, its action is necessarily confined to organisms directly exposed to the rays, and not protected by media which absorb them, such as even shallow water.

Action of Coloured Light.—When a plate culture of anthrax is exposed to the solar spectrum, the germicidal action is found to be the strongest at the blue-violet end (Ward). Janowski exposed cultures under screens of various coloured glasses and aniline dyes, and found that no action took place under brown or yellow; whereas solutions of

fuchsine (which transmits violet rays), gentian violet, and methyl blue, had but little more effect than colourless fluids.

The action of light on micro-organisms supports the opinion of specialists in hygiene, that free access of light is a great factor in relation to the health of a community.

(2) **Action of Heat.**—As we have already seen, the 'optimum' temperature for the growth of most of the bacteria is between 20° to 40° C.; while some of them can grow at the freezing-point of water, and others can grow at as high a temperature as 60° to 70° C. Generally speaking, the pathogenic organisms require a temperature of 35° to 40° C. In considering the influence of heat on the bacteria, we must take into account the very great difference in the resisting power of the vegetative cells and the spores; and the different destructive powers of dry and moist heat, as well as the time of exposure and other conditions.

Dry Heat.—If bacteria, or their spores, when in a well-dried condition, are exposed to the action of heated dry air, the temperature required for their destruction is much higher than when moist heat is employed.

Koch and Wollffhügel, in 1881, made a thorough investigation of this subject. A large number of pathogenic and non-pathogenic organisms were tested, with the following results:

A temperature of 78° to 123° C. (=172° to 253° F.), maintained for over an hour, was found not to kill, and it was found necessary to employ a temperature of 120° to 128° C. (=248° to 262° F.) for at least an hour and a half to insure the complete destruction, in the absence of spores, of all of the species tested. The spores of *Bacillus anthracis* and *Bacillus subtilis* resisted this temperature, and required to insure their destruction a temperature of 140° C. (=284° F.) maintained for three hours. This temperature is injurious to most articles requiring disinfection, such as bedding and

clothing. But the lower temperature (120° C. = 248° F.), which destroys germs in the absence of spores, can be employed for disinfecting articles soiled with the discharges of patients with cholera, typhoid, or diphtheria, as the specific organisms of these diseases do not form spores. In practical disinfection it is necessary to remember that dry heat possesses but little power of penetration. In the experiments of Koch and Wollffhügel, it was found that registering thermometers, placed in the centre of folded blankets and various packages, did not indicate a temperature sufficiently high to destroy germs, even after three hours' exposure in a hot-air oven at 133° C. (= 271° F.), and above.

Moist Heat.—The thermal death-point of bacteria in the absence of spores is comparatively low when exposed to moist heat. Thus, all the pathogenic organisms as yet isolated are killed, when free from spores, by a temperature of 60° C. (= 140° F.), or below. Some of them fail to grow after an exposure to as low a temperature as 50° C. for two or three minutes. The *Spirillum choleræ Asiaticæ* and the *Micrococcus pneumoniæ crouposæ* are cases in point.

By extending the time, a still lower temperature will effect the same result. Chauveau found the anthrax bacillus to be killed by twenty minutes' exposure to a temperature of 50° C.; and Brieger also found that he could sterilise diphtheria cultures by exposure for some hours to the same temperature.

As already mentioned, there are micro-organisms (generally known as the 'thermophilic' bacteria) that are able to multiply at a temperature of 65° to 70° C. Miquel, in 1881, found a motionless organism in the water of the Seine, which grew in broth at 69° to 70° C.

Van Tieghem has also discovered several species which grow at about the same temperature.

With regard to the resisting power of 'spores' to moist heat, those of most of the pathogenic bacteria are quickly killed by a very short exposure to 100° C. (=212° F.).

On the other hand, the spores of certain non-pathogenic species resist the boiling-water temperature (100° C.= 212° F.) for hours.

In the practical application of steam for disinfecting purposes, it must be borne in mind that, although steam under pressure is more effective than ordinary steam, it is not necessary to give it the preference, in view of the fact that all the pathogenic organisms and their spores are quickly killed by a comparatively short exposure to the temperature of boiling water. 'Superheated' steam has about the same germicidal action as heated dry air at the same temperature. This is shown by the experiments of Esmarch, who found that the spores of anthrax were killed by three minutes' exposure to ordinary steam, but were not killed by the same time in 'superheated' steam at a temperature of 140° C.

From the above facts it will be seen that for any object to be rendered germ-free, *i.e.*, sterilised by dry heat, it must be exposed to a temperature of 160° to 180° C. (=320° to 356° F.) for half an hour at least, if this object is to be attained with certainty.

If sterilisation is to be secured by the agency of moist heat, the article must be heated to 60° to 70° C. (=140° to 158° F.). As already mentioned, most spores are killed by simple boiling; but there are, on the other hand, certain resistant kinds which can support for a short time a temperature of 110° to 115° C. (=230° to 239° F.).

In order to sterilise culture media, it is necessary to heat them to 115° C. for fifteen minutes under pressure, in vessels of special construction.

Fractional Sterilisation.—This is a method of rendering

culture and other media germ-free by exposing them to a temperature of not more than 60° to 70° C. (=140° to 158° F.) for several times in succession, the operation extending over at least three days.

By the first heating the adult bacteria are killed, the spores only remaining alive; the liquid is then kept at about 20° to 25° C. (=68° to 77° F.) for about twelve to twenty hours, to allow the spores to germinate, and then again heated. All the spores that have developed into full-grown bacteria are thus killed, and in case some of the spores should not have developed, the process is repeated again on one or more successive days.

But, as has been pointed out by Miquel, absolutely certain results are not to be attained by this method, as some spores take days, or even weeks, to germinate. There is, therefore, always the chance that some such spores may be present, and may ultimately develop in a medium that was believed to be sterile.

The difference which exists between the resistance offered by organisms and their spores to heat can be made use of to obtain pure cultures of some spore-bearing organisms. To obtain, for instance, a pure culture of the hay bacillus (*Bacillus subtilis*), the spores of which resist boiling water, the following method can be employed : Hay is left in water for twenty-four hours; the resulting infusion is strained, and one part of the liquid is diluted with ten parts of water. A flask is three-quarters filled with this liquid, and the neck of the flask is plugged with cotton-wool. The contents of the flask are now heated to boiling, the liquid then being allowed to gently simmer for an hour. In this manner all other ordinary organisms and their spores are killed, the spores of the hay bacillus being alone able to withstand the heat of boiling-water for this length of time. These spores, when the liquid is allowed to stand by for a day or two,

begin to develop vigorously, the hay infusion which contains the spores being a most favourable medium for the growth of the hay bacillus. As the cotton-wool plug prevents the entrance of other organisms, a pure culture of the hay bacillus is thus obtained.

A similar method, using regulated temperatures, is employed to obtain pure cultures of the tetanus bacillus.

(3) **Action of Cold.**—Micro-organisms are extremely resistant with regard to cold. Frosch, in 1877, exposed various cultures to a temperature of – 87° C., which he obtained by means of liquid carbon dioxide, and found that most of the organisms experimented upon multiplied on being placed again under favourable conditions. Prudden has recently made some extended experiments on the influence of freezing. He found that while some organisms withstood the action of cold for a long time, others failed to grow. The *Bacillus prodigiosus* failed to grow after being frozen for fifty-one days, as did also the *Proteus vulgaris*. The *Staphylococcus pyogenes aureus* withstood freezing for sixty-five days, and the typhoid bacillus for 103 days. Sub-cultures made at intervals showed, however, a diminution in number of the bacteria. A similar diminution in number would perhaps have occurred in old cultures in which the material for growth was exhausted, independent of freezing; for the bacteria, like the higher organisms, die in time as the result of degenerative changes in the protoplasm of the cells, and continued vitality in a culture depends on continued reproduction.

Repeated freezing and thawing was found by Prudden to be more destructive to the typhoid bacillus than continuous freezing. Cadeac and Malet kept portions of a tuberculous lung in a frozen condition for four months, and found that at the end of this time tuberculosis was produced in guinea-pigs by injecting a small quantity of the material.

(4) **Desiccation.**—Cultures of various micro-organisms, when kept moist, retain their vitality for a considerable time, but this varies with the different species. Sternberg found cultures of *B. typhosus*, *B. prodigiosus*, and others to be alive after being hermetically sealed for eighteen months. Some species die quickly, but most of them retain their vitality for months. The cholera spirillum ('comma' bacillus) will remain alive for months if kept moist; but Koch and Kitasato found that a broth culture, dried in the form of a very thin film, was incapable of development after three hours' drying. Pfuhl found the typhoid bacillus, dried under the same conditions, to retain its vitality for eight to ten weeks. Löffler found that the diphtheria bacillus resists desiccation for four or five months. Cadeac and Malet produced tuberculosis in guinea-pigs by injecting material from the lung of a tuberculous cow which had been kept in the form of a desiccated powder for five months, but at a later date the virulence was lost.

(5) **Action of Electricity.**—Many workers have made experiments to determine the action of electrical currents on various bacteria, but the results hitherto obtained are very indefinite and discordant. It is possible, however, that in the future this agent, the application of which increases daily, may play an important part in the destruction of bacteria.

(6) **Action of Chemical Agents.**—Chemical agents are destructive to bacteria by virtue of their poisonous action on protoplasm. The haloid elements, mineral acids, alkalies, metallic salts, and various organic compounds, all exert a strong germicidal or retarding action on the growth and development of micro-organisms.

The Antagonism of Micro-organisms.—The mutual antagonism or influence of growth of one species upon the growth of another has been specially studied by Freuden-

reich and Sirotinin. When several organisms are associated in a liquid culture, one species may take precedence, and the other may develop later; or two or more species may develop at the same time; or the growth of one species may prevent the growth of another by either (*a*) exhausting the food material by its rapid growth, or (*b*) by producing products which retard or prevent the growth of another. The following are some of the results of Freudenreich's investigations. *Bacillus pyogenes fœtidus* prevents the growth of the *Spirillum choleræ Asiaticæ; Micrococcus roseus* prevents the growth of *Micrococcus tetragenus*. The following organisms cause a change in broth which prevents the growth of other species: *Bacillus pyocyaneus, Bacillus phosphorescens, Bacillus prodigiosus, Spirillum choleræ Asiaticæ*. The following do not cause any change in broth which unfits it for the growth of other organisms: *Bacillus typhosus, Bacillus anthracis, Spirillum tyrogenum*.

CHAPTER II.

BACTERIOLOGICAL APPARATUS—PREPARATION OF NUTRIENT MEDIA, ETC.

The apparatus used in bacteriological research—The microscope—Hot-air and steam sterilizers—Sterilization by means of chemical agents and filtration—Incubators, microtomes, etc.—Nutrient media and their preparation.

The Microscope.—This instrument is perhaps the most important piece of apparatus used by the bacteriologist. Owing to the bacteria being the smallest and simplest living forms with which we are at present acquainted, it will be seen that it is of the greatest importance to be provided with a first-class microscope. Microscopes especially designed for bacteriological work are manufactured by the following well-known firms: Messrs. Zeiss, Leitz, Powell and Lealand, Baker, Beck, Watson and others. All the instruments and lenses made by these firms are of the highest class, both in performance and workmanship. Fig. 2 shows Messrs. Watson and Son's Edinburgh Student's Microscope, completely fitted with all the accessories necessary for bacteriological work. We have found this instrument to be very suitable for bacteriological research, and thoroughly satisfactory in practice.

A microscope suited for bacteriological work should satisfy the following requirements: The stand should be absolutely rigid, and the fine adjustment should be both sensitive and precise in its action. The instrument must be fitted with an Abbé sub-stage condenser and iris dia-

phragm, also a triple nosepiece. The last, although not absolutely necessary, is a great time-saving arrangement, which not only saves the necessity of unscrewing the objectives to obtain variations in power, but also preserves the objectives from much wear and tear. For

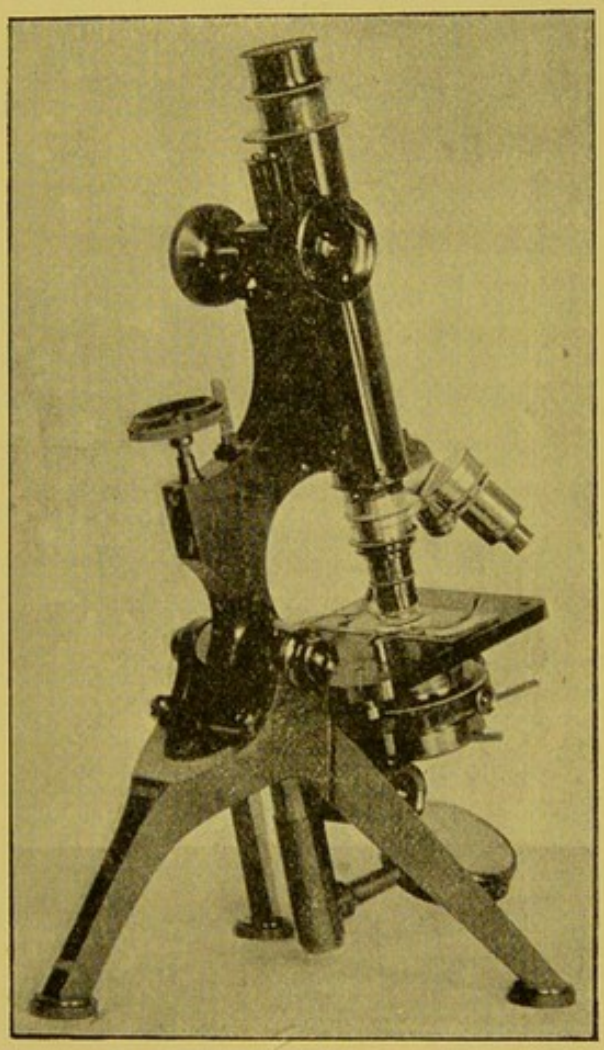


FIG. 2.—THE MICROSCOPE.

ordinary work the following objectives are required: 1 inch, $\frac{1}{4}$ inch, and a $\frac{1}{12}$ inch oil immersion. These objectives combined with an 'A' and 'B' eyepiece will give magnifications of from 50 to 1,100 diameters, which is ample for all ordinary purposes.

It is essential to be provided with a brilliant source of illumination for the examination of bacteria, particularly

when in tissues. Failing bright daylight, the most satisfactory source of light for microscopical work is, in our opinion, the Welsbach incandescent gas-light. This gives very brilliant illumination combined with perfect steadiness.

Micro-organisms are somewhat difficult to observe in liquids and tissues, being only visible through the shadows caused by the differences in the refractive power of the various structures. Hence but little light should be used, and consequently the hole in the diaphragm must be as small as possible. In the case of stained specimens, however, an open diaphragm can be used, and the preparation examined with the full power of the Abbé condenser.

After using the oil immersion objective, the cedar-oil used must be removed from the lens with soft filter-paper, and then wiped with a silk handkerchief. Should the oil have been allowed to dry on at any time, it is best removed by placing on a little fresh oil and allowing to stand a short time; this will soften the hardened oil, when the whole may be cleaned off together.

The Hot-air Steriliser.—This is an iron box fitted with double walls, with a door in front. The whole is supported on four legs. It is heated by means of a rose gas-burner from below, and the temperature of the interior is indicated by means of a thermometer inserted through a hole in the top. If necessary, a mercury gas-regulator can be inserted through a second opening.

It must be borne in mind that the temperature in these ovens is by no means uniform; care, therefore, must be taken that the objects exposed for sterilisation really reach the desired temperature.

Test-tubes, dishes, plates, cotton-wool, etc., may be thoroughly sterilised by exposure to a temperature of 150° C. for one hour. The cotton-wool is put loosely into a beaker, and placed with the tube or plates that are being

sterilised; when the cotton-wool becomes slightly browned, it may be taken as a sign that the sterilisation of the objects is complete. The platinum inoculating wires, forceps, etc., are best sterilised by passing through the flame of the Bunsen burner.

The Steam Steriliser. — This is a cylindrical vessel of copper about 1 metre high by about 30 centimetres wide, jacketed with felt or other non-conducting material, and

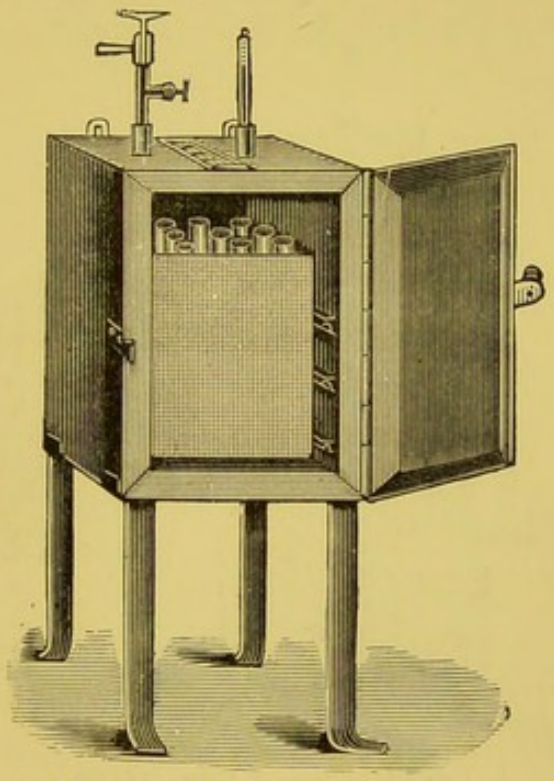


FIG. 3.—HOT-AIR STERILISER.

provided with a lid. The lid is also covered with felt, and is perforated to receive a thermometer. Inside the vessel is a diaphragm or grating about two-thirds down which divides the interior into two portions: the upper, or 'steam-chamber,' and the lower, or 'water-chamber.' This part is fitted with a water-gauge to indicate the water-level. The apparatus stands upon three legs, and is heated by two ordinary Bunsen burners, or, better, by a large Fletcher burner.

The heat must be sufficient to keep the water in vigorous ebullition, so that the steam issues freely from the top. In this way a uniform temperature of 100° C. is maintained in the apparatus. The steriliser is fitted with a wire basket or metal rack for the reception of test-tubes containing nutrient media.

This apparatus is employed for sterilising media and apparatus which cannot be exposed to temperatures higher

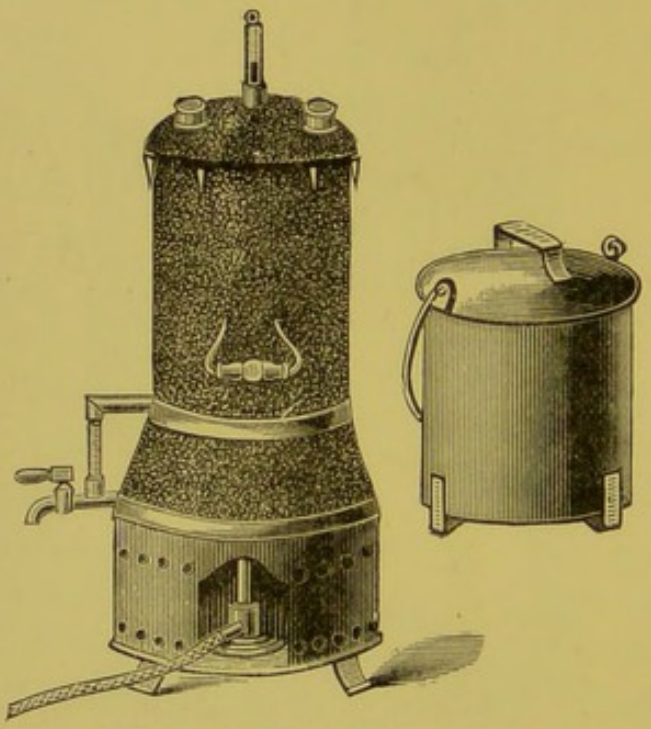


Fig. 4.—Steam Steriliser.

than that of boiling water (100° C.). Glass and other utensils may be steamed for from one to two hours; but some nutrient materials are unable to withstand the temperature of 100° C. for any length of time. Thus, nutrient gelatine loses its power of solidifying on prolonged heating at the boiling temperature. Hence it is advisable to expose media containing gelatine to a current of steam for not more than fifteen minutes on three successive days. The heating on the first day destroys all

the bacteria present and most of the spores, but some of the latter remain and develop by the next day into adult organisms; these are killed on heating the second time; any organisms that remain are finally destroyed by the third heating. The steam steriliser is also conveniently employed in hastening the filtration of nutrient agar, in preference to the use of the hot-water funnel. For this purpose the flask to receive the filtrate, together with the funnel containing the medium on the filter-paper, is wholly immersed in the steam.

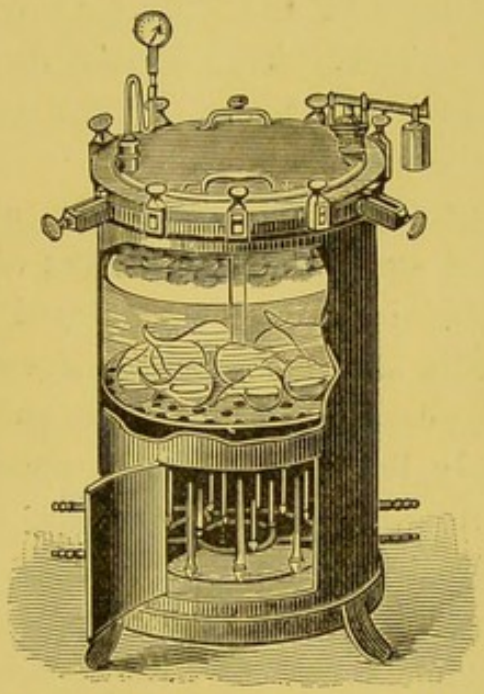


FIG. 5.—PRESSURE STERILISER.

The High-pressure Steam Steriliser.—High-pressure steam applied by means of an autoclave acts with greater rapidity than ordinary steam. Owing to the costly nature of high-pressure digesters, their employment is not to be recommended for ordinary use, as no advantage accrues from it. In certain cases, however, as in the sterilisation of soil, the high-pressure digester may be conveniently used. Globig (*Zeitsch. f. Hygiene*, iii., p. 332, 1887) found that certain spores were able to resist ordinary steaming for

three hours, whilst they were destroyed in fifteen minutes by steam at 110° to 120° C.

A number of substances cannot be heated above 100° C., in consequence of chemical changes brought about; among these may be mentioned the sugars, urea, albuminoids, etc.

Discontinuous or Intermittent Sterilisation.—In the case of certain substances, such as blood-serum, hydrocele fluid, etc., it is necessary to effect sterilisation below the temperature of coagulation of albumin. This consists of heating to a temperature of from 54° to 65° C., for three or four hours daily for about a week. This is best done in a special incubator or a bath of warm water, the heat of which is controlled by a thermo-regulator.

Sterilisation by Chemical Agents.—In addition to the usual methods of sterilisation by means of dry heat and steam, various chemicals may be employed for the purpose of sterilizing media and implements. For washing instruments, and in the case of experiments on animals for locally washing the body before making an incision, either for inoculation or dissection in the autopsy, a solution of 1 in 1,000 of corrosive sublimate or 1 in 30 solution of phenol is the germicide generally used. Chloroform is particularly suitable for the sterilisation of blood-serum, as it has a powerful germicidal action combined with a low boiling-point, so that it can be driven off with certainty after sterilisation is complete. As Globig has shown, it is impossible by heat to free blood serum from the organisms which do not grow below 50° C., and are capable of withstanding a temperature of 70° C. To sterilise by this method, the liquid under treatment is shaken up with chloroform, and allowed to stand some days, after which the mixture is freed from chloroform by prolonged heating at 62° C. The boiling-point of chloroform is 61·2° C. In all operations in which chemical agents are used for sterili-

sation purposes, great risk is incurred by traces of the germicide escaping removal, and thus destroying the organisms under examination or introducing other elements of uncertainty into the work. Great care must be taken when using such substances; in fact, it is advisable only to resort to their use under special circumstances. For ordinary purposes it is best to rely upon the careful fulfilment of all the details required in the sterilisation by the usual methods.

Probably the most ready means of sterilising plates, tubes, instruments, etc., wherever possible, is prolonged boiling in water, taking care to protect from dust when cooling.

Sterilisation by Filtration.—Air and other gases are readily freed from micro-organisms by drawing them through a tube containing a plug of dry sterile cotton-wool, or packed with sugar or sand. The application of this principle is seen in the plugging of culture tubes and flasks with cottonwool to protect the contents from aerial organisms.

In order to deprive water or other liquid, which is not too viscid, of bacteria, it is forced through cylinders made of unglazed porcelain (Pasteur-Chamberland filter) or baked infusorial earth (Berkefeld filter). When it is necessary to free water from organisms without chemical change, or for the purposes of concentration, as in the testing of water for the typhoid bacillus, or the separation of bacteria from the products of their vital activity (in the preparation of toxines, etc.), the use of these filters is invariably resorted to. Not only will these filters keep back the bacillus of mouse septicæmia, one of the smallest known micro-organisms, but putrid blood serum can be filtered, and the filtrate is rendered not only perfectly clear, but quite free from organisms.

Milk may not only be deprived of its fat by means of these cylinders, but a clear sterile serum is obtained.

For experimental purposes these filters must be sterilised and cleaned before being used. This operation does not affect the Pasteur filter, but tends to disintegrate the Berkfeld, which after a time loses its sterilising power.

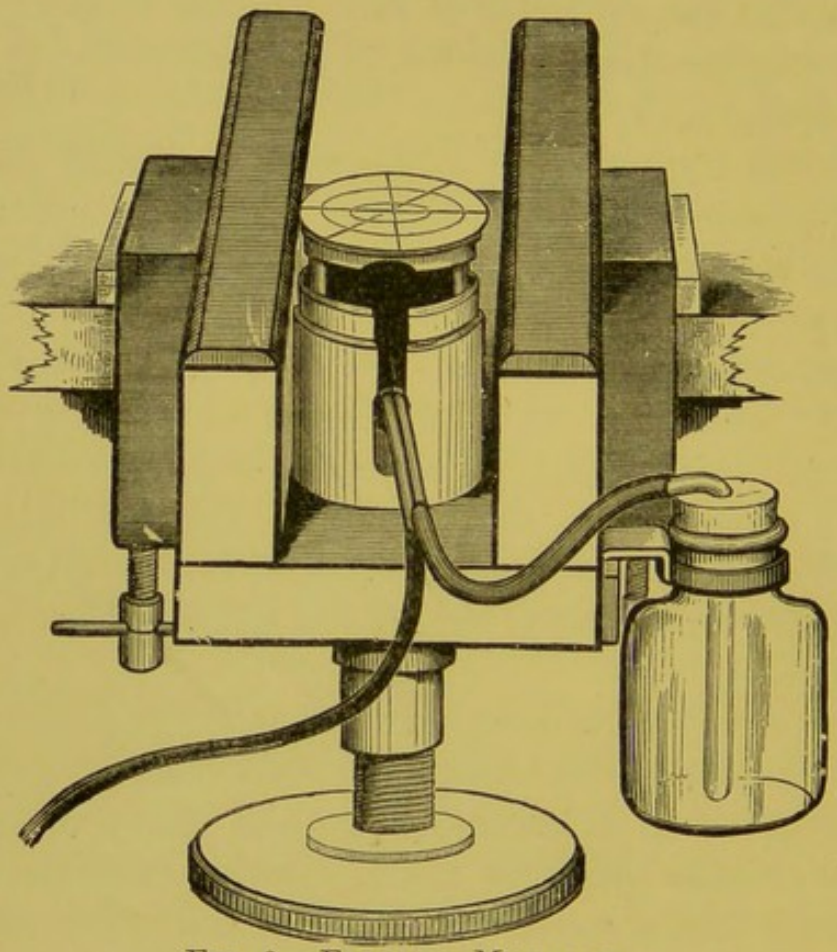


Fig. 6.—Freezing Microtome.

The application of air, compressed under a pressure of 10 lb. per square inch, to Pasteur tubes steeped in water is a trustworthy test of their bacterial soundness, as bubbles escape from any fault.

The Microtome.—A large number of machines for the cutting of sections of tissues have been introduced by various makers.

Cathcart's Microtome.—An improved form of Cathcart's freezing microtome is made by Messrs. W. Watson and Son, High Holborn, W.C., which is both cheap and convenient to use in practice. The tissue, after 'hardening' in alcohol (*q.v.*), is placed on the zinc plate of the microtome together with a little gum-water. Some ether is placed in the bottle, and the bellows worked until the gum has frozen; more gum solution is added and again frozen, and so on until the tissue is covered and frozen into a solid mass. Fine sections are then cut with a flat ground razor blade, which is kept moistened with alcohol. The bellows are worked a little from time to time to keep the mass frozen. The sections as soon as cut are transferred to alcohol.

The Rocking Microtome.—This machine is made by the Cambridge Scientific Company. It is only used for specimens imbedded in paraffin (*q.v.*), and is automatic; that is to say, it can be set to cut sections of definite thickness, and every time the handle is pulled, a section is cut and the specimen is moved forward ready for another.

Muencke's Microtome.—Dr. R. Muencke, of Berlin, makes a microtome which indicates by means of a dial the thickness of the sections being cut. This is a very useful and convenient form for general purposes.

The Incubator.—Although the greater number of the saprophytic and many of the pathogenic bacteria grow at the ordinary temperature, yet some of the pathogenic species can only be cultivated at the higher temperatures, and many of those which grow at the room temperature develop more rapidly and vigorously when kept in a warm chamber or incubator at a temperature of from 27° to 38° C. (=80° to 100° F.).

Whenever the 'ordinary' or room temperature is mentioned in connection with bacteriological work, a temperature of about 20° C. (=68° F.) is understood, while by 'incuba-

tion' temperature is meant one about the heat of the human body, *i.e.*, 37° C. (=98·6° F.).

The incubator, or warm chamber, consists essentially of an inner chamber of copper, fitted with a door; this chamber is surrounded with an outer casing, which is protected by one or more layers of felt, or some other

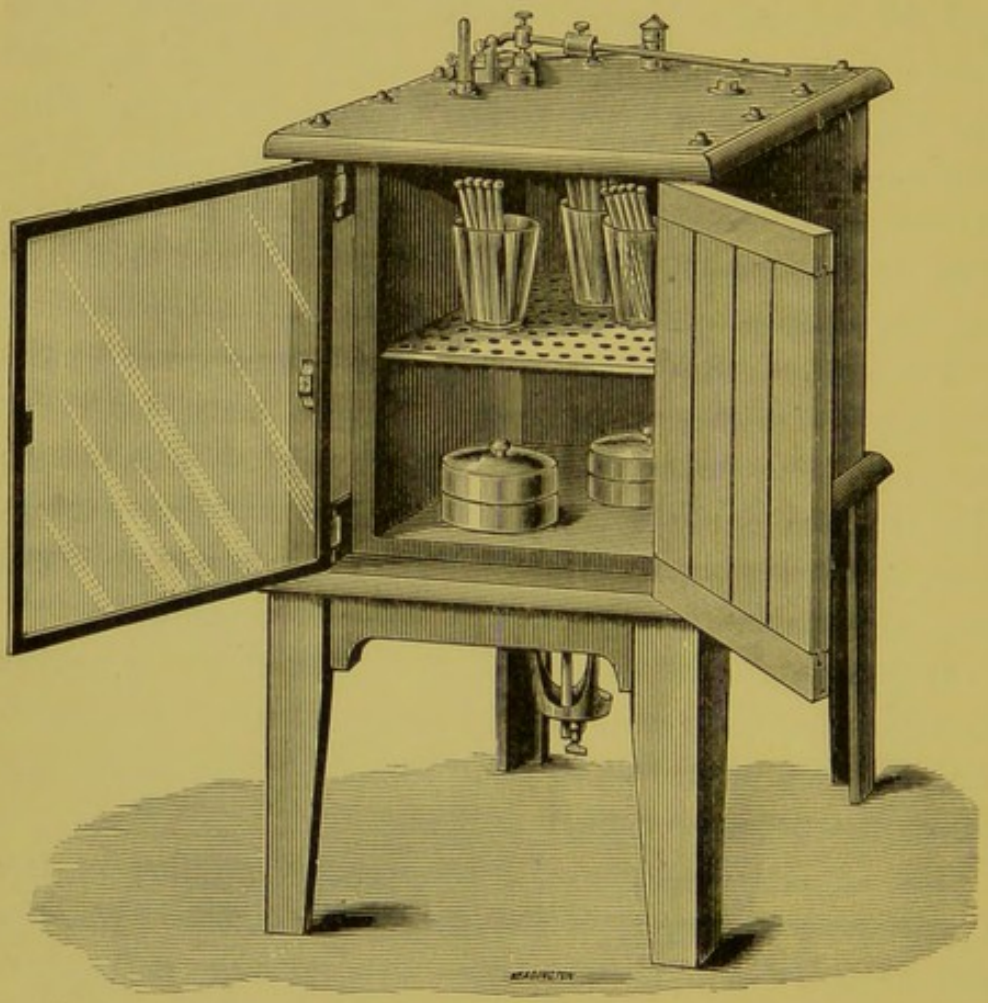


Fig. 7.—Incubator.

non-conducting material. The space between the two walls is filled with water, which is warmed to the necessary extent by means of a small gas-burner. To secure an even and regular temperature, the water or air in the inner chamber is controlled by means of a thermostat or regulator, generally a mercurial one of the Page or Reichert

type. These regulators depend upon the rise and fall of mercury upon the application of heat and cold; thus, when the temperature falls the gas-flame increases in size, since by the contraction of the mercury in the thermo-regulator more gas passes; again, when the temperature rises too much of the gas is partially cut off by the expansion of the mercury. In case the main gas opening should become quite closed by the expansion of the mercury, the thermo-regulators are fitted with a small by-pass pipe, which allows so much gas to pass as to maintain a small pilot light to prevent the flame becoming extinct. In addition to the mercury gas-regulators, of which so many types are in use, various other ingenious devices are employed to regulate the temperature of the incubators, among which may be mentioned those depending upon the differential expansion of metals, electric alarms, and the most recent invention, which is known as the Excelsior gas-valve, in which the pressure of ether and other vapours is employed, contained in a flexible envelope; this, acting upon a lever, controls the gas-supply.

The best form of incubator for bacteriological purposes is a modification of the Champion egg-incubator devised by Messrs. Hearson and Co., of 235, Regent Street, W., which is fitted with the Excelsior gas-valve. The following is a description of the Hearson incubator:

The tank which forms the water-jacket is made of stout copper, the junctions in which are effected by a means which the experience of many years has proved effectual in avoiding the local galvanic action so prejudicial to ordinary solder. The outer case is made of pine, and the space between it and the water-jacket is filled with a non-conductor of heat. The chamber is closed with an inner glass door and an outer wooden one. In the incubator, and immediately below the Excelsior valve, which occupies

the left-hand back top corner of the apparatus, is a small metallic hermetically-sealed capsule, which contains a few drops of a liquid having a boiling-point at or near the temperature which we wish to maintain the heated chamber.

The capsule lies in a little holder suspended below the tube, through which the needle under the screw P (Fig. 7 and Fig. 8) passes.

Soldered to the upper side of the capsule is a thick piece of metal, having a central depression. In this depression the lower end of the needle seen in Fig. 8 rests, and the upper end of the needle enters a short distance into the

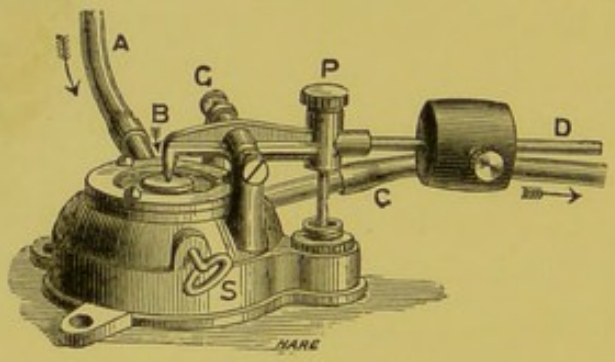


Fig. 8.—Excelsior Gas-valve.

socket end of the screw P. Communication is thus established between the capsule inside and the Excelsior gas-valve outside.

Above is shown an enlarged view of the gas-valve seen in Fig. 7.

A is the inlet for gas. C, the outlet to burner. BD, a lever pivoted to standards at G, and acted upon by the capsule through the needle which enters the socket below the screw P.

The construction of the acting portion of this valve is such that, whenever the end B of the lever BD presses on the disc below the end B, the main supply of gas is entirely cut off. At such times, however, a very small portion of

gas passes from A to C, through an aperture inside the valve, the size of which aperture can be adjusted by the screw-needle S, hence the gas-flame which burns in a little lantern below the incubator is never extinguished.

The reader will no doubt have grasped the fact that the expansion of the capsule, owing to the boiling of its contents, provides the motive force for acting upon the lever BD, and as this expansion only takes place at a predetermined temperature, the lever will only be acted upon when the critical temperature is reached, no sensible effect being produced at even one degree below that at which the capsule is desired to act. We have thus a thermostat acting at a nearly fixed and predetermined temperature, and without any further additions to the apparatus already described, we should have (were it not for slight barometric variations) an *absolutely* fixed temperature regulator.

Changes in atmospheric pressure, however, tend to make the temperature fluctuate about a degree (F.) on either side of the normal, if observations be taken extending over considerable intervals of time.

To compensate for these variations, if it be desired to do so, a sliding weight runs on the lever-rod D. But this weight serves a yet more important function.

It gives us the opportunity of retarding within certain limits the boiling-point of the capsule, and of thus adjusting the temperature at which the capsule shall expand several degrees above that at which (with the weight quite to the left) it first commenced to act.

By this means the operator is enabled to obtain a range of about 8° with any particular capsule, and as these can be made to act at any temperature from 60° to about 300° F., we are enabled to maintain any desired temperature in incubators, sterilisers, water-baths, etc.

In actual practice it is found that the temperature can be

maintained uniform within half a degree without readjustment of any part for months together, and this, too, in defiance of great changes of gas-pressure, and of air-temperature in the room in which the apparatus is working.

Instructions for starting an Incubator regulated with a Capsule and an Excelsior Valve.

These instructions must be carried out in the order in which the paragraphs are numbered.

1. On receiving the apparatus, and having examined it and found it perfect, place it where it is to stand, and make a connection between the pipe A (Fig. 8) and the gas-service, but do not light the gas.

2. Look up under the apparatus, and on the right-hand side you will see an open-ended pipe. On the bench below this place a basin to receive the overflow.

3. In like manner under the left-hand side you will find the emptying-pipe furnished with a cock, which must be closed whilst the incubator is in use.

4. Open the doors of the incubator, and do not close them again until the following instructions have been carried out.

5. Fill the tank with warm water at the filling-tube in the top of the incubator until it runs out at the overflow below, and add a small quantity weekly, if necessary, to compensate for evaporation.

The middle row of figures written on the ticket pasted inside the door indicates on Fahrenheit's scale the temperature at which the capsule begins to boil. The water used to fill the tank should be at least 5° colder than this, and the temperature should be taken before the water is poured into the tank.

6. Push the lead weight on the lever quite to the left.

7. See that the needle below the milled head-screw P rests in the depression in the lower end of the screw.

8. Withdraw the by-pass screw S and the milled head-screw P three or four turns, then turn on the gas both at the service and under the lantern, and light it.

9. With one hand lift the lever D as high as it will go comfortably, but without putting any strain upon it. With your other hand turn the screw S in again until the flame is reduced to less than the size of an ordinary pea.

10. Release the lever, and as it falls the gas will suddenly rise to its full height.

11. Turn the milled head-screw P in the direction to raise the lever, and continue to do so until the flame is reduced to the same size as it appears when you hold the lever up with your hand.

12. Now give the milled head-screw P *one half-turn* backwards, and the gas will again rise to its full height.

13. By means of the cock below the lantern, reduce the flame to about the size of the bowl of an ordinary egg-spoon.

14. Shut the doors of the incubator and gently lower the thermometer down the brass-bushed hole near the Excelsior valve.

15. Put on the short glass chimney to protect the flame from draughts.

16. The flame in the lantern will now remain constant until the lever is pushed up by the expansion of the capsule.

17. When this is the case, the flame will decrease automatically until it is reduced to that size which shall supply just the amount of heat required to keep the interior at the temperature now indicated on the thermometer.

18. If after a lapse of some hours it is found that the

temperature does not continue to rise, and that the gas in the lantern is not lowered as described, it may be concluded that the heat is insufficient, and a little more gas may be turned on at the cock under the lantern.

19. The adjustment having been thus effected, nothing remains but to bring the interior up to the temperature desired.

20. This is effected by sliding the lead weight (which up to this time has been quite to the left) along the lever-rod D, one or two inches at a time at short intervals, until the desired temperature is indicated on the thermometer.

21. To get at the capsule, hold one hand under it inside the incubator, and with the other unscrew the milled nut which you will find below the screw P. The capsule-holder with the capsule in it will now fall into your hand.

Do not attempt to regulate the temperature by any adjustments of the gas-taps, or by altering the adjustments of the milled head-screw P or screw S. The temperature can only be successfully varied by varying the load on the lever, or by the substitution of another capsule having a higher or a lower boiling-point.

If you require a temperature only a degree or two less than that given when the weight is quite to the left, you may substitute a smaller weight; but if you require a higher temperature than that given with the weight quite to the right, you must have a capsule acting at a higher temperature.

If from any cause you suspect that the adjustment of the Excelsior valve has become deranged, *open the doors* of the incubator and turn out the gas, or pour cold water into the tank until the contents are reduced 5° below the boiling-point of the capsule, and make the adjustment *de novo* in the *same order* as before.

Failure to start the incubator at the first attempt may be

due to (1) filling with water too warm; (2) leaving the by-pass too high; (3) giving the screw P more than half a turn back after having screwed it down to cut the main supply off.

Messrs. Hearson and Son have applied the above principle to an incubator which is heated by means of a petroleum lamp. This form is a very convenient one for use in places where gas is not obtainable.

The outer case of this apparatus is similar to the one already described, save only that the woodwork on the right-hand side is carried lower down to form a support for the lantern in which the lamp T burns.

The general construction of the water-jacketed chamber is also the same; but there is a large water-space below the chamber to make room for a pipe L, which conveys the heated products from the flame through the water and back again to the lantern, the lantern being furnished with a second chimney, which discharges into the open air a short distance behind the one seen in the illustration.

A is the water-jacket surrounding the chamber containing the cultures.

O is the pipe through which the water-jacket is filled with water.

N is a cock for emptying the same.

M is the overflow.

S is the capsule contained in a case attached by a tube to the lever plate outside.

D is a lever pivoted on the left, and carrying at its free end a damper F, which when resting on the chimney V effectually closes it.

P is a screw for adjusting the damper when starting the apparatus. The end of this screw is concave, and into this concavity is inserted the upper end of a wire, the lower end of which rests on the capsule.

H is a lead weight for bringing more or less pressure to bear on the capsule.

K is the thermometer, the bulb of which is inside and the scale outside the heated chamber.

The apparatus having been adjusted according to the instructions, the action is as follows:

The heated products of combustion, not being able to find any exit at the chimney V, pass along the flue L, and, parting with the greater portion of their heat *en route*, return again to the lantern by a flue behind and parallel with the one seen in the section, and are thence conducted into the open air by a second chimney placed in the lantern a short distance behind the one covered by the damper F.

The products of combustion continue to move in this direction until the water, and consequently the chamber, are sufficiently heated to distend the capsule.

When this point is reached, the wire between S and P will be pushed up by the capsule, and the lever will cause the damper to rise more or less off the chimney V. In a short time the damper will be found to hang steadily in one position, and on examining the thermometer at intervals, the inside of the chamber will be found to remain steadily at one temperature.

The precautions to be observed with this incubator are much the same as those required in working with an incubator which acts with an Excelsior valve, and the temperature is adjusted in the same manner by moving the lead weight; but to make the matter quite clear we append the following instructions for working.

Instructions for working an Incubator heated by a Lamp.

22. Fill the tank with water at least 5° colder than the boiling-point of the capsule.

23. Push the lead weight quite to the left.

24. Turn the milled head-screw P until the damper F rests on the chimney V.

25. Drop the thermometer gently down the tube prepared to receive it, and see that the whole of the bulb and a little of the stem is visible inside.

26. Shut the doors of the incubator.

27. Fill the lamp with good American petroleum oil, and trim the wick so that when burning it shall produce a flame without tails.

28. Refill the lamp every twenty-four hours, and trim the wick as required.

29. Light the lamp, put on the chimney, and turn the flame low to prevent smoke.

30. To put the lamp into the lantern, pass your forefinger through the looped handle, and press the thumbpiece as far down as it will go. Slide the lamp into the lantern, release the thumbpiece, and you will now find that the chimney touches the horizontal plate in the lantern, and so establishes a direct communication between the lamp-burner and the flue. If the communication is complete, the oil good, and the lamp properly trimmed, there will be no smell whatever.

31. Turn the flame to about half the height at which it will burn without smoking.

32. The incubator may now be left for an hour, but before doing so the present temperature should be noted. On your return observe whether the temperature has risen, and if not, raise the lamp-flame a little.

33. The flame must always be somewhat in excess of that required to keep up the temperature. If you exceed the required amount considerably it is of no consequence, as the apparatus will get rid of it; but you will be burning more oil than is necessary.

34. When the damper rises (which it will do when the interior reaches the boiling-point of the capsule), you must move the lead weight along the lever as described in paragraph 20.

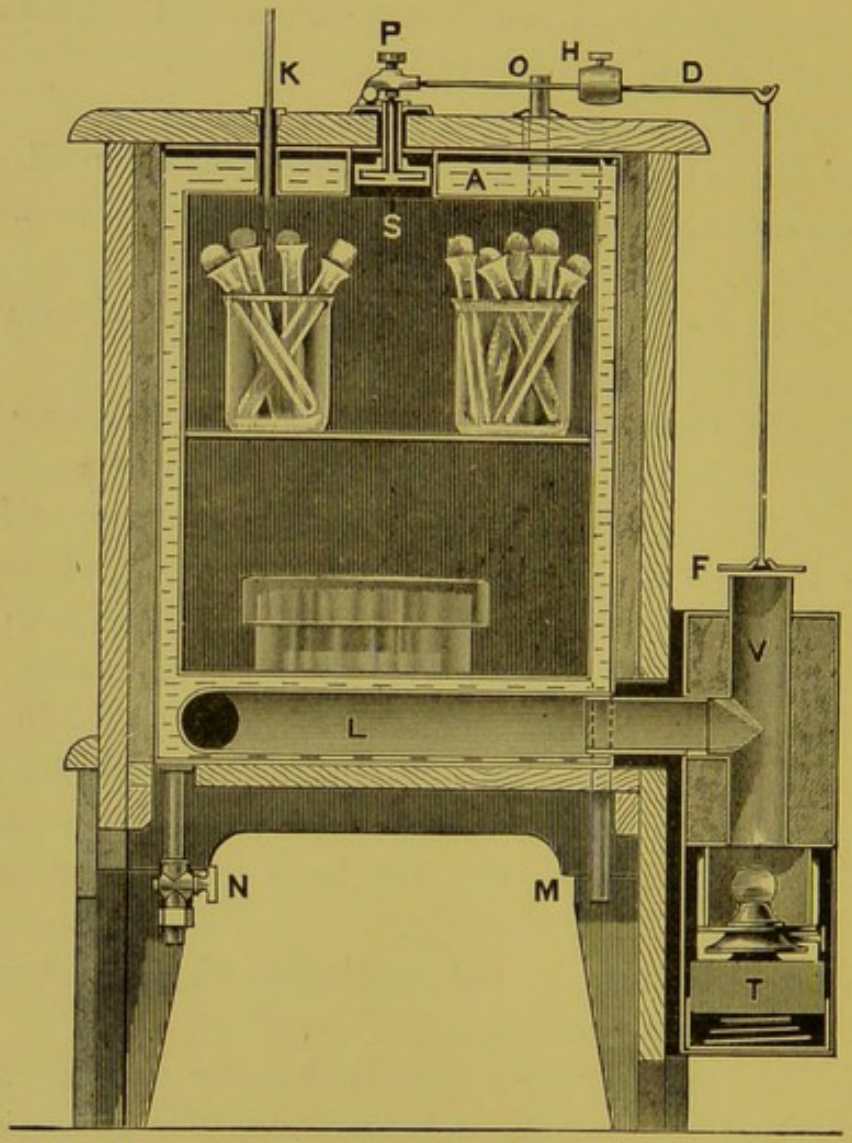


Fig. 9.—Incubator heated by a Lamp.

35. When the thermometer registers the desired temperature, clamp the lead weight to the lever by means of the milled head-screw which goes through it.

36. After having been once adjusted it will be found that the heat in the interior will remain constant, notwithstand-

ing the utmost changes of temperature occurring in these latitudes, nor will very great alterations in the size of the lamp-flame seriously alter the results.

37. The milled head-screw P must not be turned, after the first adjustment, during the whole time that the incubator is in use.

Observe the temperature before opening the door; observations taken afterwards are worthless.

The Cool Incubator.—It must be clearly understood that the ordinary incubator with the Excelsior valve is only suitable for temperatures at least 5° F. above the temperature of the room in which the incubator is used. In order to render bacteriological investigation by means of gelatine tubes and plates possible during hot summers and in tropical climates, Messrs. Hearson have devised a cool chamber or incubator in which ice is used. It consists of a water-jacketed chamber, similar to that already described, surmounted by a vessel, B, which contains ice, the whole apparatus being surrounded by a thick layer of non-conducting material and wood to protect it as far as possible from the effects of external influences.

The regulation of temperature within the chamber is effected by a small stream of water which runs continuously through the apparatus in one of three directions, the choice being automatically determined by a thermostatic capsule.

On the top of the apparatus is a lever plate and lever, M, similar to the one used in the lamp incubator already described, only in this case the damper is dispensed with. A bracket screwed to this plate supports a vertical shaft, pivoted on centres at the top and bottom, which carries a horizontal tube, C.

In the incubating chamber is a capsule in a holder, supported by a tube screwed to the lever plate.

A stiff wire communicates the motion of the capsule (as

in the other incubators) to the lever M, and this lever is so connected with the tube C that when the capsule expands the tube moves horizontally to the left.

At the side of the apparatus is a lantern containing an open boiler, F, heated by quite a small gas or petroleum lamp flame.

The bottom of the boiler is connected with the bottom of the water-jacket by a tube (not shown), so that the water in the boiler always stands at the same level as that in the water-jacket. The bottom of the ice vessel B has also an outlet which communicates with the water-jacket above the incubating chamber (not shown).

The water-jacket is provided at the top with an overflow and waste-pipe at N, through which the surplus water escapes.

The front end of the little tube C is bent downwards, and immediately under the bent end are two tubes, D and E, standing vertically side by side in an open vessel with a short interval between them.

The vertical tube E is connected with the top of the ice-box by a tube, E′, and the vertical tube D is connected with the boiler F by a tube, D′.

The valve K is connected on the right with a continuous water-supply, and on the left by means of a small india-rubber tube with the small tube C.

The apparatus having been adjusted according to the instructions, the action is as follows:

The stream of water passing the valve K flows along the tube C, down the tube D, and along the tube D′ to the boiler F, where it is heated, and thence flows into the water-jacket and increases the temperature.

After a time the capsule expands and moves the tube C to the left, thus causing the water to fall between the two tubes D and E.

In this case the water is collected in the open vessel in which the tubes D and E stand, and is conducted by the pipe H H′ to the waste-pipe N without producing any effect whatever on the incubating chamber.

If the temperature of the room in which the incubator is placed is above the boiling-point of the capsule, the horizontal tube will continue to travel towards the left, so that presently the water will run down the tube E, along the tube E′, and, passing through the ice-box, will so lower the

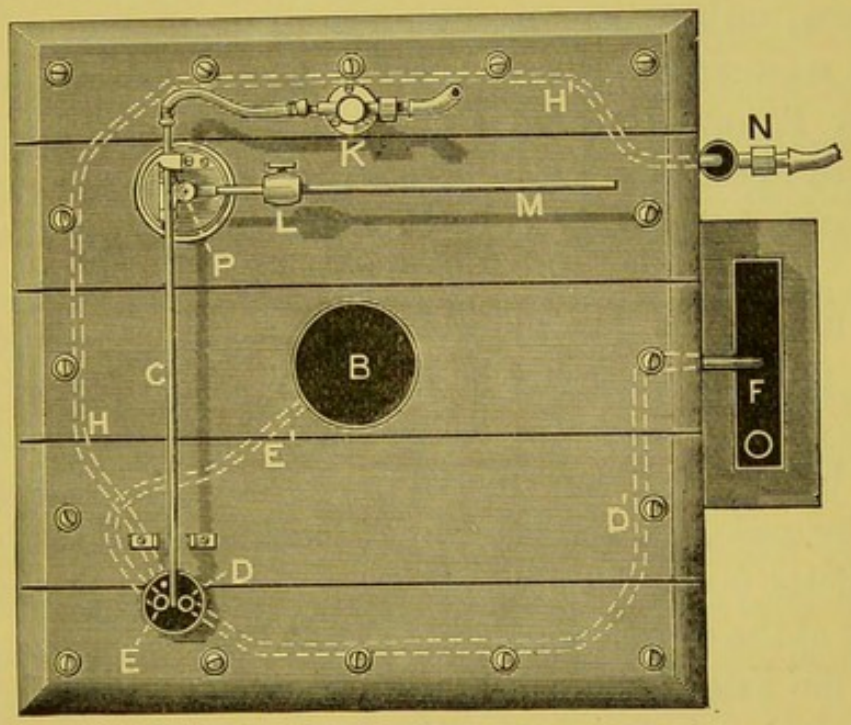


Fig. 10.—Plan of Cool Incubator.

inside temperature that the capsule will collapse a little and cause the flowing water to again take up a position midway between the two tubes.

A fraction of a degree is quite sufficient to determine which tube the water will fall into, so that the interior of the apparatus remains at a practically uniform temperature.

We have thus, so to speak, a reservoir of heat in the boiler and a reservoir of cold in the ice-box, which the

thermostat draws upon according to the requirements of the incubating chamber. When it is desired to work at a temperature below the temperature of the external air, it is essential that the ice-box B be kept supplied with ice. When the temperature required is above that of the external air, the lamp or gas must be lighted under the boiler F. If the required temperature be at or near the mean of the external air temperature, both ice and flame will be necessary to correct the gain or loss of heat due to external variations.

In starting the apparatus, water must be poured into the boiler F until it overflows at the waste-pipe. If it be desired to cool the water, ice may be placed in the boiler, or some of the water may be poured in through the ice-box.

If it be found that the water is not cool enough by the time that the incubator is filled, run some water through the ice-box. When the temperature inside the incubator is 5° below the boiling-point of the capsule, the adjustments can be made, but not before. The middle row of figures on the ticket inside the door indicates the critical or boiling-point of the capsule in degrees Fahrenheit.

If the water be cooled too much, that is of no consequence, as the object in first cooling the apparatus is to collapse the capsule.

Whilst the capsule is thus in a collapsed condition, the milled head-screw P must be turned until the stream of water runs down the centre of the tube D. In this position the running water will be directed towards the boiler, and, being heated, will in that condition pass into the water-jacket of the apparatus.

Having been thus adjusted, the milled head-screw P must not be further interfered with during the whole time that the apparatus is in use. After a lapse of two or three

hours the thermometer will be found to register a few degrees below that at which you desire to work. To bring the heat up to a proper working temperature you will now require to move the lead weight L on the lever M (which until now has been quite to the left) step by step towards the right, an inch or so at a time, and to observe the effect on the thermometer at hourly or half-hourly intervals. When the thermometer indicates the required temperature, clamp the weight to the lever.

The rate of flow of water is not very important. We have found a flow of 120 drops a minute sufficient to correct an external difference of 10°, but a fluid ounce per minute may be run through without ill effect, because as soon as the temperature for which the apparatus is adjusted is arrived at, the water falls between the two tubes and simply runs to waste.

The screw valve K on the top of the apparatus is for adjusting the water-supply, and the cock below the boiler is for emptying the apparatus.

The valve K should be permanently connected with a constant supply of water from a tank above the level of the apparatus. The usual house-supply will generally be found to answer the purpose perfectly, but it must be clearly understood that if the water ceases to flow there will be no regulation of temperature. The waste-pipe N should also be carried to a properly-trapped drain.

In climates where the external air is always above the temperature of the incubating chamber, the ice-box must be so frequently replenished that there is always ice in it, without which there is of course no cooling effect.

A lamp or glass may be used for heating the boiler, but need not be lighted unless the air is below the temperature required in the incubating chamber.

The Centrifugal Machine.—This machine has many useful

applications in bacteriological research, among which may be mentioned the separation of bacteria from liquids when held in suspension, as in the case of tubercle bacilli in milk. It can also be used to separate blood-corpuscles from fluids, fine precipitates from stains, etc. There are many types of centrifugal machines in use, but we have found the machine devised by Messrs. Leffmann and Beam for the

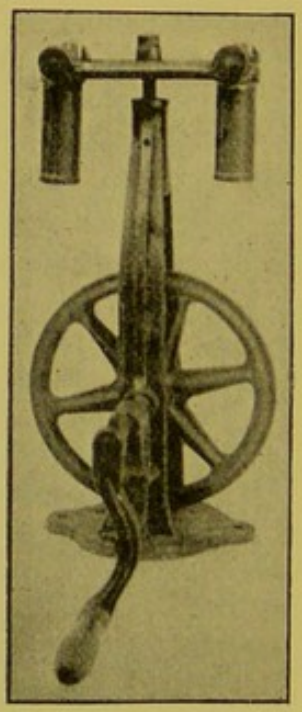


Fig. 11.—Centrifugal Machine.

estimation of fat in milk to be inexpensive, and in every way suitable for general purposes. This machine can be driven up to the rate of 3,000 revolutions per minute.

Inoculating Wires.—A number of these should be to hand, both straight and with small loops at the end. They are best made of fairly stout platinum wire, about 3 inches long, the ends of which are fused into glass rods to form handles for convenience in holding. This is easily done by means of a blowpipe flame. A looped

platinum wire is termed in Germany an *öse*, a term which, on account of its brevity, may be conveniently adopted.

In addition to the various utensils and chemicals which are to be found in every well-appointed chemical laboratory, such as scales, measures, gas-burners, saucepans, dishes, plates, flasks, beakers, funnels, filter-paper, test-tubes, pipettes, wood blocks, stands, knives, etc., and the general chemical reagents, the following apparatus and materials are required: scalpels, forceps, hypodermic syringes, gelatine, agar-agar, blood serum, cedar and clove oils, aniline, xylol, peptone, stains such as fuchsine, gentian-aniline-violet, methylene blue, methyl violet, eosine, hæmatoxylene, picric acid, carmine, paraffin and celloidine for imbedding, india-rubber caps for covering culture-tubes; and such special apparatus as cultivation-flasks, tubes, chemicals, etc., which are wanted from time to time, are necessary in a laboratory required for bacteriological research. As has already been frequently insisted upon, cleanliness in connection with bacteriological work is of the greatest importance, and therefore it follows that in the practical applications of bacteriology, particularly as applied to medicine, there is need of the utmost care in the cleaning of the hands, apparatus, and instruments used, in order to render aseptic procedure possible. The hands are best cleansed by the use of soap and a brush, followed by rinsing in 1 in 1,000 solution of corrosive sublimate, and, lastly, a rinsing in strong alcohol.

The Preparation of Nutrient Media.

It is necessary, in order to obtain a satisfactory knowledge of the biological characters of the various micro-organisms, to obtain pure cultures—that is, a culture containing one species only. The bacteria are artificially cultivated in

both liquid and solid culture media, which are prepared as far as possible similar to the natural soil on which they first grew. In the following pages will be found an account of the preparation of the culture media generally used by bacteriologists. The preparation of these nutrient media is not difficult, but it requires great care and attention to the directions in order to insure success.

Nutrient media are employed in test-tubes, small triangular (Erlenmeyer's) flasks, plates, or flat Petri dishes. All test-tubes, flasks, etc., employed in the preparation of nutrient media are thoroughly cleansed with strong nitric acid, after which treatment they are well rinsed with water to remove all traces of acid. The tubes are then allowed to drain until nearly dry, when they are finally rinsed out with a little strong alcohol, drained, and allowed to dry.

(1) **Preparation of Beef-broth.**—One pound of beef steak, freed from fat and connective tissue, is cut up and passed through a small mincing-machine. The finely-minced meat is then digested with 1,000 c.c. of water. It is then boiled, with constant stirring, for about twenty to thirty minutes in a tinned or enamelled saucepan, which is kept well covered. The broth is then strained through muslin, and then made up with distilled water to 1,000 c.c. to replace that evaporated off during the boiling. To the broth is then added 5 grammes of sodium chloride and 10 grammes of peptone. The latter is first rubbed up with a little of the broth in a glass mortar, after which it is added to the bulk.

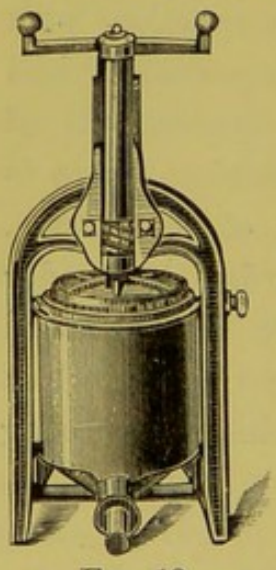


Fig. 12.
Meat Press.

The mixture is now boiled for five minutes, and then very carefully neutralised with a solution of sodium car-

bonate, making the solution very faintly alkaline to litmus-paper. The solution is again boiled for ten minutes, with constant agitation. The reaction is again tested with litmus-paper, and if still faintly alkaline, the solution is filtered into a flask through a double-pleated filter-paper. The filtered product, which should be absolutely clear and bright,* is then run into flasks (which are plugged with sterile cotton-wool and covered with tinfoil), and sterilised on three successive days in the steamer for fifteen or twenty minutes on each occasion.

(2) **Glycerine-broth.**—To every 100 c.c. of beef-broth prepared as above, 5 c.c. of glycerine is added, and shaken till thoroughly mixed. The product is run into tubes, and sterilized in the usual manner. Glycerine-broth is used for the cultivation of the tubercle bacillus.

(3) **Grape-sugar-broth.** — To each 100 c.c. of broth is added 2 grammes of commercial glucose. When the glucose is quite dissolved, the broth is sterilized as usual. Grape-sugar-broth is used in the cultivation of anaerobic bacteria.

(4) **Carbolated Broth.**—This fluid is generally known as Parietti's medium, and is used to restrain the growth of the various putrefactive and ordinary water bacteria when it is desired to isolate the *Bacillus typhosus* and *Coli communis* from water. An aqueous solution is prepared, containing 5 per cent. of phenol and 4 per cent. of hydrochloric acid. From 2 to 5 per cent. of this solution is added to ordinary sterile nutrient broth.

(5) **Nutrient Gelatine.**—This well-known and useful culture medium, on which most organisms grow, giving rise to

* If in spite of filtration the broth remains turbid, half the white of an egg is added to the cooled broth, well mixed, then raised and maintained at the boiling-point for ten minutes. The precipitated albumen is then filtered off and the filtrate sterilised as above directed.

very characteristic growths, cannot be used for temperatures above 20° C., as its melting-point is about 22° C., or a little higher. The nutrient gelatine prepared according to the formula of Koch and Löffler is the one most in use, and is made as follows: One pound of beef-steak is minced, as directed under the preparation of beef-broth, and digested with 1,000 c.c. of water, which is then slowly raised, with stirring, to the boiling-point. The infusion is then strained through muslin, after which 5 grammes of sodium chloride and 10 grammes of peptone are added; the mixture is then boiled for five minutes. The liquid is now carefully neutralised with a solution of sodium carbonate, and then rendered just faintly alkaline. Should the broth have been made too alkaline, a little dilute lactic acid is added until the proper degree of alkalinity is reached. The broth is now poured into a large flask, to which has been added previously 100 to 120 grammes of the finest colourless leaf gelatine. The gelatine is allowed to soak for an hour or so, when the flask is immersed in a water-bath until the gelatine is perfectly dissolved. The reaction of the mixture is again carefully tested with litmus, to see if it still remains just alkaline. Owing to the loss of water by evaporation, the volume of the media should be noted, and hot water added until the volume is about 1,100 c.c. The mixture is now cooled, and the white of one egg is carefully and thoroughly mixed in with the gelatine solution, which is then heated in a boiling-water bath for fifteen minutes, or until the whole of the albumin has been precipitated. The gelatine is now filtered through a double-pleated filter-paper previously moistened, and kept warm by means of a hot-water funnel; or, better, the filter can be placed in an ordinary funnel in the steam steriliser until the whole of the gelatine has run through perfectly clear. It will be found best to return the first 100 c.c. of filtrate to

the filter, when the filtrate will be found generally to run quite clear.

The gelatine thus prepared should be perfectly clear and of an amber-yellow colour, and should not become cloudy on heating. Nutrient gelatine should not be heated more

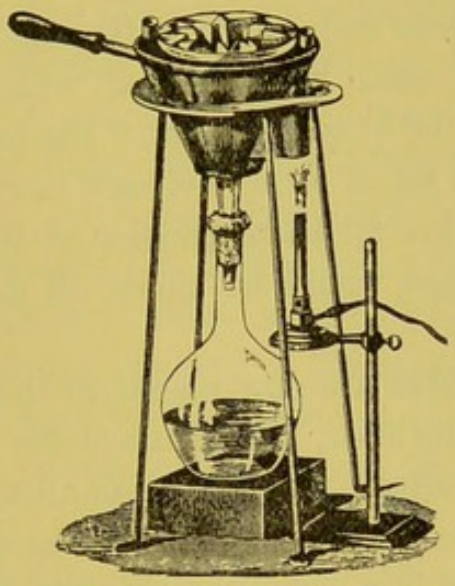


Fig. 13.—Apparatus for Filtering Media.

than necessary, since by so doing it may lose the property of setting when cold. If the medium becomes turbid after sterilisation, it is probably due to the mixture being too alkaline. Dilute lactic acid should always be used to reduce the alkalinity, and not the mineral acids, such as sulphuric or hydrochloric. The gelatine medium, prepared as above, can be sterilised on three successive days in the steam steriliser, for fifteen minutes on each occasion, or, better, run off into test-tubes at once. This is best done by melting the gelatine at a low temperature, and pouring it into a sterile separating-funnel; or a more convenient method to use after a little practice is a 100 c.c. pipette. The chemically clean test-tubes are placed in a rack, and from 5 to 15 c.c. of the media run in without soiling the edges of the tubes. When all the tubes are filled, they are plugged

with cotton-wool that has been heated in the hot-air steriliser for about an hour at 120° C. The plugs, when inserted, should be a little over an inch long, and should fit as tightly as possible. The best way of plugging tubes is to place a loose piece of wool on the mouth of the tube, which is then pressed home with a penholder with a rotary motion. The loose ends of the cotton-wool are then cut or singed off, and each tube covered with tinfoil; or the rubber caps can be at once put on, if the precaution is taken to insert a short bit of string between the cap and the edge of the tube to allow for the expansion of the air; if this is not done, the caps would be blown off during the sterilisation.

The capped tubes are now placed in the rack which fits into the steam steriliser, and steamed for three successive days for about fifteen to twenty minutes. After the last sterilisation, some of the tubes, while the contents are still liquid, are placed in a sloping position to allow the gelatine to expose as much surface as possible for streak cultures.

(6) **Carbolic Acid Gelatine.**—To every 100 c.c. of the above 10 per cent. gelatine solution is added 4 c.c. of a 5 per cent. solution of pure phenol. The tubes are then filled and sterilized as above. This gelatine is used for separating the typhoid bacillus or the *Bacterium coli communis*.

(7) **Grape-sugar Gelatine.**—Two per cent. of glucose is dissolved in the ordinary gelatine medium, and sterilised as usual.

(8) **Agar-agar.**—Twenty grammes of agar-agar* is finely cut up, and soaked in a dilute solution of acetic acid (5 c.c. glacial acid in 500 c.c. of water) for twenty minutes. This causes the agar to swell up and become more readily

* Agar-agar is a vegetable substance procured from some species of Japanese marine algæ. It is generally obtained commercially in the form of long threads, which should be cut up as finely as possible.

soluble. The agar is then thoroughly washed in water to free it from all traces of acid, after which it is well boiled with a litre of nutrient broth, prepared by the method already described, for about thirty-five to forty-five minutes, until all the agar has become quite dissolved; the water lost by evaporation is replaced from time to time. Care is then taken to see that the medium is faintly alkaline, after which it is cleared with egg-albumin, as described under the preparation of gelatine. The agar is then filtered through a damp filter, as directed for the making of nutrient gelatine. A very quick and good method of filtering agar is to use a small jelly-bag, which is suspended in the steam steriliser. Some workers prefer that the hot agar should be allowed to stand in the steam steriliser in a tall, cylindrical vessel till the flaky particles which cause the turbidity sink to the bottom, when the clear agar can be drawn off.

The agar, when filtered, is run into test-tubes, as already directed for gelatine. During the solidification of agar-tubes, a few drops of water, the 'water of condensation,' separates out, and prevents the firm adherence of the medium to the tubes. Esmarch recommends the addition of gum arabic to the medium, to prevent the slipping away from the surface of the glass. The water of condensation can also be got rid of by removing the india-rubber caps, and allowing the tubes to remain for a few days in the incubator at blood-heat.

Agar jelly has the distinctive property of remaining solid at 40° C., and only melting completely at 90° C.; hence this medium is well adapted for use as a culture medium for those micro-organisms which must be grown at the higher incubating temperatures. Nutrient agar is often quite clear when hot, but is almost always cloudy and opaque on cooling.

(9) **Glycerine Agar.**—This is prepared by the addition of 5 per cent. of pure glycerine to the nutrient agar prepared as above. This addition is particularly valuable, as it prevents the drying up of the medium, and is useful for growing the tubercle bacillus.

(10) **Grape-sugar Agar.**—The addition of 2 per cent. of glucose to nutrient agar is useful for the cultivation of anaerobic bacteria. The tubes for this purpose are filled two-thirds full.

(11) **Urine Gelatine and Agar.**—Fresh urine thickened with 10 per cent. of gelatine, or 2 per cent. of agar, with the addition of 1 per cent. of peptone and ½ per cent. of sodium chloride, is rendered feebly alkaline and filtered. The details of the method of preparation are the same as those already described for nutrient agar and gelatine. These two media are largely used in Germany, and are said to yield equally satisfactory results to those prepared from broth.

(12) **Peptone Solution.**—Ten grammes of peptone and five grammes of sodium chloride are dissolved in 1,000 c.c. of distilled water; the solution is then well boiled, and neutralised carefully in the usual manner. The solution is again boiled and filtered. The solution is then run into tubes, and steamed for fifteen minutes on three successive days. These tubes are used in the diagnosis for cholera.

(13) **Milk-tubes.**—'Separated' milk is carefully neutralised with sodium bicarbonate and filled into tubes, and sterilised as usual. These tubes are useful for the differentiation between typhoid and cholera.

Reaction of Media.—Some bacteria grow readily in a medium having an acid reaction, while the faintest trace of acid will prevent the growth of others. As a rule, the pathogenic species require a neutral or slightly alkaline medium.

(14) **Potato-tubes.**—Large and sound potatoes are thoroughly scrubbed until clean, and then with a large cork-borer cylindrical pieces are bored to fit into test-tubes. Each cylinder is cut into two halves diagonally, and each half placed in a test-tube. It is advisable to allow the cores of potato to rest on a moist plug of cotton-wool; this will keep the potato-cylinder moist. The tubes are then plugged and capped as usual, and sterilised for thirty minutes in the steam steriliser on three successive days.

Potatoes form an excellent culture medium for many organisms, and one which secures their development in a very characteristic way. Care should be taken to prevent overheating, otherwise the potatoes will lose their natural white colour, and become sodden in appearance. Potatoes possess a slightly acid reaction, so the surface is rendered faintly alkaline with sodium bicarbonate for the growth of certain micro-organisms whose growth requires alkalinity.

(15) **Blood Serum.**—Blood serum is an excellent medium for all the pathogenic organisms, all of which grow with greater rapidity on this medium than on any other. It does not matter much what animal the blood is taken from, but the blood of the horse yields the lightest-coloured serum. Blood from horses, bullocks, pigs, and sheep is generally to be obtained from slaughter-men. The blood from the jugular vein or an incised wound is allowed to run into a tall glass vessel. The vessel containing the blood is at once placed in a cool place without the least shaking, and allowed to stand overnight, when it will be found that a firm clot has formed; the clear serum is then drawn off by means of a glass siphon or large pipette. The serum is rendered faintly alkaline and run off into test-tubes, which are then plugged as usual, and laid on a slanting surface, and the serum made to set by heating in the hot-air steriliser to 75° C. The tubes can then be

sterilised in the usual way by steaming on three successive occasions. We have found the above to be the most easy method of preparing blood serum, and one which gives perfectly satisfactory results. The serum, when so prepared, should have a jelly-like consistency, and is of an opalescent, yellowish-white colour.

The serum from human blood, which is sometimes to be obtained at operations and from placentæ, is used by some workers, but its use presents no advantages over that obtained from the horse or other animals.

Modifications of Blood Serum.—The fluid obtained from hydroceles, cysts, or dropsical effusions is practically the same in composition as blood serum, and the method of preparing nutrient media from it is the same as in the case of blood serum.

(16) **Löffler's Medium.**—This medium, which is used for the rapid diagnosis of diphtheria, is prepared as follows: Two parts of blood serum are mixed with one part of nutrient grape-sugar broth. The tubes are then solidified in a slanting position, and treated as in the case of the ordinary serum tubes.

(17) **Egg Albumin.**—The albumin from birds' eggs is a very convenient and good medium for the growth of many bacteria. The albumin is carefully separated from the yolk, and treated as directed under the preparation of blood serum tubes. The white from plovers' eggs yields an almost transparent medium. Hens' eggs may, according to Hüppe, be themselves used with advantage as nutrient media. The newly-laid eggs are washed in soda solution, and then laid in 1 in 2,000 mercury bichloride solution for a short time; the eggs are now thoroughly rinsed in water that has been well boiled. The eggs are finally rinsed in strong alcohol and ether before they are inoculated. The inoculation is performed as follows: The end is pierced

with a sterile needle, and the material to be inoculated is introduced into the egg by means of a glass capillary tube, from which it is blown with great care. The hole is now closed with sterile cotton-wool. This method of cultivation is particularly well adapted for the cultivation of the anaerobic bacteria.

(18) **Bread.**—Bread and pastes formed by boiling up wheaten flour or ground rice with water are employed particularly for the growth of moulds. Slices of bread or layers of the paste are placed in Petri dishes and steamed, as in the case of other media.

(19) **Malt Extract.**—Solutions of malt extract and infusions of raisins and other fruits are extensively employed in the study of the yeasts. The foregoing materials thickened with gelatine or agar are useful for the growth of those organisms which, like the yeasts and moulds, are favoured by an acid medium.

(20) **Irish Moss Jelly.**—This medium was devised by Miquel for the study of the 'thermophilic' organisms, which do not grow at a lower temperature than 50° C. This medium, generally known as Miquel's high temperature jelly, is prepared as follows: 400 grammes of Irish moss (Carragheen, *Fuscus crispus*) are placed in 10 litres of boiling water and boiled for several hours; the liquid is then passed through a sieve, the filtrate boiled again, and strained through fine linen. The filtrate is slowly evaporated on a water-bath, and the residue dried at about 45° C. On adding 1 to 2 per cent. of the gelatinous substance so obtained to ordinary nutrient broth, a culture medium is obtained which remains solid at 50° C.

(21) **Silica Jelly.**—This novel and ingenious preparation, which is wholly destitute of organic matter, was devised by Kühne, and was used by him and Frankland in their researches on the organisms of 'nitrification,' which will not

grow on an organic medium. In this preparation the gelatinous consistency is obtained by means of dialysed silicic acid. The method of preparation is as follows: Two solutions of the following composition are prepared:

(*a*) Ammonium sulphate, 0·4 gramme.
Magnesium sulphate, 0·05 gramme.
Calcium chloride, trace.
Distilled water, 50·0 c.c.

(*b*) Potassium phosphate, 0·1 gramme.
Sodium carbonate, 0·75 gramme.
Distilled water, 50·0 c.c.

These two solutions are rendered sterile by the usual method, after which they are mixed.

A sterile solution of dialysed silicic acid is now prepared as follows: A solution of potassium or sodium silicate is poured into dilute hydrochloric acid; the mixture is then placed in a dialyser, the outside of which is kept surrounded with running water during the first day, and subsequently with distilled water, which is frequently changed until it yields no trace of turbidity with silver nitrate, thus showing the whole of the chlorides to have been extracted. The contents of the dialyser, if the solution of alkaline silicate originally employed was not too strong, will be quite clear. This liquid is then poured into a flask and concentrated by boiling until it is of such a strength that it is found that, on cooling a little of the solution and mixing it with one-third of the above mixed alkaline solutions, it readily gelatinises on standing. When the solution of silicic acid is found to give this result, it is cooled, and one-third to one-half of its volume of the mixed alkaline solutions (*a* and *b*) are added, the solutions well mixed, and at once poured into Petri dishes or flat-bottomed flasks. The medium should gelatinise in from five to fifteen minutes. The material containing the organisms for ex-

amination is introduced and thoroughly mixed, before gelatinisation takes place; or a 'streak' culture may be made on the surface after the medium has solidified.

A large number of modifications and various combinations of many of the culture media described in the foregoing pages are employed by various workers in bacteriological research. Amongst others may be mentioned: mixtures of potato-paste with gelatine; gelatine prepared with milk; mixtures of nutrient gelatine and agar; and solutions containing combinations of inorganic and organic salts, such as alkaline phosphates and tartrates, etc.

In addition to the above, various substances are added to culture media to detect by qualitative reactions the products of growth of the organisms under examination. For instance, litmus, Congo-red, and iron salts are frequent additions made to demonstrate the formation of acids, alkalies, and sulphuretted hydrogen.

CHAPTER III.

METHODS OF BACTERIOLOGICAL STUDY— STAINING, ETC.

The study of micro-organisms by means of pure cultures—Methods of staining and mounting bacteria, their spores and flagella — The imbedding and cutting of sections of tissues—The staining of micro-organisms in sections.

For the study of the growth of the bacteria, various materials, both liquid and solid, have already been described in the previous pages. The introduction of solid media by Koch in 1881 inaugurated a new era in the progress of our knowledge relating to the bacteria. It was observed by Koch that when a slice of cooked potato was exposed to the air, and afterwards kept moist and at a suitable temperature in a covered chamber, small isolated dots and patches made their appearance after a few days. The various centres or colonies may present very different characters both in shape and colour. It was found that each of these points was made up of micrococci and bacilli, and in nearly every case a pure cultivation or colony of a particular organism. Each individual organism which gained access to the potato was fixed *in situ*, and, being unable to move from the spot, commenced to grow, and in a short time the rapid multiplication of the bacteria gave rise to a colony, which soon became visible to the naked eye.

It was these observations which led Koch to devise his beautiful and simple methods of bacteriological study by means of gelatine plate cultures.

Koch found it necessary in his investigations to devise a medium that would be of such a composition that it would afford food material for the growth of the greater number of micro-organisms. It might be expected that it would be an easy task to find a food to suit all the requirements of the various bacteria, seeing that from one point of view they are all so similar in character ; but, as a matter of fact, there is the greatest diversity in their tastes, and media which are suited to the growth of one organism are totally unfit for the growth and nourishment of another. After a great deal of investigation Koch found that meat-broth, with the addition of salt, peptone, etc., thickened with gelatine, gave the best results in practice.

The methods of bacteriological study thus devised by Koch have enabled us to separate and study the morphological and biological characters of each species of bacteria free from the complications which led to such error and confusion when the employment of liquid media was the only available means of bacteriological study.

In all investigations connected with the bacteria, it must always be borne in mind by the student that our surroundings are always crowded with micro-organisms. Thus, it will be seen that it becomes of paramount necessity that every operation in connection with the study of the bacteria should be conducted in such a way as to prevent the possibility, or, at any rate, to reduce to a minimum the chance, of the introduction of foreign organisms. The precautions to be taken in bacteriological work cannot be too painstaking, and if in the descriptions of the preparation of the various nutrient media and processes too much emphasis may appear to have been laid on the necessity for the most absolute care to be taken in the sterilisation of vessels, and so on, to prevent contamination, it must be remembered that by neglecting to take the most trivial

precaution a great deal of labour may be rendered useless, and the work has to be done entirely afresh.

In the culture of the bacteria the store of nutrient material necessary for their growth becomes gradually used up by the vital activity of the organisms, and their gradual development and reproduction comes to a standstill. Some of the bacteria die from want of nourishment; while others, as has already been shown, develop permanent forms, or 'spores,' which are able to remain quiescent for long periods of time until favourable conditions of growth reappear. In order, therefore, to continue the propagation of bacteria in cultures, it is necessary to reinoculate them from time to time into fresh media.

We have already described the preparation and sterilisation of the various solid and liquid culture media, and will now give the manner in which they are used for the isolation and study of organisms, and the special advantages which they afford in particular cases.

Gelatine Plate Cultures. — Three test-tubes, containing nutrient gelatine, are placed in warm water at about 40° C. (= 104° F.) until the contents are liquid. This temperature is sufficient to keep the gelatine liquid, but is not high enough to destroy the vitality of the bacteria which are to be experimented upon. The tubes are then numbered 1, 2, and 3.

We next, by means of a platinum-wire loop, which has been previously sterilised at red heat, introduce in tube 1, after carefully withdrawing the plug, a small amount of the pure culture or mixture of organisms which it is desired to examine. Care must be taken not to introduce too much of this material, as it must be remembered that the smallest trace may contain millions of organisms. If the material added is rather too coherent, attempts must be made to separate the organisms by rubbing them with the point of

the platinum wire against the side of the tube below the surface of the gelatine. The wire is again sterilised by passing it through the flame, and when cool it is again introduced into tube 1, and a loopful of the gelatine transferred to tube 2, and the contents well mixed; after which a loopful from tube 2 is transferred to tube 3. The reason for using three tubes will now be apparent. It is usually impossible to introduce a few organisms into the first tube, so we effect our object by dilution; by the above procedure we commonly succeed in so reducing the number of organisms that only a few will develop upon the plate we subsequently make from it. It may so happen that the dilution may in some cases have been carried too far, in which event we shall obtain the plate we require from the second tube; but success in this operation is a matter of experience and judgment. When inoculating the tubes, care must be taken to hold the plug of cotton-wool between the fingers, best between the third and fourth, using the back of the hand, and thus twist it out of the tube, which must be again carefully returned into the tube after inoculation, without being allowed to come in contact with the surface of the hand or bench. During these operations some glass plates, from 8 to 10 centimetres wide and 10 to 12 centimetres long (the glass 'quarter-plates' used by photographers are a convenient size), are carefully cleaned and sterilised in the hot-air oven at 150° C. for an hour. A box made of sheet iron is very convenient for holding the plates during and after sterilisation. In the absence of a hot-air steriliser, the plates can be sterilised in an oven or over a flame by holding the plates in a pair of tongs.

In order that the liquid gelatine may be distributed evenly over the plates, the apparatus figured on p. 72 is used. This consists of a glass plate supported by a tripod. By means of a spirit-level the glass plate is adjusted to an

exactly horizontal position. The sterilised glass plate is placed in the glass tray shown in Fig. 14, and the gelatine from one of the prepared tubes quickly poured on to it, and distributed by means of a sterile wire over the surface,

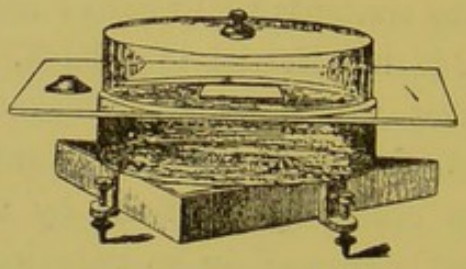


Fig. 14.—Plate Culture Apparatus.

care being taken not to bring the gelatine too near the edge. The glass cover is then lowered, and other plates can then be prepared in the same way by placing them on a metal or glass rack over the first plate.

Petri's Dishes.—The use of these has some advantages over the plate method of Koch. The dishes are from 10 to 20 centimetres wide and about 1·5 or 2 centimetres deep,

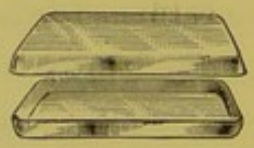


Fig. 15.—Petri Double Dish.

and have roughly-fitting covers of the same form as the dishes themselves. These dishes can be safely carried about, and do not need the levelling apparatus; moreover, the colonies may be examined and counted if desired without removing the lid, and, consequently, without the exposure to contamination to which Koch's form of plate is liable. Petri's double dishes are made both in the round and square form.

Esmarch's Roll Cultures.—A useful modification of Koch's method is that of Von Esmarch. Instead of pouring the

liquid gelatine medium upon plates or in shallow dishes, it is distributed in a thin layer upon the walls of a wide test-tube. This is done by rotating the tube upon a block of ice or in iced water. It is more convenient to turn the tubes upon a block of ice having a horizontal surface, in which a shallow grove is first made by means of a test-tube containing hot water. In the winter the tube can be revolved under the water service tap to solidify the jelly.

A little practice will enable the operator to distribute the jelly in an even layer on the walls of the tube, and as soon as they are quite solidified they are set aside for the colonies to develop. These tubes possess the advantage

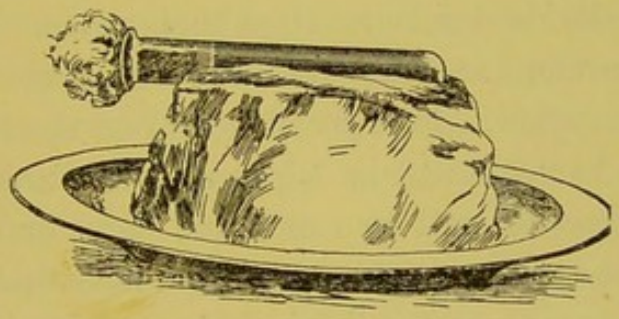


FIG. 16.—METHOD OF MAKING ESMARCH'S ROLL CULTURE.

that they are quickly made, they do not occupy much room, and are well protected against atmospheric germs. When the colonies have formed, they can readily be counted and examined by means of a lens.

Agar Plates.—The characters of the growth on nutrient agar media are not so varied as in the case of those on gelatine, and the plates are rather more difficult to manage; but this medium possesses the advantage of not liquefying at 40° C., whereby the nature of the growth can be studied at higher temperatures than is possible in the case of gelatine. Agar media are not liquefied in the manner that gelatine media are by many organisms.

The tubes containing the nutrient agar are stood in a beaker of boiling water until the contents are completely melted. After this the water in the beaker is cooled somewhat, and then allowed to stand, with a thermometer immersed in the water until a temperature of 40° C. is reached. The tubes are then immediately inoculated and the contents poured into a plate, as previously directed for the preparation of gelatine plates. It is a good plan to warm the dishes or plates to 40° C. before pouring the agar, and, above all, to work quickly, as the agar solidifies at 40° C., and after solidification has begun to take place an even distribution of the medium is no longer possible.

The gelatine plate cultures are kept at room-temperature until the individual colonies show themselves. The different bacteria develop at very different rates at the ordinary room-temperature. It is possible that on the following day colonies of bacteria will be apparent to the naked eye; but often one has to wait two or three days, or even longer, according to the special kinds of organisms that are present. Of course, growth in the summer is very much more rapid than in the winter. At first it is difficult to distinguish the colonies from small air-bubbles. As they grow, however, the different colonies may be distinguished one from the other by a great number of different characteristics. Some are spherical, and these may be transparent or opaque, or they may have an opaque nucleus surrounded by a transparent zone. Again, the outlines may be irregular, giving rise to amœba-like, rosette or star-like forms, with fringed or bushy-like margins. In the case of those which liquefy the gelatine, they will be seen to sink somewhat, and to liquefy the gelatine in more or less wide circles, while in others the liquefaction is a much slower process, and is only visible after some time. Most of the liquefying organisms liquefy gelatine in a very characteristic

way. Some only liquefy the gelatine as far as the colony extends; others form insignificant point-like colonies, which are surrounded by a ring of fluid which may extend over the whole of the plate in a few hours. In cultivations on agar-agar these characteristics are lost, as no bacteria exercise any liquefying action on this medium.

In the case of the bacteria that do not liquefy gelatine, they may raise button-like prominences upon the surface, or form drop-like collections or thick, compact masses; or they may form zone-like rings or concentric layers. Some colonies develop only on the surface of the media, others in the depth. The different colours of the colonies also afford distinguishing traits. They are only rarely colourless and transparent; as a rule, they are more or less coloured. The predominating colours are yellow and white; these occur in every possible tone. Not infrequently the colony remains colourless, while the surrounding gelatine may become coloured. In addition to the bacterial colonies proper, colonies of various coloured moulds very frequently appear, but these are never mistaken for colonies of bacteria, as they are always characteristic on account of their raised and feather-like hyphæ.

Having thus described the methods of isolating micro-organisms from a mixture by means of plate cultures, it becomes necessary to further separate and study each individual colony. It should not be overlooked that isolated colonies do not necessarily contain only one species, as they may not have developed from a single cell.

To further study the organisms thus isolated by means of plate cultures, it is necessary to inoculate from the colonies into tubes of various nutrient media to determine the morphological and biological characters of the micro-organisms under examination, and thus gain a knowledge of the class to which the organism belongs. This proce-

dure is carried out as follows: A portion of a colony is removed on the point of a sterile platinum wire and transferred to gelatine and agar in the form of 'streak' and 'stab' cultures, and in some cases to other media. There are several mechanical contrivances sold for the purpose of accurately picking out the particular colony it is desired to examine, but with a little skill the use of a simple inoculating wire is perfectly satisfactory in practice.

'Streak' Cultures.—For the 'streak' cultures we use tubes in which the nutrient gelatine, or agar, has been solidified in an oblique position, so as to expose as much surface as possible. The tube is held in a horizontal position, to prevent aerial organisms from falling into the tube, and the plug is carefully withdrawn with the third and fourth fingers of the right hand, using the back of the hand. The platinum wire (which has been previously sterilised by heating to redness in flame), with a trace of the colony on the point, is carefully passed down the tube, so as not to touch the sides, and is gently drawn along the centre of the nutrient medium, using a light but even pressure. The wire, on removal, is at once sterilised by heating, and the cotton-wool plug returned to the tube, having been previously singed in the flame. The whole operation is carried out as quickly as possible, so as to reduce the chance of outside contamination to a minimum.

The 'streak' cultures in the case of gelatine are kept at a temperature of 70° F.; when on agar, they are incubated at about 100° F. The colonies may confine themselves to the actual inoculation stroke, or they may spread themselves out until the whole surface of the medium is covered with the growth. The growth, again, may flourish only on the surface, as is generally the case, or the organisms may grow downwards into the medium in the form of hair-like or radiating runners. Some organisms will develop on the

surface of the medium in the form of isolated drops, which do not coalesce; or they may form skin-like ridges, as is the case in the growth of the tubercle bacillus.

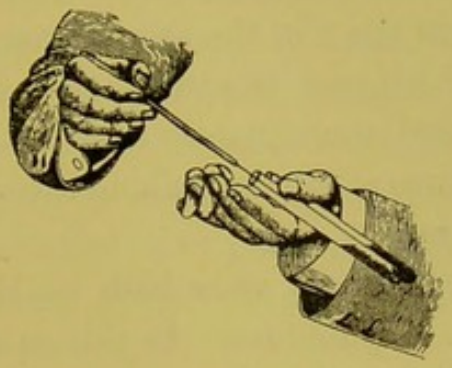


FIG. 17.—METHOD OF MAKING A 'STREAK' CULTURE.

'Stab' Cultures.—A platinum wire inoculated with the infecting material is thrust into the depth of an ordinary culture-tube containing about 10 c.c. of nutrient medium, care being taken to introduce the wire in a central line and in a direction parallel with the sides of the tube. It is

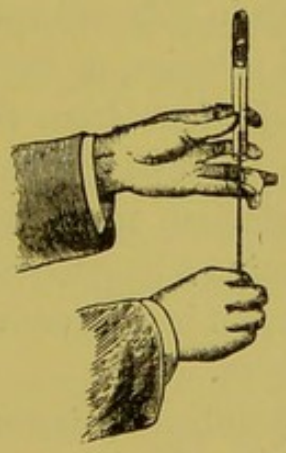


FIG 18.—METHOD OF MAKING A 'STAB' CULTURE.

best to always hold the tube during the inoculation in an inverted position to prevent the risk of contamination, and to singe the plug before returning it to the tube.

The characters of the growth in these 'stab' or 'depth'

cultures are very various. In the case of non-liquefying organisms, the growth may be entirely on the surface or only in the depth. In the first case, the organism is aerobic —that is, it requires oxygen for its growth, and will only grow in presence of this gas ; in the second case, the organism is anaerobic, in which case it cannot grow in the presence of oxygen or air, and consequently does not grow upon the surface of the culture medium or along the upper portion of the line of puncture.

The growth, again, may grow both on the surface and also along the line of puncture. In this case the organism is not strictly aerobic, but may grow either in the presence or absence of oxygen, and is thus a facultative anaerobe.

Again, we have differences as to the character of the growth both along the line of puncture and on the surface. The surface growth may be composed of a piled-up mass at the point where the rod entered the gelatine, or the growth may form a layer which entirely covers the surface of the medium. The growth along the line of inoculation in the depth differs very much in different species. We may have a number of spherical colonies, or we may have little tufts forming moss-like projections from the line of puncture. The characters of the liquefying organisms are very characteristic. The liquefaction may take place all along the line of inoculation, forming a long narrow funnel of liquefied gelatine, or we may have a broad funnel, or a wide cup-like cavity of liquefied medium.

'Shake' Cultures.—A tube of gelatine or agar medium is liquefied by heating the tube in a beaker of hot water, which is then slowly cooled until the temperature of the water is 40° C. The medium is then inoculated with the organism under examination. The plug is replaced, and the tube well shaken to distribute the organisms evenly through the medium, care being taken not to allow

any of the gelatine to touch the cotton-wool plug. The contents of the tube are allowed to set in cold water. On incubating the tube at the room or higher temperatures as the case may be, many organisms will be found to give rise to the formation of gas, the bubbles of which will become larger in size as growth increases.

Culture of Anaerobic Bacteria.—In addition to the method of studying anaerobic organisms by means of 'stab' cultures, many methods have been employed from time to time, which depend upon the withdrawal of the oxygen from the culture-tubes.

The most simple method is to place the tubes, or plates, under the receiver of an air-pump, and then withdraw the air.

Koch lays a thin sterilised plate of mica upon the gelatine or agar plate; this adheres closely to the surface of the nutrient medium and excludes the oxygen. The exclusion of air can be rendered more complete by running melted wax round the edges of the plates.

Blücher and Botkin cultivate anaerobic organisms in an atmosphere of an indifferent gas, such as carbonic acid gas or hydrogen.

Grüber effects the complete removal of oxygen by the following means. The culture material is inoculated into a culture-tube by means of Esmarch's method. The cotton-wool plug is then pushed down the tube to about an inch in depth; and above is inserted an india-rubber stopper, which must be well-fitting, and which is pierced with a hole through which passes a glass tube. The air is then exhausted from the tube by connecting the tube to an air-pump. When this is done, the glass tube is sealed by means of a blowpipe flame. The eggs of birds can also be successfully used for the culture of many anaerobic organisms by the method which has been already described.

In addition to the various means described above of keeping cultures out of contact with oxygen, various additions of deoxydizing substances are added to the culture medium, as used in 'stab' cultures. The chief substance used for this purpose is glucose (grape-sugar), which, when added to the extent of 2 per cent. to nutrient gelatine or agar, gives very good results in the case of the bacillus of tetanus. Small additions of resorcine, formic acid, and sulphindigotate of sodium have also been employed as additions to culture media for the purpose of extracting oxygen.

Hanging-drop Cultures.—With a platinum loop, a drop of sterile broth is placed on a clean cover-glass which has been passed through a flame. This drop is then inoculated with a very minute trace of the organism under examination. A 'hollow' slide is then taken—that is, one with a concave excavation ground in the centre. The outside of the well is then painted round with a narrow ring of vaseline by means of a camel-hair brush; the cover-glass, with the drop of broth, is inverted and laid on the prepared slide, and gently pressed down in such a way as to make the cover-glass adhere firmly to the glass slide, so as to make an air-tight joint, and thus prevent the drying up of the drop by evaporation.

The slide is then examined under the microscope—first with a low power to find the edge of the drop, and then with the higher powers, since, as it appears bounded by a sharp line, the organisms in the drop can be more sharply focussed. The narrowest possible aperture of the diaphragm must be used. This method of examination is exceedingly useful in the examination of bacteria in their fresh state, the progress and changes in growth and peculiarities, such as motility, mode of propagation, and so on, being most distinctly brought under observation.

Permanent Cultures.—In order to preserve cultures of the various micro-organisms in a permanent form in such a way as to be available for future reference, evaporation of the water from the nutrient medium must be prevented by hermetically sealing the tube. This is most satisfactorily effected by sealing the tube by the blowpipe just above the culture. Many cultures can be kept in good condition for a long time by simply pushing in the cotton-wool plugs for about half an inch, and then filling up the small space thus left at the top of the tube by means of paraffin or sealing wax. Praunitz pours a thin layer of strongly-carbolized gelatine over the culture in the tube, which is then allowed to set, after which the tube is corked.

Cultures can also be killed and rendered permanent by the addition of a drop or two of concentrated formic aldehyde (formaldehyde) to the tube, after which the tube is corked, the cork being waxed or varnished over.

The Indol Reaction.—This test is a useful method for the differentiation of certain organisms; among those which give the reaction may be mentioned the cholera spirillum, the *Bacillus coli communis*, the *Spirillum Metschnikovi*, etc. This test depends upon the interaction of indol (which is one of the products elaborated in some bacterial cultures) with nitrous acid, to form nitroso-indol nitrate, which is of a red colour. The test is applied as follows: To 10 c.c. of the culture in ordinary alkaline peptone broth of the organism which has been growing for twenty-four hours at blood-heat, add 1 c.c. of a solution of potassium or sodium nitrite (containing ·02 in 100 c.c.), and then a few drops of concentrated sulphuric acid. *If indol is present, a rose to a deep-red coloration is produced.*

In the case of cultures of the cholera spirillum, only the addition of the sulphuric acid is required to bring about the reaction, as the necessary nitrite is already present, having

been formed by the reduction of the nitrate which is invariably contained in the peptone used in the culture medium. This test is sometimes called the *cholera-red reaction*.

The Staining and Mounting of Micro-organisms.

Owing to the very great difficulty of observing bacteria in their natural condition, even with the best microscopes, it becomes necessary to treat them in such a way as to make them easier of observation. This is done by staining them with various dyes. Staining constitutes an indispensable aid to the study of the finer details of the various organisms, and the great advance which has been made in our knowledge of the bacteria is largely due to the many ingenious methods of differential staining which have been devised for their identification.

Owing to the avidity with which the bacteria take up certain aniline dyes, it becomes possible to recognise them amongst the tissues of the animal body and in other places where otherwise they would escape notice. Not only by the use of staining reagents is much of the internal structure and other details, as spore formation, made out, but as the behaviour of various organisms is not the same to different dyes, this property serves to distinguish between various kinds of organisms which are not otherwise to be differentiated by simple microscopical examination.

Weigert, in 1876, found that bacteria could be stained with the basic aniline dyes, but not by the acid dyes or the natural colouring matters. Koch and other workers at once recognised the value of this discovery, and rapidly investigated the matter, and devised many of the methods now in use.

All the basic aniline dyes, such as gentian violet, fuchsine, methylene blue, methyl violet, Bismarck brown, etc., have

a very strong affinity for bacteria; whereas the acid coal-tar dyes, such as magenta, eosine, safranine, picric acid, and the natural dye stuffs, such as logwood and cochineal, do not possess this property.

Gentian violet and fuchsine are the two dyes most frequently used for the staining of bacteria. These stain quicker and more intensely than any others. In order to increase the staining properties of the dyes, certain reagents are added to the stains to act as mordants. Phenol, aniline oil, and alkalies are amongst the bodies most frequently employed for this purpose. A very large number of stains and staining methods have been devised by various workers from time to time, but we will only give a few of the most approved methods which are applicable to all ordinary purposes.

Stock solutions of concentrated alcoholic solutions of stains, such as gentian violet, fuchsine, and methylene blue, are prepared by allowing a large excess of the dye to digest for some time in strong alcohol, shaking the solution from time to time. The concentrated solutions are then filtered and preserved in stoppered bottles. Most organisms can be stained by means of a simple aqueous solution of the dye, prepared by the addition of a few drops of one of the above concentrated alcoholic solutions to water in the proportion of about 1 to 4. Care should be taken not to have the staining solution too strong, as it is very easy to overstain. The solution can be tested as follows: The dye should be of such a strength that ordinary print is just visible on placing a watch-glass full of the stain upon some ordinary printed matter. This, of course, only holds as a general rule, as a stronger or a weaker solution is sometimes required.

The best results are obtained by the use of one of the following solutions:

Ehrlich's Aniline Gentian Violet.—This powerful staining solution is prepared as follows:

Saturated alcoholic solution of gentian violet	11 c.c.
Saturated aqueous solution of aniline ...	100 c.c.

The aniline solution is prepared by shaking about 5 c.c. of colourless aniline with 100 c.c. of distilled water for some time, when most of the aniline passes into solution. This solution is now filtered through a wet filter, which will prevent the undissolved aniline from passing through into the filtrate. The gentian violet in this stain can be replaced by fuchsine or methyl violet, using 11 c.c. of saturated alcoholic solution.

The solution prepared as above should not be kept for longer than about two weeks, but should be made fresh and filtered before use.

Ziehl's Carbol-Fuchsine.—This stain is much the same as the above, except that, instead of aniline, carbolic acid (phenol) is used as the mordanting agent. The solution is prepared by taking:

Fuchsine	1 gramme
Phenol	5 grammes
Distilled water	100 c.c.

The fuchsine is very finely powdered and added to the water, together with the phenol; the whole is allowed to stand, with frequent agitation, until dissolved. Frequently 10 c.c. of alcohol are added to dissolve the fuchsine more easily; but this is not necessary, and the addition reduces the staining power of the solution. The solution is filtered before use. This solution has the advantage over those prepared with aniline, that it will keep any length of time, although its staining power is not so great.

Löffler's Methylene Blue.—This solution is prepared by taking:

Saturated alcoholic solution of methylene blue 30 c.c.
Caustic potash solution (1-10,000) ... ... 100 c.c.

This solution keeps well, and is very useful for those organisms which are very apt to overstain with the two preceding staining solutions, such as the *Bacillus diphtheriæ*, sarcina, yeasts, etc.

All the above staining reagents should be preserved in the dark when not in use.

Decolourising Agents.—It is found that when bacteria are stained by any of the above methods, they can be made to give up their stain, partly or wholly, by the application of various reagents, such as acids, iodine, alcohol, etc. The great importance of this fact will be seen when the staining of bacteria in the tissues is dealt with.

Cover-glass Preparations.—A chemically clean cover-glass* is taken up with a pair of forceps and drawn through the flame of a Bunsen burner or spirit-lamp to remove any faint trace of grease remaining on the cover-glass. A small droplet of water is then placed in the centre of the cover by means of a clean glass rod. A trace of the culture or other material to be examined is taken on the end of a sterile platinum wire and mixed with the drop of water, and the mixture spread out as evenly as possible over the cover-glass. Care should be taken not to convey particles of nutrient medium with the bacterial material on to the cover-glass, as in the subsequent treatment these are apt to become

* Mere rubbing with a cloth is not sufficient to clean cover-glasses. They are best cleaned as follows: The cover-glasses are first cleaned by rinsing in water, and wiped with a clean rag, after which they are heated for ten minutes in a mixture of strong sulphuric acid and bichromate of potash. After rinsing them in distilled water, they are immersed in dilute ammonia, and after that polished on a clean linen rag which is quite free from grease. The clean cover-glasses are best preserved in strong alcohol, or in a clean stoppered bottle, so as to be kept free from dust.

detached and carry the bacteria off the cover-glass with them. Only the smallest possible quantity of material should be used, otherwise the bacteria will be found to be too crowded; the right quantity to use is soon found by a little practice. The cover-glass is now allowed to dry either spontaneously, taking care to protect from dust, or by holding between the fingers some distance over a flame.

Now, in order to prevent the bacteria from becoming detached from the cover-glass during the subsequent washing, it is necessary to 'fix' the layer containing the bacteria. To 'fix' the bacteria, the cover-glass is held in a pair of forceps, and is passed (the side on which the bacteria are uppermost) through the flame of the Bunsen burner three times, at the same rate as the swing of the pendulum of an ordinary clock.

This *fixing* must be done with great care; if the cover-glass is not sufficiently heated, the bacteria come off during the washing, and if, on the other hand, the cover-glass is overheated, the bacteria lose their power of absorbing the stain.

The preparation is now stained by transferring a few drops of the stain on to the cover-glass by means of a pipette; or the cover-glass is laid face downwards upon the surface of the stain, which is contained in a watch-glass or small dish, in such a way that the cover-glass floats upon the surface of the liquid. It is best to hold the cover-glass by the edge between the thumb and first finger, and then to bring it as close as possible to the surface of the stain, and drop it suddenly.

The second method gives the best and most evenly-stained preparations after a little practice, but the first is somewhat easier. When using the first method, the cover-glass should be quite covered with the stain. If it is

desirable to quicken the staining process, as is necessary in the case of some organisms, by using hot staining solution, the cover-glass, well covered with the staining reagent, is held by means of a pair of forceps over a low gas-flame until steam just begins to rise from the liquid; when this happens, the source of heat is removed. This treatment is then repeated at frequent intervals. A better method is to float the cover-glass face downwards upon the staining liquid, which has just previously been heated in a small dish, until the steam begins to rise. Great care must be taken not to allow the staining solutions to boil, as this causes a precipitation of colouring matter which renders the preparation useless.

The stain should, as a general rule, be filtered just before using, particularly gentian violet. The staining reagent is allowed to act for from three to ten minutes, the time varying according to the organism or particular stain being operated upon. The cover-glass is then well rinsed in running water until no more colouring matter comes away. When the washing is found to be complete, the cover-glass is held between the fingers and dried by very gentle warming over a low flame, or, better, it is allowed to dry spontaneously.

A small drop of a thick solution of Canada balsam in xylol is placed in the centre of a clean glass microscopic slip, and the cover-glass, preparation downwards, deposited on the drop of balsam, which then spreads out, and finally extends over the whole under-surface of the cover-glass. The preparation can now be observed by placing a drop of cedar-oil on the top of the cover-glass, and examining with the oil immersion lens. After examining, the cedar-oil on the cover-glass is carefully absorbed with filter-paper.

After a few days the balsam will harden, and become very hard after a few weeks.

If a permanent preparation is not required, the cover-glass can be examined immediately after washing off the excess of stain by placing on a glass slip, taking care to dry the top surface of the cover-glass before applying the drop of cedar-oil.

Smear Preparations.—In cases where micro-organisms are found in the blood and tissues of the body, their presence may be demonstrated by making a smear preparation. A drop of the blood is spread in a very thin layer over a perfectly clean cover-glass, or it may be brought in contact with the freshly-cut surface of the organ, such as the liver or spleen. Another method is to press the material between two cover-glasses, which are then separated by sliding them apart, thus leaving a thin layer of the material on each cover-glass. This method is particularly applicable to blood and sputum. The cover-glasses are now air-dried and stained, as described under cover-glass preparations.

'Impression' Cover-glass Preparations. — These preparations are frequently known as 'contact' preparations. They are made as follows: A cover-glass, cleaned as already directed, is held with a pair of forceps over a colony (which for this purpose should be a young one, not exceeding 2 millimetres in diameter), and placed with one edge resting on the nutrient surface, in a slanting position; the cover-glass is then allowed to sink gradually down over the colony, and very gently pressed. The cover-glass is now carefully lifted with a needle, and allowed to dry spontaneously in the air. The preparation is now 'fixed' and stained, as described under the preparation of ordinary cover-glass preparations. By this method many very beautiful preparations are yielded by a large number of bacteria growing in plate cultures, and which show very clearly the manner of growth and the arrangement of the organisms.

Staining of Spores.—The spores of microbes differ from the fully-formed organisms in the resisting power they offer to staining solutions. When ordinary cover-glass preparations, stained in the usual way, are made of some organisms—say, for instance, of *Bacillus anthracis* or *Bacillus megatherium*—bright unstained spots are sometimes seen, which may be isolated or in the middle or ends of the organisms. These are the spores which have resisted the colouring matter of the stain. All unstained spots in preparations are not necessarily spores, as many causes may give rise to this irregular appearance, among which may be mentioned faulty staining due to air-bubbles, the use of old staining solutions, or in the case of old cultures the organisms may have become degenerate and broken down ; these and many other causes may give rise to unstained spots which may be mistaken for spores.

Heat Method.—If an ordinary cover-glass preparation is passed through the flame about twelve times instead of three, as is usual in 'fixing,' stained for a few minutes with warm Ehrlich's gentian violet or Ziehl's fuchsine solution, and then well washed in water, the spores will be found to be deeply stained, whereas the bacilli will be found to be only faintly stained or colourless. The heating destroys the power of the organisms to take up the stain, thus leaving only the spores stained.

Neisser's Method.—The cover-glasses, prepared in the usual way, are stained with warm carbol-fuchsine solution for about thirty minutes. For this purpose it is best to float the cover-glasses on the surface of the stain contained in a small dish on a sand-bath, which is kept warm with a very small flame. The cover-glass is removed, washed in water, and then decolourised for a few seconds in a 3 per cent. alcoholic solution of hydrochloric acid. The cover-glass is now well washed in water, and counter-stained

with Löffler's methylene blue for three minutes, washed in water, blotted, dried, and mounted. Examined with a $\frac{1}{12}$ inch oil immersion lens, the bacilli will be found to be stained blue and the spores red.

This method gives very satisfactory and pretty preparations. The spores of *Bacillus megatherium* and *Bacillus filamentosus* are more easily stained than those of *Bacillus anthracis* or the hay bacillus (*Bacillus subtilis*).

Fiocca's Method.—This method is also very successful and rapid. About 1 c.c. of a saturated alcoholic solution of fuchsine is added to 20 c.c. of a 10 per cent. ammonia solution contained in a dish. The solution is heated until steam just commences to rise. The prepared cover-glasses are then immersed in the solution for from three to ten minutes, removed to a 20 per cent. solution of sulphuric acid to decolourise, washed in water, and finally counter-stained, as in the method given above.

Staining of Flagella. — The flagella or organs of motion which are attached to many bacilli and to some micrococci are much more difficult to demonstrate by staining than spores. In order to reveal the presence of these delicate, whip-like processes, it is necessary to have recourse to a complicated and ingenious method of staining, as they cannot be stained by any of the methods already described, as the flagella do not possess any affinity for dyes unless they are previously prepared with a 'mordant,' or a fixing material to enable the flagella to subsequently fix the dye. The following points must be carefully attended to in order to obtain satisfactory results: The cover-glasses must be absolutely clean, in which case a drop of water will spread itself evenly over the surface, and will not run back or refuse to adhere to any portion of it. The smallest particle of grease or dirt will absolutely prevent any satisfactory result being arrived at. No trace of nutrient medium should

be transferred to the cover-glass, or the result will be the same as if the cover-glass were dirty; and, owing to the particles becoming stained, they may completely conceal the faintly-stained flagella. Great care must be taken not to have too many organisms on the cover-glass. The best way to proceed is as follows: A trace of the microbic layer from a fresh agar culture is mixed very carefully and quickly with a drop of ordinary tap-water upon a slide, rubbing the material as little as possible against the slide, as the organs of movement, which are extremely delicate, may be broken off. Small drops of water are placed on a number of perfectly clean cover-glasses, and each of these inoculated with a trace of the dilution, which is gently spread over the cover-glasses. Better results are obtained by diluting a broth culture about twenty-four hours old. Tap-water should always be used for diluting, as the micro-organisms are so extraordinarily sensitive that they often cast off their flagella if placed in distilled water.

The prepared cover-glasses are now allowed to dry in the air spontaneously. The 'fixing' must then be done with great care by passing through the flame three times, by holding the cover-glass in the fingers instead of the forceps, as the temperature which is endured by the fingers does not injure, but is quite sufficient for the purpose. The preparations are now treated by one of the following methods:

Löffler's Method.—It is to Dr. Löffler that we are indebted for the following method, which he devised as the result of a long and tedious investigation. This process, when carefully carried out, gives very fine results. Löffler found that some organisms require an alkaline, and others an acid, mordant; and, again, the exact amount of alkalinity or acidity varies according to the particular organism under investigation. To render the mordant alkaline, he recom-

mends the use of a 1 per cent. solution of caustic soda, while for the acidification of the mordant he employs a dilute solution of sulphuric acid of such a strength that a given volume is exactly neutralised by the 1 per cent. soda solution.

The following is the composition of the mordant: Solution of tannin (20 parts tannin + 80 parts water); to 10 c.c. of this tannin solution add 5 c.c. of a cold aqueous solution of ferrous sulphate and 1 c.c. of a concentrated solution, either aqueous or alcoholic, of fuchsine.

The prepared cover-glasses, dried and fixed in the manner already described, are now treated with the mordant. The simple mordant, as above, can be used for some species, but in most cases, as already mentioned, it must be rendered alkaline or acid, to an extent which varies with the various organisms. The following are the additions of acid and alkali respectively made to the mordant as recommended by Dr. Löffler for particular organisms:

22 drops = 1 c.c.

Spirillum choleræ Asiaticæ	1 drop of acid to 16 c.c. of mordant.
,, *rubrum*... ...	9 drops ,, ,, ,, ,,
,, *Metchnikoffi* ...	4 ,, ,, ,, ,, ,,
Bacillus pyocyaneus ...	5 ,, ,, ,, ,, ,,
Spirillum concentricum ...	0 ,, ,, ,, ,, ,,
Bacillus mesentericus vulgatus	4 drops of alkali to 16 c.c. of mordant.
Micrococcus agilis	20 ,, ,, ,, ,, ,,
Typhoid bacillus	22 ,, ,, ,, ,, ,,
Bacillus subtilus	29 ,, ,, ,, ,, ,,
Bacillus œdematis maligni	36 ,, ,, ,, ,, ,,
Bacillus of symptomatic anthrax	35 ,, ,, ,, ,, ,,

The mordant, with the proper amount of acid or alkali added, is run from a pipette on to the cover-glass, and the latter is gently warmed over a flame with constant movement until steam just begins to form. On no account should it be allowed to boil, for if bubbles are once formed

the preparation is spoiled, as a fine precipitate of mordant is produced, which becomes stained later on and obscures flagella.

The heating must only last for from half to one minute; the liquid is poured off, and the cover-glass thoroughly washed with water; it is allowed to dry in the ordinary way. The preparation is then stained with a few drops of 5 per cent. aniline-water solution of fuchsine. The cover-glass may be gently warmed for about a minute, after which the stain is washed off very thoroughly with water; the cover-glass is now air-dried and mounted in balsam.

If the preparation is a successful one, the bacteria are of a very dark-red colour, and much thicker than when stained by the usual methods. In the ordinary processes of staining, only the protoplasmic body of the organism is coloured, the outer sheath-like covering but rarely taking up any dye at all; the above process, however, stains both the cell-wall and the protoplasmic contents, thus making the organism appear thicker than when stained in the ordinary manner. The flagella should be seen as a number of very fine curved threads, stretching out in an irregular manner from the bacilli, more or less intensely stained.

Van Ermengem's Method.—This method is easy, and gives good results in careful hands. The following solutions are prepared:

(*a*) Osmic acid (2 per cent. solution), 1 part.
Tannin (10 to 25 per cent. solution), 2 parts.
To each 100 c.c. of the tannin solution add 4 or 5 drops of acetic acid (glacial).

(*b*) Nitrate of silver (·25 to ·5 per cent. solution).

(*c*) Gallic acid, 5 grammes.
Tannin, 3 grammes.
Fused acetate of soda, 10 grammes.
Distilled water, 350 grammes.

The cover-glasses, prepared and fixed as already directed for Löffler's method, are covered with the osmic acid solution, which is allowed to act for half an hour. The cover-glass is washed in a large excess of distilled water, and then in alcohol. It is now dipped for three to five seconds in the nitrate of silver solution (*b*), and then, without washing, passed quickly through the gallo-tannic acid solution (*c*).

The preparation is now washed again in a fresh quantity of the silver nitrate solution (*b*), moving the cover-glass about gently, and withdrawing it when the solution begins to turn black. It is then washed thoroughly in several changes of water, and mounted first in water and examined with the $\frac{1}{12}$ inch oil immersion lens. If the preparation be satisfactory, float off the slide, carefully air-dry, and mount in xylol balsam.

If the flagella are not sufficiently stained, the cover-glass is again passed quickly through the gallo-tannin solution, and then treated with the silver nitrate solution as directed above. Care must be taken to change the nitrate of silver solution as soon as any precipitation shows itself.

Pitfield's Method.—Dr. Pitfield, of Philadelphia, has published* the following process for the staining of flagella, which consists of the use of but a single solution which is at once mordant and stain :

The solution should be made in two parts, which are filtered and mixed—(*a*) saturated solution of alum, 10 c.c., and saturated alcoholic solution of gentian violet, 1 c.c.; and (*b*) tannic acid, 1 gramme, and distilled water, 10 c.c. The solutions should be made with cold water, and immediately after mixing the stain is ready for use. The cover-slip is to be carefully cleaned, the grease being burned off in a flame, and after it has cooled the bacteria are spread upon it, well diluted with water, care being taken to exclude

* *Medical News*, Philadelphia, No. 10, vol. lxvii.

culture medium. After the preparation has been thoroughly dried in the air, it should be held over the flame with the fingers, as Löffler has directed. Afterwards the stain is gradually poured on the slip and heated gently, the fluid being brought almost to boiling-point; the slip, covered with the hot stain, should then be laid aside for one minute, washed in water, and mounted. Upon examination, the bacteria, both isolated and in clumps, will, if motile, be found to have the flagella clearly and delicately defined. In the middle of the cover-slip, as well as round the edges, the bacteria will be found equally well stained, the clumps being surrounded by a zone of delicate fringing flagella, each being well stained and distinctly outlined from its fellows. If a clear preparation is desired, the stain, after mixing, may be filtered; but Dr. Pitfield has found that the most reliable method is to use the unfiltered stain. In the case of the former, a clear fluid is produced without the detritus, etc., being precipitated on the glass around the micro-organisms, and all the flagella are stained, but not so distinctly as with the unfiltered solution. If the filtered stain is used, a second stain of aniline water, containing gentian violet, had better be used, which should be applied but a moment and then washed off, thus leaving a clean field, showing only the bacteria lightly stained, with their flagella still more lightly coloured. In examining the different bacteria, Dr. Pitfield found that the bacillus of typhoid fever, the colon bacillus, the cholera bacillus, and the bacillus of hog cholera, each stained well by this method, and without the addition of any acid or alkali to the mordant such as Löffler uses. The bacillus of typhoid fever showed the flagella most beautifully, and there seemed one flagellum to each cell that stained more deeply than the others and appeared larger and stronger.

The Examination of Organisms in Sections of Tissues.

The examination of bacteria in the tissues of the animal subject, it is needless to say, is of vast importance in medical research. Not only is much learnt of their position in the tissues, but also of the manner by which the organisms gain access to the body. In order to examine the tissues or organs of the animal body for the presence of micro-organisms, it is necessary to first prepare the thinnest possible sections of the organ or tissue in question. To prepare these, the tissues are first 'hardened' by the application of various reagents, and afterwards cut into sections with some form of microtome.

Freezing Method.—This is a very rapid method of preparing sections. This is done by means of an instrument known as a freezing-microtome.

A small piece of the fresh tissue is laid on the roughened plate, and then frozen hard by means of the ether-spray apparatus. Sections are now cut of the required thickness by means of the razor-blade attached to the apparatus. This freezing process frequently destroys delicate tissues, owing to the ice which forms bursting the cells of the tissue, so it is usual to harden the tissues before cutting the sections.

Hardening of Tissues.—The most satisfactory hardening reagent for bacteriological purposes is absolute alcohol. Small pieces of the tissue are cut about a cubic centimetre square; these are immersed for about forty-eight hours in absolute alcohol, which is changed frequently. A good plan is to place the alcohol in a wide-mouthed bottle, in the cork of which are fixed several needles. The pieces of tissue are placed on the needles in such a manner that, when the cork is fixed in the mouth of the bottle, the pieces of tissue are just beneath the surface of the alcohol.

The alcohol gradually abstracts the water from the tissue, and as that containing the water sinks to the bottom, fresh alcohol constantly comes in contact with the material. Tissues containing much water are, of course, more difficult to harden than those containing little.

Another method of hardening tissues is to soak them for thirty minutes in 5 per cent. solution of mercury bichloride (corrosive sublimate), kept at about 70° C.; after which treatment they are transferred to alcohol, when, after remaining about twelve hours, they are generally sufficiently hardened.

Imbedding.—After hardening the tissue is imbedded, in order to prepare it for the section-cutting machine. The simplest method is to soak the pieces of tissue in a strong solution of gum arabic for a few minutes, after which they are fixed to a cork. When dry or nearly dry, the whole is immersed for some time in alcohol, which abstracts the water from the gum, thus rendering the mass sufficiently firm to be cut. The cork, with the pieces of tissue firmly attached, is fixed in the clamp of the microtome. Sections of the material are now cut from ·02 to ·05 mm. in thickness. Great care must be taken to keep both the tissue and the knife-edge wet with alcohol. The sections so cut are carefully transferred to alcohol by means of a needle and brush.

Imbedding in Paraffin.—This method, although seldom employed, gives very good results. The material, after hardening in absolute alcohol, is placed in a mixture of equal parts of absolute alcohol and chloroform for twenty-four hours, and finally for the same length of time in pure chloroform. After this it is laid in paraffin dissolved by heat in chloroform, and remains in this solution for three hours at about 35° C. A paper mould or small cardboard box (such as a pill-box) is about one-third filled with

melted paraffin-wax; the prepared pieces of tissue are laid on the centre of the wax layer, then more melted paraffin-wax is poured on in such a way as to enclose the material in the centre of a small block of wax. When set, which may be hastened by immersion in cold water, the block is trimmed to a suitable form with a knife to fit the clamp of the microtome. The sections are cut without any moistening fluid.

The sections are transferred to xylol in order to dissolve out the paraffin, after which they are placed in absolute alcohol, and thence into water. If they do not sink in water, the paraffin has not been properly removed; in this case they are put back to soak in alcohol and xylol.

Imbedding in Celloidine.—The alcohol-hardened portions of tissue are fixed to bits of cork by means of a solution of celloidine in a mixture of alcohol and ether; and then, after the celloidine has set, they are immersed in absolute alcohol for some time (about twenty-four hours), when they become of a suitable consistence for cutting. The pieces are now soaked in a mixture of alcohol and ether, and finally in a celloidine solution of medium consistency, in which they remain for about twenty-four hours. The prepared pieces are now allowed to dry in the air for a short time, and then immersed in 30 per cent. alcohol for three or four days. The celloidine becomes first cloudy and then converted into an opaque, milk-white mass, which is of a sufficient consistency to be cut by the microtome. By this method the sections are saturated, so to speak, with celloidine, which is capable of taking up the stain in the same manner as the actual tissue.

Very fine sections are obtained by this method even with the most refractory materials; for instance, satisfactory preparations of actinomyces in sections can be obtained

by this process, which cannot be obtained by any other method.

The Staining of Bacteria in Sections.—There are a great number of different methods published for the staining of bacteria in sections of tissue. The following procedure is common to most of the published methods: The sections are transferred from the alcohol to water; they are then subjected to the action of the stain, which varies from a few minutes to several hours. The time is in some cases shortened by warming the staining solution. The sections are washed, and then decolourised by some suitable reagent; the sections are again washed, then counter-stained if necessary. The sections are now dehydrated with alcohol, and then cleared with xylol or oil of cloves. Xylol is preferable to oil of cloves as a clearing agent, as it has no solvent action on the stains, does not resinify on exposure to the air, and evaporates without leaving a deposit. Great care must be taken to remove all the water from the section by means of alcohol before transferring to the xylol, otherwise the section will not properly clear. After remaining in the xylol for about five minutes, the section is removed by means of a section-lifter, and then laid out flat by careful manipulation with two small glass rods on a clean microscope slip; the excess of clearing agent is removed by careful blotting with two or more thicknesses of filter-paper. A drop of thick solution of Canada balsam in xylol is dropped on the section, and a cover-glass laid on in such a way that the drop of balsam covers up the section, and extends over the whole under-surface of the cover-glass, as in the case of simple cover-glass preparations. The preparation is now ready for examination with the oil immersion lens.

Löffler's Method.—The sections are stained in Löffler's methylene blue for from ten to twenty minutes. Super-

fluous colour is removed by immersing the sections in diluted alcohol, or in a 0·5 per cent. solution of acetic acid for a few seconds. The sections are now dehydrated in absolute alcohol, cleared in xylol or oil of cloves, transferred to the slip, blotted, and mounted as usual.

Kühne's Method.—The object of this method is to prevent the removal of the colour from stained bacteria in sections during the treatment which such sections usually receive before they are ready for mounting, *i.e.*, during the washing and dehydrating processes usually employed. For staining Kühne prefers a methylene blue solution prepared as follows: 10 c.c. of a saturated alcoholic solution of methylene blue is added to 100 c.c. of 5 per cent. solution of carbolic acid (phenol). The sections are placed in this solution for thirty minutes, then washed in water, and decolourised in very dilute hydrochloric acid (2 drops strong acid in 100 c.c. water). This decolourising operation must be very carefully conducted, as very thin sections will only require to be immersed for two or three seconds, after which the sections are at once transferred to an alkaline solution prepared as follows: 10 drops of a saturated solution of lithium carbonate in 10 c.c. of water. The sections are then washed in water for a few minutes, dehydrated in absolute alcohol, which Kühne colours with a little methylene blue. The sections are now placed in aniline oil, which also contains a little dissolved methylene blue.* The sections are now washed in colourless aniline, then in xylol, and lastly mounted, as usual, in balsam.

Ziehl-Neelsen Method.—This important method is used for the diagnosis of tubercle and leprosy. These are the only two organisms ordinarily met with which withstand the

* The aniline oil blue solution is prepared by shaking an excess of methylene blue with colourless aniline, when, after standing with frequent agitation, the coloured oil is filtered off.

decolourising action of the strong sulphuric acid used. The following is the method employed for the examination of tuberculous sputum and the bacilli of tubercle and leprosy in sections. In the case of sputum, a very thin layer is spread over the cover-glasses with a platinum wire or a fragment of wood; the cover-glasses are then dried and fixed in the usual way. The cover-glasses are now floated, the prepared side downwards, on a warm Ziehl's carbol-fuchsine solution for ten minutes (three minutes in the case of sputum). The stain must be warmed until steam just rises; it must on no account be allowed to boil, otherwise a precipitation of the colouring matter will take place. If preferred, the cover-glass can be held in a pair of forceps, and a few drops of the warm stain dropped on from a pipette; the stain is kept warm by very gentle heating over a flame. The cover-glass is now well washed in water, and then held in 25 per cent. sulphuric acid until just decolourised; the cover-glass is again well washed in water, and then counter-stained in Löffler's methylene blue for thirty seconds, again washed in water, dried between filter-paper, warmed slightly until quite dry, and, lastly, mounted in balsam.

The tubercle bacilli will be stained red, and the lung débris dark blue.

The method for staining the tubercle and leprosy bacillus in section by this process is as follows: (1) Stain the sections in warm carbol-fuchsine solution for ten minutes. (2) Rinse in water. (3) Decolourise in 25 per cent. sulphuric acid and water, transferring from one to the other alternately until decolourised. (4) Rinse in water. (5) Counter-stain in Löffler's methylene blue solution for three minutes. (6) Dehydrate in absolute alcohol. (7) Clear in xylol or oil of cloves for five minutes, transfer to slide, blot off excess of clearing agent, and mount with a drop of balsam.

Gram's Method.—This is one of the most valuable and widely-used differential staining processes. Gram's method is used as an aid to the diagnosis of a large number of micro-organisms. The process can be applied equally well to cover-glass preparations and to sections. The cover-glass or section is first stained with aniline gentian violet, and then decolourised with iodine solution. A precipitate is formed with the colouring matter, which adheres to the organisms, but can be easily washed out of the tissues. The bacillus of cholera, typhoid, glanders, the spirilla of recurrent fever, the gonococcus and the *Pneumococcus Friedlanderi* are amongst the organisms which yield up their colour, and therefore do not stain by Gram's method.

The process is as follows: (1) Stain the cover-glass or section in Ehrlich's aniline gentian violet for ten minutes. (2) The section is then immersed without washing in iodine solution (1 gramme of iodine and 2 grammes of potassium iodide are dissolved in 300 c.c. of water) for one to two minutes. (3) Wash in alcohol until no more colour comes away. (4) Counter-stain in an aqueous solution of eosine. (5) Dehydrate in alcohol. (6) Clear in xylol or oil of cloves, transfer to the slide with the section-lifter, lay out flat, blot off the excess of oil, add a drop of balsam, and mount.

Gram-Günther Method.—Günther has modified Gram's original method by giving the preparation a washing with a 3 per cent. solution of hydrochloric acid for a few seconds after the first alcoholic washing. By this treatment cleaner and brighter preparations are said to be obtained. Botkin recommends that the section should be washed in aniline water, after staining with gentian violet, and before immersing in the iodine solution.

It is very important to note that every pigment is not suitable for this method. Fuchsine, methylene blue, and

Bismarck brown cannot be used, but only the so-called pararosanilines, to which class belong methyl violet, gentian violet, Victoria blue, etc., the strong affinity which these colouring matters have for iodine being, according to Unna, the cause of the remarkable action of Gram's method.

CHAPTER IV.

METHODS OF SPREAD OF DISEASE—DISINFECTION—IMMUNITY.

The methods of spread of infection—Measures to prevent the spread of diseases—Vaccination—Quarantine—Notification—Practical disinfection—Disinfection of rooms—Disinfection by sulphur, chlorine, heat, steam, etc.—Immunity and susceptibility—Hypothesis of immunity—The exhaustion or pabulum, antidote or retention, and acquired tolerance hypotheses—Antitoxin treatments—Putrefaction and oxidation.

The Methods of Spread of Infection.—The principal methods of infection are:

1. Pulmonary infection, the bacilli or spores being inspired.

2. Intestinal infection, the organisms being swallowed with food, water, or dust.

3. Inoculation through a wounded or unwounded surface of the skin or mucous surface.

4. Infection by contagion, fomites, etc., in which the manner of entrance of the virus into the body is not precisely understood.

We will now proceed to briefly consider to which of the above classes some of the various diseases belong:

Actinomycosis.—Very rare in man, usually caused by the chewing of ears of cereals infested with the fungus. Possibly infection is also by inspiration. Two or three cases are on record where infection has been conveyed from animals to man. Class 3.

Anthrax.—Conveyed either by inspiration from infected hides or by inoculation through traumatic injury. Classes 1 and 3.

Cholera.—Conveyed only by swallowing the specific organism in food or dust. Class 2.

Diphtheria.—Conveyed in food or dust containing the organism coming into contact with a mucous membrane. Class 3.

Erysipelas.—Infection usually through a wounded or damaged surface of skin or mucous surface. Class 3.

Influenza.—Probably Class 1.

Glanders.—Class 3.

Gonorrhœa.—Class 3.

Leprosy.—The method of conveyance is unknown. Class 4, and possibly Class 2.

Malaria.—Class 4.

Measles.—Class 4.

Pneumonia.—Classes 1 and 4. The micrococcus of Sternberg is probably very widely distributed in an attenuated condition, in which it has little virulence.

Rabies.—Conveyed by inoculation. Class 3.

Relapsing Fever.—Class 4.

Scarlet Fever.—Classes 2 and 4.

Small-pox.—Conveyed aerially to great distances. Class 4.

Syphilis.—Classes 3 and 4.

Tetanus.—Class 3.

Typhoid.—Class 2.

Tubercle.—Classes 1, 2, and 3, the first being most common. The second probably occurs chiefly in young children; in the case of the third, inoculations (*i.e.*, post-mortem wounds) usually remain as local lesions only.

Whooping-cough.—Class 4.

Yellow Fever.—Class 4.

With regard to diseases generally, the general measures

taken to prevent spread of disease may be divided into two classes:

1. (*a*) Vaccination; (*b*) Quarantine; (*c*) Notification and isolation.

2. Disinfection of the person, clothes, home, and discharges of the patient.

It would be obviously out of place to deal with general preventive measures here, but the considerations involved in practical disinfection may fairly be included, and will be considered in detail.

By far the greater number of pathogenic organisms given off ultimately from any case are destroyed by what we may term 'natural disinfection'; for example, by the action of light and air, or by meeting with conditions of soil and temperature unfavourable to their growth; or, again, they may be crowded out by saprophytic bacteria that are more capable of life under the existing conditions. Again, a certain number of organisms, varying in different cases, are required to produce a *toxic* dose—that is to say, to make headway as invaders against the healthy tissues; the number of organisms thus required doubtless varies with the age and condition of the subject, the state of the tissues, and the condition of virulence or *attenuation* of the organism, while hereditary tendencies and other influences must not be neglected.

It is in many cases advisable to attend to careful disinfection of the body of the patient—for example, in the case of small-pox, measles, scarlet fever—while after diphtheria the throat should be disinfected by means of a suitable gargle till the Klebs-Löffler bacillus can no longer be found on inoculation of serum-tubes. For disinfecting the skin or the hands previous to an operation, a solution of mercuric chloride (1 in 1,000) is convenient, the skin having been well cleaned with soap and water, ether, turpentine, or other suitable grease solvent.

Clothes, hangings, and bed-linen from infectious cases should, if possible, be sent to a steam-disinfector, as they are in no way injured by the process, and are rendered perfectly sterile.

Excreta should be received into a solution of mercuric chloride (1 in 2,000), or into a 4 per cent. solution of bleaching-powder (6 ounces to the gallon). It must be remembered that, when we employ mercuric chloride, any albuminous matter in solution will form an insoluble precipitate, which is useless as far as germicidal action goes. If solutions of permanganates are employed, they will certainly part with their oxygen to oxidizable organic matter before the organized and resistant cell of the bacterium is attacked. The 'disinfection' of closets, except in cases where they are used to receive excreta from infectious cases, is neither necessary nor advisable; in a properly-managed closet there is nothing to disinfect, and the use of any agent to wash or destroy effluvia will only lead to obscuring the ready perception of the in-leakage of sewer gas, or the necessity of proper ventilation. The disinfection of sewers and street-gullies is useless for the same reason, and if bad gases are given off, it is because proper ventilation has not been provided, or because of the stagnation of the sewage. These remarks do not apply to the flushing of sewers, which is in many places essential to their maintenance in proper condition.

Practical Disinfection of Rooms.—It is utterly useless to attempt the disinfection of the air of rooms which seems to be sought after by some, but the floor, walls, ceiling, hangings, furniture, etc., should be dealt with.

As by far the greater number of bacteria must be on the floor, it is important to destroy them first, and not to allow them to be stirred up into the air by the movement of those engaged in the subsequent operations. To ensure this, the floor and carpet should be liberally sprinkled with

sawdust mixed with 10 per cent. by weight of crude carbolic acid (Calvert's No. 5), or with a solution of mercuric chloride (about 1 in 1,000).

A fire should then be lighted in the room, both to cause the air in the room to leave it by the chimney, and to be available for burning anything that is sufficiently valueless to be destroyed.

All hangings, bedding and clothes should then be removed to a steam-disinfector, and the walls and ceiling washed down by means of a whitewashing brush and a solution of mercuric chloride (1 in 2,000) or bleaching-powder (6 ounces to the gallon); the furniture should be taken out of doors and scrubbed. The wall-paper is stripped and burned without being taken out of the room, and the carpet taken up, and (if in the country) beaten out of doors, or if in town sent to the disinfector.

The sawdust should be rubbed on the bare boards, so that the bacteria may stick to it, and then swept up and burned; after this the floor should be well scrubbed with hot soap and water, the ceiling limewashed, and the walls repapered before the room is reinhabited.

Disinfection by Sulphur. — The burning of sulphur in rooms is probably entirely without effect, unless everything has first been made thoroughly damp by boiling away a quantity of water in an open vessel, and the same is probably true of chlorine. Both of these procedures cause injury to metal-work, and hence we give preference to the method indicated above, which would certainly be far more effective as regards destroying the vitality of the greater number of bacteria.

Those who advocate the use of burning sulphur for the disinfection of a room consider that 1 pound of sulphur should be burned for every 1,000 cubic feet of space; this will produce a little over 1 per cent. of sulphurous acid gas in the

air. Instead of burning sulphur, liquid sulphurous acid may now be bought. It is sold compressed in tins, with a small metal pipe that can be cut off with a knife, thus allowing the gas to vaporize slowly.

Disinfection by Chlorine.—According to Koch, very large quantities of chlorine are required to be effective, as much as 15 pounds of bleaching-powder being necessary for 1,000 cubic feet, while to liberate the chlorine from this we should need either 22 pounds of hydrochloric acid or 7 pounds of sulphuric acid. The sprinkling or spraying of infected rooms with germicidal solutions is more frequent abroad, and is a more scientific method to employ than the liberation of gases, as we are not dealing with an unknown virus any longer in the case of most diseases, but with numberless small organized vegetable structures, the death of all of which can be assured if they are brought into contact with the proper reagents.

Apart from the low specific disinfectant value of such gases, they must in practice become diluted to an uncertain extent, which increases continually during the operation; the process of diffusion by which they penetrate to various parts of the room under treatment gives a disinfectant atmosphere of varying and uncertain composition; and the presence of any slight mechanical obstacle, such as a little dust or flue, may be sufficient to protect organisms from being disinfected. A disinfectant spray, on the other hand, has a known initial strength, which continually increases during drying; it is brought directly in contact with the organisms on the surface to be disinfected; and when applied by a suitable apparatus, such, for instance as the Equifex sprayer, it is projected on to the surface with a sufficient velocity to penetrate obstacles which would protect against the action of a gas or vapour.

Disinfection by Heat.—Hot air does not kill all organisms

at any temperature which can be endured by any ordinary fabric, except horse-hair, even when the organisms are exposed on the surface. It is still less efficient in regard to organisms below the surface; for the penetration of the heat is effected by the slow processes of conduction and convection, and the external temperature cannot in practice be obtained at any substantial depth.

Wide variations of temperature occur within the disinfecting chamber, owing to unequal diffusion of the gases and radiation from the heated surfaces. These facts were notably demonstrated by Koch in 1881. Cambier has recently shown that the temperature and exposure for merely superficial disinfection by dried heat is at least two hours at 156·5° C., or one hour at 180° C.

Hot air must therefore never be used for disinfection of fabrics. Where steam is not available they may be boiled for an hour in water, or an alkaline solution such as potash or soda. Care must be taken to obtain and keep the temperature at the boiling-point throughout the mass of the water and of the objects of the treatment. Before applying any process of heat disinfection, stains of blood, etc., should be well moistened with potassium permanganate solution to prevent them from being fixed by heat.

Heat can be most effectively applied for the disinfection of fabrics by causing steam to condense in their pores. More steam is sucked in to fill the place of that which has been condensed, and is in its turn condensed; and the process goes on till the interior of the fabrics becomes so hot that no more condensation takes place; that is to say, that the temperature of the entire contents of the vessel is equal to that of the incoming steam.

Steam at any temperature or pressure which can condense without cooling is called 'saturated steam.' Thus, steam from a kettle or in a boiler is saturated. When in

any way, such as, for instance, by contact with hotter surface or by being derived from a saline solution, its temperature is raised above that at which it can condense under its existing pressure, it is called 'superheated.' The process described in the last paragraph does not occur with steam so long as it is superheated, its heating effect while in that condition being due only to its being cooled by conduction, and amounting to a very small fraction of that exerted by condensation. The disinfectant value of strictly superheated steam is about the same as that of hot air. In practice, the extent of superheat present in a disinfector is usually not sufficient to prevent the steam from being rapidly reduced to saturation, and acting as saturated steam. It is only in the latter stages of a disinfection that the risk enters of the objects being too hot to cool the steam to saturation, and of organisms on the surface thus escaping disinfection. The extent to which this risk is of practical importance varies with the design of the stove, and has not at present been accurately determined for the types of disinfectors used in this country. A more certain objection to the use of superheated steam is that its temperature, not being determined solely by its pressure, cannot be read off on a pressure gauge. The first proposal of the use of superheated steam for disinfection was made by Koch, who, in 1881, suggested raising steam from saline solutions of boiling-points above 100° C. Disinfectors were made on the Continent, working respectively with solutions of salt and of calcium chloride, but were found unsatisfactory.

Steam is used either confined under pressure or as a current with or without pressure. The advantage of some amount of pressure of saturated steam, however small, is that it gives a real control over the temperature of steam, which in a well-designed disinfector is practically uniform

throughout. What the temperature should be is still a matter of discussion. Many common disorders, such as typhoid, diphtheria, and cholera, are with certainty disinfected by almost momentary exposure to temperatures below 100° C. This is not the case with all; dried tuberculous matter, for instance, having been known to resist over three hours' boiling, and our knowledge of the organisms producing many diseases, for example small-pox and scarlet fever, is at present insufficient to justify a definite statement of the temperatures necessary for their disinfection. The latest researches (Miquel and Lathrage) conclude that twenty minutes' exposure to a temperature of 110° C. should be allowed in all cases. A description of the best known forms of steam disinfectors will be found in the Appendix.

Immunity and Susceptibility.—In considering the action of disease germs on animals, one cannot fail to be struck with the remarkable differences which the same organism produces when injected into different animals.

The organism that invariably produces a fatal disease in one animal will, when introduced into another animal, either produce a mere local affection of no particular moment, or possibly no effect whatever.

For example, a virulent culture of the *Bacillus tuberculosis*, if inoculated into a guinea-pig, will produce general tuberculosis, resulting in the death of the animal; but in man, a local tubercular infection, as, for instance, a post-mortem wound, usually produces only a slight local lesion, which after a time heals up completely.

When an organism is capable of producing specific disease in an animal, that animal is said to be 'susceptible' to that disease.

The 'susceptibility' varies greatly in degree, even for the same kind of animal, the following being some of the most important factors regulating the degree of susceptibility:

1. The age of the animal, young animals being often affected by injections that would not affect full-grown animals of the same species.

2. The condition of the animal's health. When in robust health, infection in whatever way presented is not so easily taken as when the system is debilitated; for example, an attack of typhoid often occurs after exposure to sewer gas, which does not itself contain the organism, but, by lowering the general tone, causes the virus, which might otherwise have been inactive, to take effect.

3. The manner in which infection is presented, whether aerially, or in food, or by traumatic inoculation, or by contagion.

4. Lastly, in the case of infection by artificial cultures, the age of the culture and the medium on which it has been grown.

As an example of the last case, it is well known that a culture of the tubercle bacillus, which has been subcultured through many generations on artificial media, will lose its virulence or infective power to a considerable extent, so that a much larger dose will be required to produce an effect on a susceptible animal, but that by passing the organism through an animal its virulence may be restored.

The virulence of an organism may be decreased, or 'attenuated,' artificially; for example, by exposing cultures of anthrax to a temperature of 40° C. for some time, they become attenuated to such a degree that they will kill nothing larger than mice.

The effect on animals of an organism may be greatly enhanced by the injection along with it of some other organism that has not pathogenic properties, but which in some way that we do not yet understand adds greatly to the virulence of the pathogenic organism which it accompanies.

Apart from the variations in degree of susceptibility mentioned above, we find that, taking the case of a particular pathogenic organism, the *Bacillus mallei*, for example, while many animals exhibit more or less susceptibility, some are incapable of being affected at all; such animals are said to possess a 'natural immunity' to glanders.

Anthrax, again, is very fatal to ordinary sheep and many other animals, while Algerian sheep enjoy a complete immunity.

The lower animals possess a complete immunity to several important diseases to which man is susceptible, namely, leprosy, syphilis, gonorrhœa, cholera, etc.

The carnivora are remarkable in enjoying a considerable degree of immunity against the organisms of septicæmia, to which the herbivora are far more susceptible.

Confining our attention more particularly to the diseases affecting man, we find that in the case of several, notably small-pox, measles, mumps, whooping-cough, and scarlet fever, it is comparatively rare for the same person to be attacked twice by the same disease. That is to say, one attack is 'protective,' and in the above-mentioned diseases the 'protection' usually lasts a lifetime.

On the other hand, an attack from certain other diseases does not confer this protection, but rather predisposes the patient to the second attack of the same disease. This is true of influenza, diphtheria, pneumonia, and malaria.

To return to the group of diseases before mentioned, in which protection is conferred by an attack, this protection extends only to that particular disease, and does not in any way protect against other diseases; while in the second group, in which no protection is afforded, we find that there may be not only predisposition to a second attack of the same disease, but even a distinct predisposition to

attack by other diseases—thus, diphtheria and scarlet fever mutually predispose to one another.

Hypotheses of Immunity.—Before examining in detail the chief theories that have been put forward to account for the phenomenon of immunity, we may first consider certain special cases of immunity which merit individual attention.

It has been found experimentally that frogs, which are naturally immune to anthrax, may be infected if they are kept at a temperature of 37° C.; fowls, on the other hand, may be made susceptible to the disease by being immersed in water so as to lower their temperature. This would appear to point to the presence of some means of resistance normally present in frogs and fowls which ceases to be effective when their normal conditions of existence are interfered with.

It has been found by several observers that the blood of various animals possesses decided germicidal powers; for example, the blood of rats is able to destroy the vitality of anthrax bacilli, though this property is not impossibly due to its excessive alkalinity.

The blood of animals immune to anthrax has been found to have the action of conferring protection against a dose of anthrax bacilli that would otherwise be fatal if injected with it, or within a certain interval of time before or after injections. Blood serum from animals immune to a disease may, however, have the effect of conferring a power of resisting the action of the toxalbumens produced by that organism without having any germicidal action on living organisms themselves; thus, diphtheria antitoxin serum injected into a suitable animal will neutralize the effect of the poisonous toxalbumen in diphtheria cultures, but it has not a germicidal effect on the bacilli, as diphtheria antitoxic serum may be used as a culture medium for the Klebs-Löffler bacillus.

Four hypotheses have been proposed to account for the immunity produced by an attack, which we will consider in order:

1. The exhaustion or pabulum hypothesis.
2. The retention hypothesis.
3. The hypothesis of acquired tolerance, or acclimatisation.
4. The phagocytosis hypothesis.

1. **Exhaustion or Pabulum Hypothesis.**—The supporters of this theory hold that the bacilli by their action abstract from the blood some chemical compound necessary for their growth, the consequence being that, once this pabulum is exhausted, it must be re-formed before a second attack is possible. On turning to the higher branches of the vegetable kingdom, we have no difficulty in finding analogies. It is a matter of experience that, if a crop of wheat, for example, is grown on the same soil year after year, it will abstract the particular elements required by the wheat-plant to such an extent that wheat will not grow so satisfactorily till the land has rested or the abstracted elements have in some way been restored. To carry the analogy still further, we find that, though the land may be exhausted in respect of wheat, it still can produce a full crop of potatoes or roots; while as regards the human body, after an attack of small-pox there is a very strong protection against a second attack of that disease, but none at all against influenza.

In order to admit the truth of this hypothesis, we must believe that each disease organism requires a special pabulum which is present in the blood of every susceptible animal at birth, and that the varying degrees of immunity that are produced are due to the ease or difficulty with which the pabulum for that particular disease is reproduced.

The Antidote or Retention Hypothesis.—This hypothesis

assumes that after the first attack the micro-organisms leave behind them some product of metabolism that is inimical to their existence, and this theory is capable of receiving support from various experimental facts.

It is on this hypothesis, and the theory of *acclimatisation*, that the antitoxin treatments which are just now receiving so much attention are based. By antitoxin treatment we endeavour to arm the body against the growth of the specified organisms or the formation of their metabolic products, by putting into it, ready-made, those products that would be produced naturally in convalescence, and by their action would bring the disease to a successful termination. Accounts of the employment of antitoxin treatments are to be seen in every issue of the medical papers, but up to the present date the only diseases which have been extensively treated in this way are diphtheria and cholera, in both of which a decided measure of success has been met with.

Acclimatisation Hypothesis.—According to this hypothesis, it is assumed that the cells of the body become used to the products of the organisms, and that at last they cease to have any injurious influence on them. From analogy this seems a tenable theory, as we see in both the animal and vegetable kingdoms numerous examples of successful acclimatisation.

The Phagocyte Hypothesis. — This hypothesis was first put forward by Metschnikoff, who when experimenting with virulent anthrax bacilli on frogs, which are normally insusceptible to anthrax, found that the white corpuscles put out two processes to surround the bacilli, which were ultimately absorbed into the centre of the corpuscle.

He also held that if in the case of a local infection the first number of leucocytes that hurried to the spot were not sufficient to repel the invaders, more and more were brought

forward, till at last they were so numerous that the organisms could not make any headway, and were thus destroyed. The attraction possessed by living or dead bacteria for the leucocytes is remarkable, while they are equally repelled by the presence of certain bodies, such as quinine, chloroform, etc.

The term *chemiotaxis* has been applied to this phenomenon; when the leucocyte is attracted towards a body, the chemiotaxis is said to be positive, and when it is repelled, negative.

The above are the principal hypotheses advanced; the truth probably lies not with any one alone; all four play their part, one predominating over the others in different cases. They are each based on some amount of experimental evidence, and more than one may, in fact, play a part in the phenomena which they seek to explain. But it cannot be said that any one of them has at the present time been sufficiently verified to be regarded as an experimental theory. For the time being it can only be stated that *acquired immunity is a capacity either to prevent the growth of disease-organisms, of which the pathogenic action may lie in their intercellular tissue or their metabolic products, or to neutralise the toxic action of such products.*

Putrefaction and Oxidation.—The bacteria play the principal part in causing the disintegration and dissolution of dead animal and vegetable matter, of which the molecules are, so to speak, in a condition of unstable equilibrium, and by abstracting the small portion of nutriment which they require for their own development destroy the balance and bring about the resolution of the animal or vegetable tissues into simpler inorganic bodies, the chief products being water, carbonic acid, and ammonia, together with smaller quantities of other products, some of them of particularly evil odour and poisonous properties.

The bacteria of putrefaction are for the most part

anaerobes, and are therefore found at some little depth in the soil. In order that the soil should be kept in proper condition for producing crops, it is essential that not only these bacteria shall be present (in the lower layers), but that in the upper layers there shall exist oxidation bacteria that shall change the carbonaceous matter into carbonic acid and the ammonia into nitrates. Plants cannot absorb ammonia direct; their nitrogen must be in the form of nitrates before it is capable of assimilation. Hence we see the necessity of maintaining due porosity in the soil, and of not unduly loading it with more organic matter than the organisms can successfully deal with. The failure of this process of oxidation is well seen in a sewage farm where sewage matter, either liquid or in the form of sludge-cake, has been applied too freely to the land. Instead of large crops being obtained, the reverse is found to be the case, and the land is said to be 'sewage-sick.'

The oxidation bacteria also play a very important part in the purification of water artificially.

The filter-beds at the waterworks are constructed of a layer of large stones with unjointed pipes placed at intervals at the bottom; smaller stones are placed above these, then gravel and rough sand, and lastly fine sand.

The action of the bed is twofold. First it acts as a mechanical strainer; and its efficiency in this respect is increased by the formation of a slimy layer derived from the water which has been filtered, and consisting principally of organic detritus and living bacteria. This layer in a great measure prevents organisms from being washed through into the filtrate. The second function of this layer is to serve as a culture ground for oxidation bacteria, which to a large extent tend to prevent the multiplication of the other bacteria, and consequently their growth through the filter. It is a matter of common experience among

waterwork managers that a far better effect is obtained from the action of a new filter-bed after it has been at work for a few days (*i.e.*, after the layer of slime has had time to form) than when quite fresh. Again, in summer, when the temperature is more favourable to the growth of the organisms, the purification is more complete than in winter. In practice the layer of slime ultimately, at periods varying with the nature of the water and rate of filtration, becomes too dense to permit the passage of sufficient water, and loses its capacity to prevent the passage of organisms. The bed should before such periods be put out of operation, and its surface should be scraped; a fresh layer of slime being allowed to form before the filtrate is again used.

The experiments of James Buchanan Young on the soil of graveyards show that they are very rich in micro-organisms, particularly those of a liquefying type, the *Proteus vulgaris* being present in great numbers. Their action is so effective that he found no notable quantities of organic carbon and nitrogen in the upper layers, while that in the lower layers was not so very much in excess of that found in virgin soil.

In addition to the two classes of oxidizing and putrefaction-causing bacteria, there are the important organisms discovered by P. F. Frankland and Winogradsky, which have the power of abstracting nitrogen from the atmosphere and fixing it for the use of plants. The absolute need for the presence of these organisms in soil intended for crops has been shown by experiments of Professor Nobbe on plants, which have been made to grow on soil that has been sterilised by heat, but which in all respects has its constituents well fitted for their growth. Similar plants have been grown in other portions of the same earth which had not been sterilised, and while these afforded good luxuriant

growths, those in the sterile earth scarcely rose above the ground. Experiments have also been made as to the effect of adding to soil substances which inhibit the growth of bacteria, with effects precisely similar to those mentioned above. It was for a long time doubted that atmospheric nitrogen could be assimilated by plants; but even though the means were not understood, it seems strange that scientists should have doubted that nitrogen was in some way assimilated, as had it not been so, our stock of combined nitrogen would be on the decrease, whereas the reverse is rather the case.

As an instance of the utilisation by man of the action of the nitrifying organisms, we may cite the process of making artificial nitre formerly largely practised on the Continent. A large heap of earth containing old mortar, chalk, and organic matter was made, and protected from the rain by a roof, but exposed to the prevailing winds. It was then watered with stale urine, and dug over to expose fresh surfaces. From time to time a portion at one end was removed and mixed with wood-ashes and lixiviates, whereby a crude solution of nitrate of potash was obtained. After lixiviation the exhausted material was mixed with fresh organic matter and returned to the heap. It was found that earth which had been used for this purpose always worked better than fresh earth.

An account of the nitrifying organisms will be found in the chapter on fermentation.

CHAPTER V.

TUBERCULOSIS.

The bacillus of tuberculosis: discovery and morphology of the organism —Growth on artificial media—Bacteriological diagnosis—Staining of the bacilli in sputum and in sections—Pastor's cultivation method—Number of bacilli in sputum—Occurrence and distribution of tuberculosis—Resistance of the bacilli to desiccation—Pathogenesis—Special regulations in force in New York and Germany—Koch's tuberculin treatment of consumption—Preparation of tuberculin—Practical disinfection.

The discovery of the *Bacillus tuberculosis* was first announced by Koch in 1882, though it had been shown in 1865 by Villemin, and in 1877 by Cohnheim, that on inoculation with tubercular sputum, guinea-pigs died from general tuberculosis.

The bacillus of tubercle is a slender rod, varying from 2·5 to 4·0 μ long, and about 0·2 μ thick; the bacillus is non-motile, it grows at blood-heat, and only on special media, its growth even on these being very slow. On repeated subculture the organism becomes longer and thicker, and as it develops a saprophytic habit, its virulence becomes reduced, but may be restored on passing through an animal.

The thermal death-point of the organism is 70° C.; that of the spores is higher, and appears to vary, not only with the particular races, but also with the condition of dampness or desiccation. Dried sputum has resisted boiling at 100° C. for over three hours. The growth of the organism

is about the same whether oxygen is supplied or withheld, but is prejudicially affected by light, diffused daylight being fatal to a culture in four or five days, while direct sunlight is fatal in a few hours, the time necessary to kill the bacilli varying according to the thickness of the culture.

Growth on Media.—Koch first succeeded in cultivating the bacillus on solidified blood serum, on which it grows very slowly. In 1888 Nocard and Roux showed that excellent growths are obtainable on agar containing 8 per cent. of glycerine in half the time required to produce a similar characteristic growth on blood serum. Typical growths appear on blood serum in three to four weeks, while fourteen days suffice for a culture on glycerine agar. The growth on either medium appears as a peculiar whitish wrinkled skin on the surface, somewhat resembling the 'casts' formed by earthworms in soft mould.

It is stated by Wurtz that broth to which a slight addition of glycerine has been made serves well for the growth of the tubercle bacillus, which develops as a floating skin on the surface.

In the lungs invaded by tubercle the bacillus is found in greatest numbers round the circumference of the 'giant-cells.' Matter derived from the middle of these will nevertheless produce tuberculosis on inoculation into a guinea-pig, the explanation given being that the bacilli in the centre of the cell have disappeared, leaving spores behind them.

Spores are formed by the bacillus whenever it finds itself under conditions unfavourable to its growth, such as want of nourishment, moisture, or a suitable temperature.

A pure culture is readily obtained by inoculation of a guinea-pig with tuberculous sputum; after two or three weeks the animal is killed, and its lungs opened with antiseptic precautions: a streak is made from an affected part (in which the bacillus exists as a pure culture) on to media.

Another method of obtaining a pure culture would be to use Pastor's method, described on page 125 ; this obviates the necessity of employing an animal, and does not require the use of a microscope.

Bacteriological Examination of Sputum and Sections.

We may employ one of two distinct methods: (1) *Direct staining;* (2) *demonstration of the presence of the tubercle by culture.* In either case it is advisable to examine the sputum for particles of broken-down lung tissue, which are noticeable as minute yellowish specks of caseous matter, best seen by pouring the sputum into a flat glass dish placed on a piece of black paper. The viscous masses of sputum are then 'teased' out with a couple of match-ends. To obtain a sample of sputum the patient should be directed first to rinse the mouth well with distilled water, and then to expectorate into a test-glass; the first expectorations in the morning should be secured if possible.

Method of staining the Bacilli in Sputum.—The cover-glass films prepared in the ordinary way after 'fixing' are stained by the Ziehl-Neelsen method as follows:

1. *The cover-glass is treated with warm carbol-fuchsine solution for three minutes.*
2. *Decolourise with* 25 *per cent. sulphuric acid.*
3. *Wash in water.*
4. *Counterstain in methylene blue for three minutes.*
5. *Wash, dry and mount in xylol balsam.*

Sections of tissue are stained as under:

1. *The sections are treated with hot carbol-fuchsine solution for ten minutes.*
2. *Decolourise with* 25 *per cent. sulphuric acid, dipping the sections for about a minute or so into the acid and then water alternately.*
3. *Wash well in water.*

4. *Counter-stain in solution methylene blue for three minutes.*

5. *Wash slightly, then soak in absolute alcohol for two minutes.*

6. *Clear in xylol or oil of cloves.*

7. *Transfer to glass slip with a section-lifter, blot with filter-paper, and mount in xylol balsam.*

By this method the bacilli are seen as bright-red slender rods, which are on the average about three-quarters the diameter of a blood corpuscle. The blue counter-staining is not absolutely necessary, but it throws the bacilli into greater relief. Ribberts has proposed a method of reducing the troublesome viscous character of tuberculous sputum by a short boiling with a 2 per cent. solution of caustic potash. This process has yielded satisfactory results in our hands.

It must be remembered that the bacilli in the sputum in cases of incipient tuberculosis are often few and far between, so that *till ten or a dozen slides have been made and carefully examined, we cannot safely assert that the bacillus is absent.*

Pastor's Cultivation Method.—A gelatine tube is inoculated with a fragment of a caseous particle, or, failing this, with some sputum, well shaken and poured into a plate.

After three or four days all the organisms except the tubercle will have developed sufficiently to render them visible; when this has taken place, some of the clear spaces of gelatine between the colonies are cut out and melted on the surface of a glycerine-agar plate and incubated; if after twenty-one days no colonies appear, the tubercle is not present.

Some observers consider it worth while to attempt a rough estimation of the number of bacilli present in sputum, with a view of forming an opinion as to the rapidity with which the caseous degeneration is proceeding.

Bollinger has estimated that the daily expectorations of a phthisical patient when caseation is far advanced may contain twenty million bacilli.

In cases where phthisis is suspected, an examination of the sputum should always be made, particularly as phthisis, when taken in time, is very amenable to treatment. In examining the urine in cases of suspected tubercular affection of the bladder, care must be taken in the collection of the specimens to avoid contamination, as there is an organism termed the *Smegma bacillus*, which is similar to the tubercle, and behaves in the same manner to Gram's stain. It is not, however, capable of growth on ordinary media, and hence the application of Pastor's method as described above would be conclusive.

Occurrence and Distribution.—The disease is found all over the globe, but is much more prevalent in cold and temperate climates than in the tropics. The mortality due to tuberculosis is highest in March and April, and lowest in August and September.

The operatives in certain trades are especially liable to be attacked by phthisis, particularly those in which there is excessive moisture or gritty particles.

The bacillus is conveyed by air, in the shape of dust; by food, such as milk, and possibly by meat.

In the recently-issued Local Government report it has been shown by feeding experiments that milk from cows with tubercular disease of the udder is very infective, also that the tubercle bacillus is not destroyed, if in the centre of a joint of meat over six pounds in weight, by the ordinary method of cooking.

As has already been stated, the tubercle bacillus retains its virulence for a considerable period of time on desiccation. Messrs. Cadeac and Malet produced tuberculosis in guinea-pigs by injecting material from the lung of a tuberculous

cow which had been kept in the form of a dried powder for five months, but they found in this particular case that at a later date the virulence was lost.

The ability of the bacillus of tuberculosis to form spores, and the obstinacy with which they retain their vitality in dried sputum, amply compensates for its inability to grow outside the body (except on special media), and makes it the most fatal and prevalent disease in these Northern climates. In observations and experiments made independently in Germany, Italy, and France by Kossal, Brouardel and Picini, the disease was found latent, post-mortem, in forty to sixty per cent. of persons who had disclosed no symptoms during life.

Pathogenesis.—Localised tubercular affections may occur in almost every part of the body. The bacilli or spores, having been inspired and entering into the circulation, invade the weakest part. A local traumatic injury may thus determine the onset of the disease in that portion of the body affected. Many diseases predispose to phthisis, as, for example, whooping-cough, pneumonia, diphtheria, scarlet fever, typhoid, syphilis, etc. It is observable that in the case of hospital patients at least 50 per cent. will be found to have a tubercular history. The bacillus has occasionally been found in the fœtus, but not often enough to afford evidence that hereditary transmission is common. When we consider, however, that, as above stated, quite 50 per cent. of patients have a phthisical history—that is to say, are born of those already weakened by the disease, and have, perhaps, been brought up in an atmosphere teeming with the specific virus—it does not seem hard to account for the run of the disease in families, or, as is sometimes noticed, in particular habitations. The warty excrescences which sometimes follow post-mortem wounds, and are apt to appear on the hands of those often occupied

in handling dead bodies (dissecting porters' warts), are of tubercular origin. Fortunately, in man these lesions rarely spread, and remain local or heal altogether, while in susceptible animals (rats, mice, guinea-pigs) an inoculation produces general tuberculosis in the course of a few weeks.

In some cases of leprosy the tubercle bacillus is associated with the *Bacillus lepræ*, while lupus, scrofula, and possibly scurvy, are all due to the *Bacillus tuberculosis*.

The partial immunity enjoyed by the Jews is remarkable, nor has any sufficient reason yet been assigned for it; it is perhaps partly due to the care taken of their meat-supply, and partly to the fact that much of their food is cooked in oil or fat.

Dr. Marshall Ward has proved that tubercle bacilli are present in the atmosphere of a hospital for phthisical patients by suspending glass plates covered with glycerine in an extraction-shaft of the Brompton Hospital. Other observers have demonstrated the presence of the bacillus in the dust of rooms occupied by phthisical patients. In health-resorts much frequented by phthisical patients, the chances of infection by inspiration of tubercle containing dust are considerable, and it often happens that thoughtless or ignorant persons needlessly expose themselves to the risk of tubercular infection. Medical men should make a point of impressing on phthisical patients and their friends the infective character of the disease, which is not fully recognised, principally on account of the long time for which, as pointed out above, the disease may be latent.

In Germany and New York special regulations are in force, and the disease is classed among those subject to compulsory notification; it will be a matter of deep interest to see what success attends the somewhat stringent measures which are being carried out in New York.

Koch's Tuberculin Treatment. — This remedy, though it

has not fulfilled what was expected of it, has at any rate been the means of bringing to notice the possibility of combating disease by injecting into the patient toxins ready-made which are liberated in the ordinary course of disease, and has led the way to the investigation of anti-toxin treatments in diphtheria, cholera, etc.

It has also been found of use in the diagnosis of tubercle, and in the treatment of lupus. If, however, it be injected into a patient in whom phthisis is dormant, it is very apt to cause the old trouble to break out afresh.

In the diagnosis of tuberculosis in cattle it is very valuable, the percentage of failures being less than 1 per cent.

Preparation of Koch's Tuberculin.—This is a liquid containing in a concentrated form the metabolic products of the growth of the tubercle bacillus. He grows the tubercle bacillus in glycerine-peptone-broth in flat-bottomed flasks for six or eight weeks at blood-heat. A thick white skin is formed, which first floats on the surface, but afterwards sinks to the bottom. The cultures are subsequently examined microscopically for impurities, and then gently evaporated on a water-bath to about an eighth of their bulk, then filtered through a Pasteur-Chamberland filter. In this way he obtains the metabolic products of the organism alone, without their intracellular contents. The concentrated liquid so produced contains 40 to 50 per cent. of glycerine, and the presence of the glycerine prevents the growth of any saprophytic organisms that may accidentally gain access to it. From this liquid the tuberculin may be precipitated by the addition of absolute alcohol.

Practical Disinfection.—The sputa should be received into 5 per cent. carbolic or mercuric chloride, and never permitted to dry on handkerchiefs. Phthisical patients should not expectorate in the streets, but should carry a proper receiver with them to receive the sputum. Where a vessel

into which to expectorate is not at hand, it may be received into rags, which should be burned without being allowed to dry. Handkerchiefs of paper are now made at a trifling cost, specially intended for the use of phthisical patients.

Rooms and wards occupied by phthisical patients should be disinfected either as previously described or by rubbing them with half-baked bread, and precautions should be taken to prevent the accumulation of dust and its dissemination throughout the atmosphere; that is to say, when sweeping or dusting is to be carried out, the floor should be liberally sprinkled with wet tea-leaves. There should be no unnecessary ledges or hangings on which dust can accumulate.

Some medical officers in this country are now making it a practice to disinfect after cases of phthisis, and this procedure is greatly to be recommended.

LEPROSY.

Discovery and morphology of the organism—Staining in sections—Distribution of the bacilli in the body—Growth on artificial media—Conveyance of disease—Leprosy in India Commission Report—Occurrence and distribution—Pathogenesis—Preventive measures.

The *Bacillus lepræ* was first described by Hansen in 1880. The organism is a small rod, the length of which is half to three-quarters the diameter of a blood-corpuscle. The bacillus is straight or slightly bent, with more or less pointed extremities, and it is not quite so large as the tubercle bacillus. Within the protoplasmic contents may be seen clear spaces (which some authorities believe to be endogenous spores), surrounded by a delicate membrane.

It is devoid of motility. The leprosy bacillus is stained in the same manner as the tubercle bacillus by the Ziehl-Neelsen method, as follows :

1. *Treatment of the section of tissue or film, fixed upon a cover-glass, with warm Ziehl-Neelsen carbol-fuchsine solution for twelve minutes.*

2. *Decolourisation of the specimen in* 25 *per cent.* H_2SO_4.

3. *Washing in* 60 *per cent. alcohol.*

4. *Washing in distilled water.*

'Cover-glass specimens are at once examined in water or after drying in xylol balsam. Sections are treated with absolute alcohol, and removed to clove-oil before mounting in balsam. A saturated solution of acetate of potash is the best medium in which to mount specimens, as the colour disappears less rapidly.'

By this method the bacilli of leprosy are isolated and distinguished as bright red rods (Baumgarten). They may also be differentiated from those of tubercle by treatment with potash solution (1 in 12). The bacilli then appear as clear, rather thick rods. If a drop of watery methyl-violet be now added, the leprosy bacilli alone are stained.

The grouping of the bacilli together in clumps and masses is also a differentiating characteristic of the bacilli in tissues, and does not resemble in any way the arrangement of tubercular bacilli in giant-cells.

The distribution of the leprosy bacillus within the body is now known to be general in most of the tissues and viscera, though it occurs more in the liver and spleen than in the kidneys and brain. Kobner is the only pathologist who claims to have found it in the blood. The bacilli are found in cutaneous and other tubercles, and in the discharges therefrom.

Leprologists almost universally agree that the direct implantation of leprous material upon solid nutrient media gives negative results. Bordoni-Uffreduzzi claims, however, to have cultivated the bacillus on peptone-glycerine-serum,

at a temperature of 35° to 37° C., on which it forms 'a light yellow stripe with irregular edges along the needle track.' The serum is never liquefied, and no growth ever occurs in the condensation water. On glycerine-agar plates, both on the surface and deeply in the medium, colonies may be seen with a power of 100 diameters which are gray net-like growths with irregular edges.*

Bordoni-Uffreduzzi found that the organism grew only with difficulty at blood-heat on the serum, but after repeated subculture appeared to adapt itself more readily to a saprophytic condition of life, and finally could be subcultured on the gelatine. The inoculation of leprotic culture into animals seems to produce no effect at all, which would differentiate it from tubercle, and also appear to indicate that leprosy is exclusively a human disease. It is unknown how the bacillus is conveyed: and the *causa vera* of leprosy still remains unsettled. There seems to be much evidence to prove that it is not spread by contagion or heredity, though there are examples which appear to favour both. This matter was so carefully investigated by the Leprosy Commission in India that it may be well to repeat here their conclusions (Leprosy Commission in India Report, p. 384):

(1) 'Leprosy is a disease *sui generis*; it is not a form of syphilis or tuberculosis, but has strictly etiological analogies with the latter.

(2) 'Leprosy is not diffused by hereditary transmission, and for this reason, and the established amount of sterility among lepers, the disease has a natural tendency to die out.

(3) 'Though in a scientific classification of diseases leprosy must be regarded as contagious, and also inoculable, yet the extent to which it is propagated by these means is exceedingly small.

* Leprosy Commission Report, p. 425.

(4) 'Leprosy is not directly originated by the use of any particular article of food, nor by any climatic or telluric conditions, nor by insanitary surroundings; neither does it peculiarly affect any race or caste.

(5) 'Leprosy is indirectly influenced by insanitary surroundings, such as poverty, bad food, or deficient drainage or ventilation, for these, by causing a predisposition, increase the susceptibility of the individual to the disease.

(6) 'Leprosy in the great majority of cases originates *de novo*, that is, from a sequence or concurrence of causes and conditions, dealt with in the report, and which are related to each other in ways at present imperfectly known.'

Occurrence and Distribution.—During the Middle Ages this disease was prevalent in England, and many leper houses, or hospitals, were established all over the country, some of the largest being at Burton, Thetford, St. Giles's (London), Sherburn, etc. It is probable that many other skin diseases were misdiagnosed as leprosy. It became finally extinct in the eighteenth century. Doubtless its extinction was largely due to its tendency to die out under favourable circumstances.* Endemic leprosy still exists in Iceland, Norway, Spain, India (100,000), Japan, the Cape, the West Indies, and the Sandwich Islands. Generally speaking, it shows signs of decline rather than increase.

Pathogenesis.—One of the different forms of the same disease generally predominates—either the nodular or 'tubercular' (lepra tuberculosa), in which the new formation has its seat in the skin or mucous membrane; or the anæsthetic (lepra anæsthetica), in which the nerves are chiefly affected. In the skin variety the hands and face are mostly affected, and larger or smaller swellings appear

* For further information see the 'Decline and Extinction of Leprosy in the British Islands' (Newman), p. 109.

(red or blue in colour), which become hard. These tubercles consist of granulation tissue, and may ulcerate and cicatrize, producing great deformities. In the anæsthetic form the nerve-stems become the seat of the granulations in the interstitial connective tissue. The spindle-shaped swellings compress and separate the nerve-fibres. Besides the anæsthesia, other evidences of interference with nerves, such as vesicular eruptions and alterations in pigmentation and ulceration, frequently occur. The peculiar and characteristic lesions of the disease gave rise to the following descriptive terms: Elephantine, leonine, tygria, alopecia; the meanings of which are sufficiently clear.

Preventive Measures.—From the earliest times it has been the practice to insist on compulsory isolation or segregation of lepers. This action was, of course, based on the common belief that the disease was spread by contagion. It appears that strict segregation was never systematically carried out in England (Newman), and it is evident that other agencies caused its decline. In Norway also, and in many other leprous localities, segregation is not strictly enforced. Voluntary isolation should be arranged, the sale of articles of food by lepers should be prohibited, leper colonies discouraged, leper asylums established, and sanitation persistently improved.

ANTHRAX.

Discovery and morphology of the organism—Growth on media—Staining of the bacilli—Resistance of the bacilli and spores to external influences—Pathogenesis—'Attenuation' of the organisms—Practical disinfection.

In 1849 Pollender observed in the blood of animals which had died of anthrax certain rod-like bodies. These were afterwards seen by Royer and Davaine, 1850, and by

Branell in the blood of a man in 1857. Davaine worked with this organism from 1863-73 ; Koch in 1876 succeeded in growing it outside the living body and establishing its pathogenicity.

The anthrax bacillus is the largest of all pathogenic bacteria ; in length it varies from 5 to 6 μ, in breadth from 1 to 1·5 μ. It is aerobic, although not strictly so, for it will grow without the presence of free oxygen, viz., in the blood of animals and in ' stab ' cultures. It is not motile, is usually straight, and has square ends, which are very characteristic. In the blood, where it occurs singly or in short chains, the ends of the bacillus are very slightly convex, and when stained sometimes show a central longitudinal mark more deeply stained than the rest of the protoplasm. In wool-sorter's disease the organism occurs in the fluid of the lungs in long threads, generally without any appearance of segmentation.

When growing, the bacillus elongates, and then gradually divides transversely in the middle, the two bacilli thus formed being enclosed in a common sheath. Under favourable conditions this process may continue until chains of great length have been formed. When the bacilli have a good supply of oxygen, and the temperature is between 24° or 26° C., spores are developed. In sporulation the protoplasm first becomes granular, and clear spaces occur, which soon take a definite oval shape and become highly refractive. The substance of the bacilli will then gradually break down and dissolve, leaving the spores free. Whenever a free spore finds itself in a suitable medium for its development, it elongates and loses its high refractivity, and the protoplasm bursts through the membranous wall and escapes as a bacillus.

When spores develop in a chain of bacilli, they do so at fairly regular intervals. These chains of bright spores

have been aptly described by various authors as resembling chains of pearls. A variety, first obtained by Behring, which is sporeless for many generations, is produced by heating the culture above blood-heat for some time, or by cultivating several times on nutrient gelatine containing 0·1 per cent. of phenol.

In speaking of the organism as it occurs in blood, it was stated that the ends were slightly convex; on cultivating they become slightly concave, but neither of these modifications is ever so great as to interfere with the characteristic squareness of appearance. This concavity is regarded as indicating an attenuated virulence. Involution forms are often seen in old and attenuated cultures.

Growth on Media.—On the gelatine plate small spherical colonies develop in the depth, which consist of closely-twisted bands of bacilli chains. When the growth reaches the surface, chains of bacilli at once begin to spread out over the surface in the most beautiful wavy convolutions, liquefying the gelatine. This stage is usually reached in two days, and is most characteristic. In the gelatine stab, growth takes place along the needle track, fine branching filaments often growing out into the gelatine. Liquefaction commences at the top of the stab, proceeding downwards in a horizontal plane, upon which a mass of bacilli rest, leaving the gelatine above clear and liquid. No pellicle is formed on broth cultures. On agar-agar a thin, gray-white growth takes place, and on potato a considerable white growth, both usually containing a large number of spores. Blood serum is slowly liquefied. An alkaline reaction is generally favourable to the growth of this organism, but it will be noted above that it grows well on potatoes, which are normally acid. The bacilli stain well and easily with aniline dyes, and are not decolourised by Gram's staining method. The spores have great resisting powers to all reagents, and can

only be stained by heating for twenty minutes or more on warm carbol-fuchsine, or by first 'flaming' nine to a dozen times.

The spores will retain their vitality unimpaired for years if kept dry and not much exposed to the light.

Direct sunlight has an inhibitory and injurious effect on both bacilli and spores. It is stated by Schild that the spores are destroyed in one hour by a 0·1 per cent. solution of formalin. Boiling kills the bacilli in a few seconds, while the spores may be able to resist this treatment for ten minutes or more. The spores will live in a 1 per cent. solution of phenol for a week, whereas the bacilli may die in two minutes, and a 5 per cent. solution will only kill the spores in about twenty-four hours. In the interior of a body dead of anthrax, the specific bacilli are killed off by a putrefactive organism in about a week. The spores of anthrax, being among the most hardy of the common bacteria, are often used in the testing of disinfectants, but it must here be remembered that the spores from various sources are not uniform in their powers of resistance.

Pathogenesis.—It occurs in great numbers in the blood of animals which have died of anthrax. In one instance it was found in the mud at the bottom of a well in Southern Russia. Animals drinking at this particular well had become infected, and a search was accordingly made for the specific organism.

Both bacilli and spores remain in fleeces, and may thus transmit the disease to those engaged in handling them.

The *Bacillus anthracis* produces anthrax or splenic fever in cattle and man, and malignant pustule, or wool-sorter's disease, in man. It is pathogenic to the following animals, which are arranged roughly in order of their susceptibility: mice, guinea-pigs, rabbits, cattle, horses, human beings,

etc., while Algerian sheep, dogs, frogs,* and white rats, are immune. If mice are inoculated with the smallest possible quantity of a culture of anthrax bacilli, they die within twenty-four hours.

With other animals the fatality or severity of the attack depends upon the age and weight of the animal, and the virulence and quantity of the culture administered. Young animals are more susceptible than old, and the fatal dose also varies proportionately with the weight.

Animals dead of anthrax present no marked peculiarities to the naked eye; the spleen is considerably enlarged, and is dark and soft, the liver may be enlarged, and there may be bloody discharges from the orifices of the body.

In susceptible rodents the subcutaneous connective tissue may be distended with blood serum of a gelatinous consistency. Considerable inflammation extends from the point of inoculation in the guinea-pig. If the tissue is examined microscopically, the blood is found to be full of bacilli, which in some places may have so distended the capillaries as to have ruptured them and escaped into the surrounding tissue. Anthrax once introduced may become endemic in a field in the following manner: The infected animal dies, the bacilli in the bloody discharges that come in contact with the air develop spores, which may be blown about on to the surrounding soil, where the organism can lead a saprophytic life. Animals feeding on grass growing about this spot would be liable to infection. The bacilli might be killed in the stomach, but the spores could withstand its action and enter the circulation.

People engaged in the woollen industries—wool-sorters, etc.—are liable to pulmonary anthrax (malignant pustule) from breathing the spores which have been shaken out of wool. Wool-sorter's disease is often associated with

* Unless the frog is heated to 37° C.

pleurisy. The virulence of this organism becomes attenuated when :

(*a*) *Cultivated in the blood of a non-susceptible animal.*

(*b*) *When cultures are allowed to remain some months before subcultures are made.*

(*c*) *After the organism has been subcultured a considerable number of times.*

(*d*) *When exposed to sunlight.*

Cultures which are so attenuated that their injection into guinea-pigs is not fatal, may have their virulence restored by passing two or three times through young mice.

Immunity, which, however, according to Petermann, is transitory, seldom lasting more than a few months, may be conferred upon susceptible animals by successive injections into their blood of either—

(*a*) *Attenuated cultures ;*

(*b*) *Filtered cultures (bacilli-free) ; or*

(*c*) *The blood serum of immunised animals.*

When protected animals are inoculated with a virulent culture, the bacilli do not enter the circulation, and only local suppuration occurs.

Practical Disinfection.—Any animal dead of anthrax must be buried deep in the ground, and then the putrefactive organisms will kill the anthrax bacilli, and no spores will be found. Discharges from an infected animal are highly dangerous to man and other animals, so that stables polluted with infective discharges should be washed out with a strong solution of bleaching-powder (8 ounces to the gallon), and harness, if possible, disinfected. It is best and safest to destroy the carcase by burning in a 'destructor.'

CHAPTER VI.

TYPHOID.

Discovery and morphology of the organism—Method of staining—Growth on media—Occurrence and distribution of enteric fever—Conveyance of typhoid by water, milk, dust, shell-fish, vegetables, etc.—Pathogenesis — The bacteriological diagnosis of enteric fever—The serum treatment of typhoid—Löffler's and Abel's researches on the immunising substance in blood serum—Practical disinfection.

The bacillus of typhoid or enteric fever (*Bacillus typhi abdominalis*) was first described in the year 1883 by Eberth, who stained it in sections of the intestine of patients who had died of typhoid; in the following year Gaffky obtained pure cultures of the organism, which is now known as the Eberth-Gaffky bacillus.

The bacillus is 2·5 to 4·0 μ long by 0·5 μ thick, which is somewhat shorter and thicker than the tubercle bacillus.

The Eberth-Gaffky bacillus is not killed by drying, nor by exposure to a low temperature. Its thermal death-point is 55° C. According to most observers, it does not form spores. Like all the pathogenic organisms, it is prejudicially affected by light, diffused daylight being sufficient to prevent its development, while direct sunlight is fatal in five hours. The organism grows equally well both under aerobic and anaerobic conditions. The microscopic appearance alone is not enough to distinguish it from several other organisms; in fact, it is not uncommon to find some

stained specimens which have a curved appearance exactly like Koch's 'comma' or the Finkler-Prior bacillus.

If a fragment of a recent culture of the typhoid bacillus is rubbed up with a drop of water (or, better, a twenty-four-hour broth culture), and examined with a $\frac{1}{12}$ inch objective, the bacilli will be seen in active movement, this motility being due to the great number of hair-like flagella by which the organism is covered. The best methods of demonstrating these flagella by staining are given on p. 90, *et seq.*

Methods of Staining.—The typhoid bacillus stains well with all the ordinary aniline dyes, although somewhat more slowly than usual. It is decolourised by Gram's method of staining.

Growth on Media.—The true Eberth-Gaffky bacillus is readily distinguished from all others by its characteristic growth on the various culture media. Repeated subculture, as in the case of many other organisms, produces longer and abnormal forms. Very lengthened forms of the bacillus, which are somewhat characteristic, are sometimes seen in cover-glass preparations; these long bacilli are known as 'leptothrix' forms.

The *Bacillus coli communis*, which is always present in the intestines of both man and animals, closely resembles the typhoid bacillus. It is slightly shorter than the typhoid bacillus, and, like it, owes its power of motion to flagella, but it never possesses the profusion usually seen in the case of the Eberth-Gaffky bacillus.

The following table shows the main points of difference between the typhoid bacillus and the *Bacillus coli communis*, for which it might be mistaken:

MEDIA.	BACILLUS TYPHOSUS.	BACILLUS COLI COMMUNIS.
Gelatine plates.	The colonies on the surface form large grayish-white expansions with irregular edges, and after a time become somewhat yellowish-brown. The depth colonies are darker, with regular edges. Under a low power the colonies exhibit a characteristic woven structure. The gelatine is not liquefied.	The colonies are round and oval, with smooth-rimmed margins. The surface colonies form dirty-white expansions, which on magnification exhibit a furrowed appearance. The colonies later become dirty yellowish-brown in colour. The gelatine is not liquefied.
Gelatine streak culture.	Produces a grayish-white expansion with irregular edges. The growth has a tendency to keep to the inoculation streak, and often has a bluish iridescence.	Dirty yellowish-white expansion, which spreads all over the surface of the media, and often has a bluish iridescence when viewed by transmitted light.
Gelatine shake culture.	No gas bubbles.	Copious gas formation.
Agar-agar streak culture.	Grayish-white expansion, which covers the surface of the medium.	Dirty yellowish-white expansion, which spreads over the surface of the medium.
Potatoes.	Generally a faint grayish-white growth; the growth varies, however, on different potatoes.	Slimy yellowish growth.
Milk.	Turns faintly acid. No coagulation takes place.	Curdled after one to three days.
Broth.	Rendered turbid, and gives no indol reaction.	Rendered turbid, and gives a well-marked indol reaction after from twenty-four to forty-eight hours.
Formalin broth (1 in 7,000).	No growth.	Growth.

The milk, potato, and broth tubes are examined after

three days' incubating at blood-heat, the gelatine shake culture after three days at about 20° C.

It is worthy of note that in gelatine streak cultures typhoid has a tendency to be confined to the inoculation streak, while the growth in the case of the *Bacillus coli communis* spreads all over the nutrient medium.

As a further means of distinction, the *Bacillus typhosus* and *Bacillus coli communis* are amongst the limited number of organisms that can grow on media that contain small quantities of phenol (carbolic acid). These two organisms will grow in gelatine or broth containing 0·05 per cent. of phenol, whereas the growth of the other pathogenic and putrefactive bacteria is inhibited.

Pseudo-Typhoid Organisms.—A number of organisms have been described by Cassedebat, Babès, Booker, Klein, Springthorpe and others, which were obtained from cases which were clinically identical with enteric fever and other sources, which resembled the Eberth-Gaffky bacillus, but were shown to present slight but constant differences in their cultural characters.

They are only to be differentiated from the true typhoid bacillus by a very careful comparison of cultures made side by side on various media.

Cassedebat found three species of pseudo-typhoid bacilli in the Marseilles water-supply during the great typhoid epidemic in that town during 1891. They all corresponded with the Eberth-Gaffky bacillus in their growth upon gelatine, potato, blood serum, etc., and they all gave a negative indol reaction. Like the typhoid bacillus, they grew in milk without causing the coagulation of the caseine, but two of them produced an alkaline reaction in this medium, while the third corresponded with the Eberth-Gaffky bacillus in producing a decided acid reaction.

Occurrence and Distribution.—Admitting typhoid fever to

be caused by a specific pathogenic organism, we must look for the cause of every case in the specific contagion given off by a previous case, and as the virus appears to be air-borne (in this country) only for very short distances, if at all, its chief means of propagation must be either through the organism becoming endemic on the soil in close proximity to the patient's abode, or through its finding its way into water or milk, or adhering to articles of food, such as vegetables or shell-fish.

More than once carbon filters have been found to be contaminated with typhoid, and thus become a veritable poison-bottle. It seems at least probable that the typhoid bacillus may, like the vibrio of cholera, become endemic for a considerable period of time, since the bacilli will live in the fæces, and may even thrive on the surface of the soil. This is borne out by the recurrence of cases of enteric fever close to previous cases, and is well illustrated by the maps contained in the Local Government Board reports, which show recent cases marked by red dots, while cases of the previous year are marked in black. In those cases where enteric fever has been communicated from patient to nurse, it is probable that the infection has been carried by particles of undisinfected excreta becoming dried, and thus forming part of the floating dust of the atmosphere, or still more directly through particles of excreta coming into contact with the hands, and thus being conveyed directly into the system. It still seems to be an open question as to whether the lower animals are capable of transmitting typhoid fever to human beings; some observers think that cows, for example, are capable of suffering from and transmitting typhoid fever; if this be so, it is surprising that they do not more often suffer from it, as it is a comparatively rare thing not to find a herd drinking from water constantly polluted with their own and other animals' excreta.

Klein has very recently shown that the Eberth-Gaffky bacillus may be inoculated into calves, and may grow and multiply within the inguinal lymph glands.

Enteric fever appears to be distributed fairly evenly throughout the world. The influence of season is very considerable, the greatest number of cases occurring in the month of October. The case mortality is about 15 per cent. In the Registrar-General's returns, enteric fever, typhus, and ill-defined forms of fever, are classed together, and the mortality due to them is about 1 per cent. of the total death-rate. Typhoid fever is one of the diseases that are subject to compulsory notification, and being in its nature eminently amenable to sanitary control (that is to say, the specifically infectious material is easily destroyed or removed by proper means), the mortality due to it is decreasing.

The Conveyance of Typhoid Fever by Water.—It is now universally acknowledged that polluted water is the most important agent in the conveyance of enteric fever. Although water contaminated with sewage has been, and is still, drunk by a large number of people with impunity, so far as the appearance of enteric fever is concerned, yet the slightest contamination of a water-supply with the dejecta from a case of typhoid has in many well-authenticated cases caused widespread epidemics of the disease, which generally was confined to those persons who had used the particular polluted water-supply.

In many of the recorded cases of water-borne typhoid, the amount of organic matter accompanying the specific pollution was so extremely small that the water-supplies have been repeatedly proved by chemical analysis to be of high organic purity. Moreover, it has been shown that the organism which is the cause of enteric fever may, when introduced into potable water of good quality, not only

retain its vitality for a considerable period of time, but may multiply almost indefinitely. Therefore the slightest contamination with the alvine discharges from a case of true enteric fever may serve to render dangerous millions of gallons of drinking water. Thus, it will be seen that the virulence of typhoid-contaminated water is not necessarily dependent upon the organic impurity of the water, but upon the specific pollution. If this is granted, and experimental proof may be easily applied,* it will be admitted that under certain circumstances the question may arise, Has the epidemic of enteric fever now in progress in a given community had its origin in the water-supply?

It must be admitted that the proof of specific pollution in a number of the epidemics of water-borne typhoid rests on a somewhat incomplete basis, as will be seen from the perusal of an interesting series of papers by Dr. E. Hart, which have recently appeared in the *British Medical Journal*.† The bacillus of typhoid fever has, however, been isolated by many competent observers from water that had conveyed and caused the disease. Some doubt attaches to the identification of the organism by some of the earlier investigators owing to the almost constant presence in the waters of other organisms so closely resembling the typhoid bacillus that their differentiation is a

* A drop of a broth culture of *B. typhosus* (twenty-four hours old) was well diluted with sterile water. One c.c. of this diluted culture was added to 200 c.c. of the ordinary tap-water. The number of organisms was then estimated by an ordinary gelatine-plate culture, when 1 c.c. of the water was found to contain approximately 900,000 organisms. This amount of pollution was not sufficient to raise the amount of albuminoid ammonia appreciably. The tap-water previously contained only 200 organisms per cubic centimetre.

† 'Water-borne Typhoid: a History Summary of the Outbreaks in Great Britain and Ireland, 1858 to 1893,' by Dr. E. Hart, *British Medical Journal*, June 15, 22, 29; July 6, 13, 20; August 17, 1895.

matter of great difficulty. The organism which has given rise to much confusion is the *Bacillus coli communis*. This bacillus is a constant inhabitant of the intestinal tract and the fæces of both man and animals, and therefore is almost invariably found in all polluted waters.

In order to ascertain whether the typhoid bacillus is present in any given water, care must be taken that the *B. coli communis* is not mistaken for the former. This is a very difficult matter, as the vitality of the *B. coli communis* is much greater under all conditions than that of the typhoid bacillus. The object is generally attained by the addition of various chemical substances to the nutrient media, which effectually inhibit or destroy the growth of organisms other than the colon and typhoid bacilli. As pointed out by Frankland, such additions have frequently destroyed the typhoid bacillus and left the *B. coli communis*, owing to its greater power of resistance, alone, master of the field.

According to many authorities, notably Messrs. Roux and Rodet, there is reason to believe that the *B. coli communis*, under certain conditions, such as growth in sewage, etc., assumes a pathogenic character, and gives rise to a disease which is clinically undistinguishable from enteric fever. This view is borne out to a great extent by the fact that water contaminated with fæcal matter may be instrumental in causing typhoid fever without the actual access of the specific bacillus, as cases are on record where water long known to be polluted has acquired the property of conveying typhoid without the previous known contamination from a specific case of the disease. This is in accordance with the well-established fact that in some places enteric fever, once endemic, has disappeared upon the substitution of a pure for a contaminated water-supply, or the provision of adequate bacterial filtration.

Messrs. Demel and Orlandi* show that Roux and Rodet's statements as to the near relationship of the *B. typhosus* and the *B. coli communis* are borne out by the physiological and pathological effects of the metabolic products of the two organisms. Animals rendered immune to one are also rendered immune to the other bacillus, the virus of the *B. coli communis* having a higher therapeutic value than that of the *B. typhosus*. Germano and Maurea,† after a very prolonged investigation, have isolated no less than thirty varieties of typhoid-like bacilli, and they consider this fact supports the theory that the *B. coli communis* may, under certain conditions, develop into the *B. typhosus*. Nicolle,‡ after a very careful investigation, could only find the *B. coli communis* in a typical case of enteric fever, the blood and spleen being particularly examined. From the above facts, it will be seen that the possible dangers to be derived from the drinking of sewage-polluted waters are greater than previously supposed.

It is worth recording, however, in connection with the above, that Chantemesse has called attention to the fact that during the typhoid epidemic in Paris in 1894, the soldiers who drank the polluted water supplied to the Menilmontant barracks all escaped typhoid, notwithstanding the fact that the water was swarming with the colon bacillus.

Dr. Klein has recently studied§ the *B. typhosus* and *B. coli communis* as to their stability as separate species in culture, and in the process of transference from animal to animal. On the one hand, bacilli of both kinds, derived in each instance from human sources, were tested by him as to their vitality, and as to the retention of their dif-

* *Centralb. für Bakteriologie*, xvi., p. 246. † *Ibid.*, xv., p. 60.

‡ 'Annales de l'Institut Pasteur,' 1895, No. 1.

§ 'The Twenty-third Annual Report of the Local Government Board,' supplement containing the Report of the Medical Officer, p. 459.

ferential characters in waters of different composition and quality. He also took the two organisms derived from sources outside the human body (namely, from excrementally polluted water-supplies). These were passed from subculture to subculture, and were passed from peritoneum to peritoneum in separate series of guinea-pigs, they being cultured through no less than thirty generations. Whatever the source of the bacilli, and whatever the experimental conditions in the laboratory or the animal body to which they were exposed, each organism retained unimpaired its differential characters, and at no time showed the least tendency to depart from the characters generally accepted as being exhibited by these organisms. Incidentally during the course of these experiments, it appeared that the persistence in a water medium of both the typhoid bacillus and of the *Bacillus coli* is largely governed by the chemical constitution of the water.

Conveyance by Milk.—It is believed by some observers that cows may suffer from enteric fever and transmit it in their milk. Whether this is so or not, it is certain that epidemics may and do arise from the washing out of the churns with polluted water, or when the milk is adulterated with polluted water. The bacillus of typhoid multiplies in milk enormously faster than in water, and a vessel left damp with moisture containing only a few organisms would be capable of infecting its entire contents of milk in a few hours. It would be to the public interest if the adulteration of milk by the addition of water was made a more serious offence than it is at present, seeing what far-reaching consequences it may have. The establishment of creameries in many parts of the country is likely to prove an additional danger in the dissemination of milk-borne disease. At these creameries the milk of a considerable number of farmers is received twice a day, mixed

in a large tank, and passed through a centrifugal separator, whereby the cream is collected. Thus, it is evident that if the milk of one farmer was contaminated with typhoid, it would be the means of the conveyance of the disease over a large area. The only method of prevention of the spread of infection by the contamination of milk would be proper sterilisation, which would have to be systematically carried out all the year round.

An epidemic of typhoid fever due to the milk-supply will exhibit some or all of the following features: (1) The outbreak is sudden, and many of the attacks are simultaneous. (2) A large proportion of the households attacked have a common milk-supply. (3) The incidence of the disease will be greatest on the principal consumers.

Conveyance by Vegetation.—Enteric fever has been known to be conveyed by vegetables grown on sewage farms, and also by watercress grown in sewage-polluted streams.

Conveyance by Shell-fish.—Oysters, mussels, etc., which have come from water contaminated by drainage, may be a source of infection. Foote has recently been carrying out a series of interesting investigations on the vitality of typhoid bacilli when inoculated into oysters. For the first fourteen days after introduction, the typhoid bacilli multiplied, but after some time a steady decline in the number of microbes took place. Thirty days after the bacilli were first introduced into the oyster their presence was still demonstrable, they having been preserved in the stomach of the oyster, where they retained their vitality unimpaired.

In some experiments the water containing the oyster was infected with the bacilli, and it was found that they actually lived longer in the body of the oyster than they did in the water containing the latter, which seems to distinctly point to the possibility of contracting typhoid through the consumption of the bivalve.

Conveyance by Dust.—It is improbable that the soil in this country is a great factor in the conveyance of typhoid, but the soil in India seems to give peculiar facilities for the spread of the disease, and to play a somewhat different *rôle* to what it does in England. The soil for the great part of the year is very dry, and becomes converted into dust. All excreta, whether from sick or healthy persons, are buried in the ground according to the shallow system, and the soil is thus converted into a nursery for the growth of the bacilli. Since dust-storms are of very common occurrence, especially in hot and dry weather, it is not very difficult to understand how columns of fine dust whirl across the country, loaded with fæcal débris, and in time of epidemics with pathogenic organisms. Thus dust-storms become a fertile means of spreading the disease, since the typhoid bacillus has considerable vitality; wells and water-supplies at distant stations become contaminated, and the disease is thus spread far and wide. One means of preventing some of the epidemics of typhoid fever now so prevalent in India would be to insist that all excreta from typhoid cases should be disinfected or burnt.

In connection with the above information, which was supplied to us by Surgeon-Lieutenant Birdwood, of the Indian Medical Service, it is interesting to compare a report by Dr. H. Henrot, of Rheims (*Lancet*, February 1, 1896), respecting an outbreak of typhoid which occurred amongst two regiments of cavalry quartered in the above town during some manœuvres. The men rode over some land which had been recently manured with night-soil, and the weather being very dry, much dust was produced, which was of necessity both inspired and swallowed by the troopers. Attention was also directed to the bad smell which was prevalent at the time. Inquiry was directed as to whether the outbreak could be attributed to the water-

supply, but this did not appear to be the fault, as civilians using the same wells were not attacked. This case seems to give additional probability to the theory advanced by Dr. Birdwood, which was communicated to us some weeks before the publication of Dr. Henrot's report.

Pathogenesis.—In persons affected with typhoid fever, the bacillus is present in immense quantities in the fæces and intestines; it sets up an inflammation and suppuration of the Peyer's patches, forming ulcers which may become so deep as to lead to perforation. The bacillus is easily demonstrable in the fæces, but not so readily in the inflamed portions of the intestine, in which it occurs rather sparingly. In the fæces the bacillus is rarely found before the eighth or ninth day of fever, and from observation made by Dr James Richmond in the pathological laboratory of the Owens College, it appeared that only a few days (from six to ten) after the cessation of the fever the typhoid bacilli were no longer present in the fæces.

The Eberth-Gaffky bacillus is also found in the mesenteric glands, the spleen, and sometimes in the blood; in the intestine it is associated with streptococci and other organisms, and the inflammation set up originally by the typhoid bacillus may be continued by these other organisms after the typhoid bacillus can no longer be found in the fæces.

Occasionally the sputum (in some cases of pneumo-typhoid) and the urine may contain the typhoid bacillus.

The injection of the bacilli into the aural vein of rabbits causes death in from twenty-four to twenty-eight hours. Guinea-pigs into which the cultures are introduced by the mouth are also killed.

The *Bacillus coli communis* is not found with the Eberth-Gaffky bacillus in typhoid lesions, but it does frequently produce a secondary infection. The two organisms cannot

be cultured together in large numbers, as the products of their vital activity are mutually inimical.

It is a matter of frequent experience that cases of typhoid fever occur after exposure to sewer gas, or vitiated air, which does not actually carry the bacillus, but may produce such irritation as to render it possible for the bacillus (from some other source) to gain headway.

During epidemics the greatest incidence of attack is always heaviest upon houses or districts in which drainage defects exist. These facts should always be taken into consideration in connection with outbreaks of enteric fever.

It is still open to question how far we are compelled to acknowledge the *absolute* 'specificity,' of the Eberth-Gaffky bacillus, and whether we cannot conceive of varying degrees of 'specificity.' It seems not impossible that the pseudo-typhoid bacilli may be the true Eberth-Gaffky in transition stages. If we determine that enteric fever can be caused by the Eberth-Gaffky bacillus alone, and refuse to admit the possibility of varying degrees of 'specificity' we shall have great difficulty is assigning the cause of those cases of typhoid fever which present all the characteristics of true typhoid, in which no possible connection with any previous case can be traced, and where we have, to all appearances, true typhoid arising *de novo*.

To clear up these very interesting and important points, detailed information must be collected respecting cases in which no connection with previous cases can be traced, and further experimental work is needed with respect to the organisms above referred to as pseudo-typhoid bacilli, particularly as to their effects on animals.

The Bacteriological Diagnosis of Typhoid Fever.

Dr. Elsner, of Berlin, has recently published* the results of an investigation made to ascertain the possibility of the early recognition of enteric fever by the bacteriological examination of the stools. He has been able to recognise the Eberth-Gaffky bacillus, in some cases in so short a time as forty-eight hours after starting the culture. The following is an abstract of Dr. Elsner's paper.

The author went over the existing methods for the separation of *B. coli* and *typhosus* from other organisms and from each other, with no better results than have been previously obtained. In all cases but one he found that either persistent organisms other than those sought to be isolated would grow to an extent sufficient to spoil the plate (*e.g.*, *B. proteus* or *ramosus*), or else the *B. coli* would develop to an extent capable of preventing the recognition of the typhoid bacillus. The exception was potato gelatine, slightly acid, and mixed with 1 per cent. of iodide of potassium. The process recommended is to boil potato decoction (0·5 kilogramme to 1 litre water) with ordinary gelatine, add normal sodium hydrate solution till a definite acidity is obtained, filter and sterilise; adding 1 per cent. potassic iodide before using, inoculating into the medium, and pouring plates from it. The method of Holz is recommended for the reduction of acidity; thus, 2·5 to 3·0 c.c. of decinormal sodium hydrate solution is added to 10 c.c. of gelatine until only a faintly acid reaction is left, which increases on sterilisation, and must, of course, be again tested after that operation, litmus being recommended as the indicator. With this medium the author examined all the waters he could obtain, and he found that even the *B. proteus* and *ramosus*, which on carbolised gelatine would always grow,

* *Zeitschr. f. Hyg.*, xxi., 1.

either never occurred on his medium or were rapidly overgrown by the *B. coli*. The *B. coli* grew in twenty-four hours, presenting the usual appearance of that organism on acid media; the *B. typhosus* was scarcely visible in twenty-four hours, but in forty-eight hours appeared in small, shining, very finely-granulated colonies, like little drops of water, which contrasted strongly with the larger, much more coarsely granulated, and brownish colonies of *B. coli*. The *B. coli* only acquired the appearance of the typhoid colonies when a very large quantity was used in an inoculation, and many, therefore, grew without finding room for their proper development. In secondary plates, or in plates made with weaker inoculation, it was almost impossible to mistake one for the other. By this method the author examined thirty different colon and typhoid cultures, and in each case obtained the same result as with Pfeiffer's method, though with all other practicable methods it had in several cases been impossible to identify several of the organisms. Similar results were obtained with fæces, suitably diluted, contaminated with artificial cultures of the two organisms. Subsequently, on the outbreak of a typhoid epidemic, Dr. Elsner repeatedly examined the fæces of seventeen patients, and in fifteen cases, at various times between the seventh day and sixth week, he isolated the typhoid bacillus, which after isolation was completely identified as the Eberth-Gaffky bacillus.

The colonies had in each case developed in forty-eight hours so as to be easily identified; in those which were made by taking a loopful of stool and diluting, nothing had grown except the *B. coli* and the small typhoid colonies, with here and there a few liquefying colonies or easily recognisable yeasts.

Dr. Chantemesse, of Paris, has recently investigated Elsner's method for the diagnosis of typhoid. The stools

of healthy people, those of typhoid patients, and those of patients suffering from the various forms of fever, were submitted to Elsner's method of examination.

They are divided into three categories: (1) fever patients in the height of the evolution of the fever; (2) the convalescent stage; (3) healthy subjects. In pyretic cases of typhoid, Eberth's bacillus was always present in the stools. Elsner supplies seventeen cases, Lazarus five, and Brieger ten. Among the convalescent, the Eberth-Gaffky bacillus was found thirteen times in eighteen examinations; it was also detected in the stools of a male nurse in perfect health who attended typhoid patients. M. Chantemesse's personal observations are as follows: Eberth's bacillus was not detected in a case of erysipelas, nor in the two others of influenza accompanied with fever. In thirteen cases of typhoid the specific bacillus was found. This occurred each time the examination was made; the bacillus thus detected settled the diagnosis, which by clinical examination had not been clearly established. Lazarus has detected the typhoid bacillus in the stools of a patient forty-one days after the temperature had fallen to the normal point. The fact that a man in good health can carry in his intestines Eberth's bacillus, and thus disseminate it, throws much light on the so-called 'spontaneous' origin of typhoid fever.

The Serum Treatment of Typhoid.—In the course of a communication to the Paris Société de Biologie on February 22, M. Chantemesse said that he had succeeded in immunising several horses against the virus of typhoid fever. He obtained the serum of such strength that one-fifth of a drop inoculated into a guinea-pig twenty-four hours before infection protected it against a dose of typhoid virus fatal to animals not previously injected with the pro-

tective serum. It was ascertained also that injections of the serum produced no injurious effects upon a healthy man. M. Chantemesse stated that he had since employed injections of serum in three cases of typhoid fever. The temperature showed a regular fall from the time the first injection was made, and seven days after the commencement of the injections all three patients were quite free from fever, and had commenced to convalesce. M. Chantemesse added that the cases were not yet sufficiently numerous to permit of any trustworthy conclusion being drawn.

Löffler and Abel, in a recent paper,* give the details of an investigation upon the specific properties of the protective substances in the blood of animals immunised to *B. typhosus* and *coli communis*. For those details we must refer the reader to the original paper; here we can only give their conclusions. They are as follows: (1) By treating dogs with increasing doses of virulent cultures of *B. typhosus* or *B. coli*, substances appear in the blood of these animals which possess a specific protective property only against that kind of bacillus which has led to their formation. (2) The serum of normal animals protects against the fatal or lower multiples of the fatal dose of typhoid or *coli communis*. The strength of the dose supportable bears a certain ratio to the amount of previously injected serum. (3) The specific efficacy of the protecting substances in the blood of previously treated animals first becomes manifest if doses of the particular bacterium are given to the animal to be protected which are multiples of those doses against which normal serum confers immunity. (4) The specific protective action of the substances also shows itself on injection of a mixture of the bacteria and the serum. (5) Typhoid serum protects

* *Centralbl. f. Bakt., Paras. u. Infekt.*, Bd. xix., 1896, p. 51.

against a somewhat larger dose of *B. coli* than normal serum, and coli serum protects against a somewhat larger dose of typhoid bacilli than normal serum. By this somewhat increased protection the family resemblance of the two kinds of bacilli is manifested. (6) The specific sera do not protect against the substances contained within the bodies of dead bacilli to a greater extent than does normal serum. (7) By injection of normal serum into the abdominal cavity of guinea-pigs, and twenty-four hours later twice the fatal dose of dead bacilli, guinea-pigs may be immunised within two weeks against a hundred times the fatal dose of living typhoid bacilli. (8) If less than the fatal dose of typhoid bacilli be given at the first injection, and afterwards increasing multiples of the fatal dose be given, guinea-pigs may be made within forty-eight hours to withstand a hundred times the fatal dose (forced immunisation). (9) By injection of 0·5 to 1 c.c. of a powerful typhoid serum, animals which have been inoculated intraperitoneally with thrice the fatal dose of typhoid bacilli may be rendered immune to an infection that kills the control animal in twenty hours, even if the injection of the protecting serum have been delayed eight hours.

Practical Disinfection.—The stools should be received into a solution of mercuric chloride (1 in 500) or into a solution of bleaching-powder (6 ounces to the gallon), and all particles of excreta should be removed from the anus with cotton-wool moistened with one of these liquids. Soiled linen must be soaked in the solution for an hour, and then well rinsed in clean water; *the disinfection of the nurses' hands should be rigidly insisted on, and they should eat all their food only with knife, fork, and spoon, touching nothing with the hands.* This last precaution is most important, and must be rigidly adhered to in all cases.

DIPHTHERIA.

Discovery and morphology of the organism—Growth on media—Bacteriological diagnosis—Method of staining—Other organisms accompanying diphtheria—Distribution and occurrence—Pathogenesis—Antitoxin treatment—Preparation of the antitoxic serum—Results and advantages of the treatment—Dr. Washbourn's report upon the serum treatment—Practical disinfection.

The bacillus causing diphtheria was first described by Klebs in the year 1875, but was not universally regarded as the true cause of the disease till Löffler succeeded in obtaining pure cultures in the year 1884. The Klebs-Löffler bacillus is a short rod devoid of motility, sometimes apparently pear-shaped at the ends when stained. When grown from the throat in the early stage of the disease, the organism is usually seen as a short rod, about 3 μ long and 1 μ thick; but after subculturing through several generations, it grows out into rods twice this length and thickness; the protoplasm then often takes the stain unevenly.

When specimens are examined from a case in which the patient suffered from true diphtheria and is now convalescent, it is generally found that the bacilli are present as long and short rods together. The bacillus does not form spores, but is not killed by drying; dust containing the bacillus retains its virulence for months. The organism is aerobic, and its thermal death-point is 58° C.

Growth on Media.—The bacillus grows readily on gelatine, if slightly alkaline, but more rapidly on agar at blood-heat, but the colonies so produced have no special characters. On potato there is hardly any visible growth, unless the potato be first moistened with beef-broth, in which case the growth is both rapid and visible.

On blood serum or glycerine-agar it grows with great rapidity, but the best medium of all is that devised by Löffler, who uses equal parts of serum and broth, with 8 per cent.

of grape-sugar (this mixture is, of course, sloped and heated to 'set' it before use).

The growth appears as a cream-coloured streak along the line of the inoculation, and is so rapid as to be plainly visible to the naked eye twelve hours after the inoculation, provided the tube is kept at blood-heat.

In addition to the actual streak produced where the wire touched the medium, there will be noticed a number of small isolated dots, near to, but not touching, the actual streak. This appearance, as well as the rapidity of the growth, is characteristic of the Klebs-Löffler bacillus, but neither can be relied on as a certain indication till confirmed by a microscopic examination of stained specimens.

Some observers state that it is possible, by rubbing a sterile wire on an inoculation streak only four hours old, long before any visible growth has appeared, to obtain sufficient material to permit a correct microscopical diagnosis, even when the bacillus could not be demonstrated by direct staining of the membrane.

In the false membrane the Klebs-Löffler bacillus is associated with several organisms, the principal being the staphylococci and the *Streptococcus pyogenes*. This latter organism has so often been found in the false membrane as to be believed by some observers to be the specific cause of diphtheria. It not infrequently happens that in the early stages of diphtheria the bacillus cannot be demonstrated, and any case that presents the clinical symptoms of diphtheria should be treated as such, whether the bacteriological examination enables us to detect the bacillus or not. On the other hand, bacteriological diagnosis will often insure the recognition of a case of true diphtheria, in which the clinical symptoms are ill defined, and which might, though slight in itself, give rise to severe cases. After an

attack the bacillus frequently persists in the throat for weeks, and as infection may be transmitted in this way, strict isolation of convalescents should, where possible, be insisted on, until the bacillus has disappeared. The bacillus is usually found in such cases in involution forms, such as long and short rods together. These are rarely found before the disease has run its course.

A person frequently entering diphtheria wards, as a nurse or medical man, may very often have the bacillus in the throat without contracting the disease.

Method of Staining.—The diphtheria bacillus is readily stained with the usual aqueous basic aniline dyes. Löffler's methylene blue is the best general staining reagent for cover-glass preparations. It is also stained by Gram's method.

Bacteriological Diagnosis of Diphtheria.—A suitable 'outfit' for this purpose consists of a small box containing two stout glass test-tubes, both cotton-wool-plugged and sterilised; the one holds a cotton-covered iron wire, the other contains Löffler's medium, duly sloped.

With one end of the cotton swab, the suspected portion of the throat is rubbed and the infection transferred to the surface of the medium, taking care to rub lightly, so as not to abrade the surface.

The tube is now plugged, and either posted to a laboratory or placed in the incubator, or it may be incubated on the person by placing in the waistcoat pocket and buttoning the coat over it. Better results are obtained by using an ordinary platinum wire to inoculate the tube with, instead of the cotton-covered swab, and when a platinum wire is used, three streaks should be made on the medium, after touching the throat once only. By this means a part of one of these three streaks will probably be a pure culture of the Klebs-Löffler, if it be present.

After twelve hours' incubating the tube is examined, and if the streaks are found to show whitish colonies, many of them separate from the actual line of inoculation, and presenting on staining the appearance of an immense number of small short rods, slightly clubbed at the ends, there is little doubt but that they are the true Klebs-Löffler bacilli.

Klein has described a pseudo-diphtheria bacillus of wide distribution, which might be confused with the Klebs-Löffler, but can be distinguished from it by growing the two organisms on gelatine, when it will be found that the pseudo-diphtheria bacillus grows much more slowly. Its occurrence is, however, so rare that it is usually neglected.

Dr. R. T. Hewlett and H. Nolan, of the British Institute of Preventive Medicine, have published (*British Medical Journal*, February 1, 1896) the results of the examination of 1,000 tubes of Löffler's blood serum medium inoculated from suspected cases of diphtheria. Of the 1,000 cases examined, 587 were found to contain the diphtheria bacillus, in 409 cases it was not found, and in 4 cases bacilli were observed, as to the identity of which with the Klebs-Löffler bacillus, or the distinction therefrom, they were unable to satisfy themselves. Thus 58·7 per cent. of the cases were true diphtheria. In 40·9 per cent., or about two-fifths, of the cases the diphtheria bacillus was not found, and the majority of these were probably not diphtheria. In 25 cases no growth appeared on the surface of the blood serum. In 600 cases they kept notes as to the other organisms present in the cultivations. In 216 cases they found the Klebs-Löffler bacillus present alone, while in 247 it was absent, and in the remaining 137 cases they found the true diphtheria bacillus associated with other organisms, micrococci, not streptococci, predominating.

Messrs. Hewlett and Nolan also draw attention in their

paper to the possibility of error owing to the swab having been rubbed on a small area of the throat, also to the 'crowding out' of the bacilli, which may occur owing to the presence of common saprophytes, or, again, to the destruction of the bacilli by the use of antiseptics.

Distribution and Occurrence.—Diphtheria seems to be distributed more or less widely all over the globe, but is far more prevalent in cold and temperate climates than in the tropics. It is one of those few diseases in which a distinct increase (which cannot be attributed entirely to improved diagnosis) has taken place in the past few years. Some twenty-five years ago the disease was far more prevalent in rural districts than in towns, but during the past few years, while it has decreased in rural, it has increased in urban districts. Its tendency to reappear at intervals in particular districts would point to the specific organism having either a saprophytic tendency, or to its retaining its vitality in dust, and thus rendering the site of previous cases more or less permanently infective.

Its distribution does not appear to be affected by other diseases; its mortality is highest during the last quarter, and lowest in the summer months. There is no evidence of any influence being exercised by race or sex, but the mortality is highest at ages below five, and rapidly diminishes after ten years of age.

Transmission of the disease may take place by direct infection, as in kissing, by the use of infected spoons, cups, etc., or by inhaling the breath of a patient, or sputum or discharges which have been permitted to dry without having been disinfected. It would also be safest to treat the bowel discharges as infective. The organism, if it finds its way into milk, either from infection from an employé or through the disease of cows themselves, multiplies with great rapidity, and epidemics may thereby be occasioned.

Faulty sanitary conditions may assist in the spread of this disease by preparing the throat for the bacillus, and may in this way apparently give rise to cases which would never have arisen had it not been for the existence of such conditions.

It is also a matter of common experience that an epidemic of true diphtheria is sometimes preceded by a prevalence of 'sore throat,' which seems to gather in intensity till cases arise of which the clinical character shows them to be undoubtedly true diphtheria. No doubt the systematic bacteriological examination which is now being undertaken in several districts will do much to increase our knowledge of these obscure points.

It has been found that during diphtheria epidemics, dogs, cats and cows may all suffer from a disease which appears to be identical with human diphtheria.

Pathogenesis.—The incubation period varies from two to seven days, but is usually from about two to four days, while the mortality due to diphtheria is about 0·20 per cent. of the total death-rate.

In a typical case of diphtheria a white membranous coating is found covering the fauces, tonsils, and uvula, from which it may be spread into the larynx and trachea. Traumatic diphtheria may arise through the organism coming into contact with an abraded surface.

In diphtheria we have to deal chiefly with a poison elaborated by the growth of the bacillus; and therefore, whether antitoxin or any other form of treatment is to be applied, it is of pressing importance to circumscribe its growth by antiseptic treatment as far as possible.

An attack of diphtheria affords little or no protection against a second, and, as in many other diseases, the mortality is greatest at the beginning of an epidemic.

Antitoxin Treatment of Diphtheria.—The theory of arti-

ficially-induced immunity is discussed on p. 112 *et seq.*; but as much interest is now being taken in this method of treatment as applied to diphtheria, we give in detail one method of its preparation and employment.

To obtain antitoxin serum, it is first necessary to prepare a toxin of known strength. This, on injection into suitable animals, causes some substance which we term an 'antitoxin,' of opposite properties, to be produced; and when the blood serum of an animal so immunised is used for injection into patients suffering from diphtheria, we introduce into their circulation the same kind of substances that are present at convalescence, and neutralise the poisonous products of the organism.

We first prepare the diphtheria toxin by growing a pure culture of the Klebs-Löffler in alkaline beef-broth at 37° C. in flat-bottomed flasks supplied with a regulated current of sterile air. The growth first causes the liquid to become cloudy, and a sediment eventually develops at the bottom of the flasks, the liquid becoming clear again. As the alkalinity of the medium becomes neutralised by the acid metabolic products, the growth slackens, and the operation is usually complete at the end of three weeks. It is then filtered through a Pasteur-Chamberland filter, and its strength has next to be ascertained, which is done experimentally by finding what volume is required to produce the death of a guinea-pig weighing 500 grammes in twenty-four hours. This should be effected by $\frac{1}{10}$ centimetre, and if at or near this standard, the toxin is ready to begin the process of immunising an animal. Horses are used for this purpose, since they are able to yield quantities of serum without injury to their health. A quantity of 10 c.c. is injected into a horse which is (so far as can be ascertained by careful examination) in perfect health, the place chosen for inoculation being the apex of the

shoulder. A slight swelling makes its appearance, and after a few days subsides again, when the operation is repeated, using a larger quantity, and a slight swelling may again appear; and when this has in turn subsided, further injections are made, till it is possible to inject so large a quantity as 200 centimetres of the toxin without injury to the animal's health.

When this point is reached, the horse is immunised against the poison of the Klebs-Löffler to such a degree that it could bear the injection even of living bacilli, and is now ready to furnish supplies of antitoxin serum. For this purpose, all that is necessary is to collect blood from the animal with due antiseptic precautions, and after the clot has separated to preserve the serum for use. This is done by placing a sterile cannula in the jugular, and drawing off the required quantities of blood into sterile glass bottles. When the clot has separated, the serum is put up in small stoppered bottles, or sealed up in tubes for sale. One of the firms supplying this article evaporate the serum to dryness, and send it out in the form of golden-yellow scales, which dissolve in three or four parts of water. The injection of the antitoxin serum into patients is usually made into the subcutaneous tissue of the flank, and if more than one injection is considered desirable, they should be made on opposite sides.

Results obtained by the Treatment.—Experience has shown that most benefit is obtained by injecting the remedy as early as possible—that is to say, on the second or third day, after which the remedy is of less value.

In mild or very severe cases, not much advantage is gained by the treatment; hence the remedy is—at present, at any rate—more adapted for use in large institutions, where cases suitable for antitoxin treatment can be picked out, than in private practice.

Advantages of the Treatment.—In many cases the injection is followed by a speedy reduction in the severity of the symptoms, and a rapid separation of the membrane, in cases where it was causing obstruction of the air-passages, thus diminishing the number of cases which would otherwise require tracheotomy.

Disadvantages and Difficulties.—One of the principal drawbacks to the antitoxin treatment is the difficulty of judging the quantity of injection that will be most beneficial, and of late much larger quantities have been employed, with more satisfactory results. It is hardly necessary to remark that the greatest care must be taken to ensure sterility of the injecting syringe, and if the whole of a bottle of serum is not used at once, it must be thrown away.

It is at present somewhat early to form very decided opinions of the value of the treatment, but we may be satisfied that in very many cases distinct benefit has resulted. The treatment is, however, not suitable for every case, and is, except in experienced hands, not entirely free from danger, and should preferably be employed not later than the third day of the disease.

Injections of serum are often followed by the appearance of various rashes, sometimes erythematous, at other times urticarial, and in a few cases not at all unlike the rash of scarlet fever or of measles. These rashes usually come on in from seven to ten days after the injection; sometimes the rash is accompanied by more or less pyrexia, and in a small number of cases by pains, and even effusion into some of the joints. It has been alleged by some that the injection of serum has actually been the cause of nephritis, but this is contrary to the experience of those who have had the best opportunities of observing the effects of serum in an extended number of cases.

Louis Corbett (*Journal of Pathology and Bacteriology*,

January, 1896) has arrived at the conclusion that the blood serum of normal horses may possess antitoxic power, and that there are two distinct therapeutic agents present in the blood of immunised horses. He also finds that there is a gradual diminution of antitoxic power in the serum yielded by horses, even though they continue to receive doses of toxins. He is of opinion that the best method of obtaining antitoxic serum is to begin with injecting the horses with a culture of living bacilli.

The following is an extract from an interesting and important report by Dr. J. W. Washbourn upon the serum treatment of diphtheria, issued to the Medical Congress held in London during August, 1895:

'The value of the antitoxin treatment has been tested in two ways: the one is the clinical method, the observation of the effect of antitoxin upon the course of the disease; and the other is the statistical method, the comparison of the mortality of a series of cases treated by antitoxin with that of similar series treated in other ways.

'In order to arrive at a correct conclusion by either method, certain precautions must be taken, and it will be my duty to point out the fallacies that may arise.

'Let us first consider the statistical method; and here I may say that I only propose to treat the matter from a general point of view, for I have been given to understand that the details of the statistics are to be discussed in another section. We know that all statistics are open to fallacies if the number of cases is not sufficiently large, and if other precautions are not taken into account.

'In diphtheria the age of the patient is a most important factor in determining the mortality. The statistics of the Metropolitan Asylums Board clearly demonstrate this point. Out of 4,435 patients under 5 years of age admitted into the Board hospitals between the years 1888 and 1894, the

mortality was 49·9 per cent.; of 3,723 patients between 5 and 10 years it was 28·1 per cent.; of 1,330 patients between 10 and 15 years it was 10·6 per cent.; and of 1,972 patients between 15 and 40 years it was 4·6 per cent.; the mortality in each quinquennium between 15 and 40 being, roughly speaking, the same. It is thus obvious that in comparing statistics of mortality the age of the patients must be stated.

'New methods of diagnosis may also lead to errors in statistics. The diagnosis of the cases treated by antitoxin has been verified by a bacteriological examination, while in former times this plan has usually been omitted. We must consider what effect this has upon the statistics.

'A bacteriological examination enables us now to exclude from our statistics many cases of angina and croup which would formerly have been included. These cases are less severe than cases of true diphtheria, and on this account the older statistics of mortality are lower than they should be. On the other hand, a bacteriological examination sometimes enables us to recognise as diphtheria mild cases of angina which in former days would not have been included in the diphtheria statistics. I have no doubt that among hospital patients, at any rate, this class of cases is decidedly less frequent than the former class, consequently the mortality of cases in which the diagnosis has been verified by bacteriological examination should, *cæteris paribus*, be higher than that of cases in which the examination has been omitted.

'Another point to consider is the varying severity of the epidemic. It is not common to meet with series of mild or severe cases occurring at irregular intervals. The only way to avoid this fallacy is to take either a large number of cases in each series, or to take a large number of series for comparison.

'Since the introduction of the antitoxic treatment, a large number of cases have been recorded in England, Germany, France, Austria, America, and other countries. Many of these are of but little value from the statistical point of view. Either the cases are isolated, or the age has not been noted, or previous statistics have not been recorded, or they have been rendered useless for comparison by the lack of some precaution or other. Nevertheless, there remain over 3,000 cases recorded by different observers, in which the mortality has been compared with that of cases not treated, and in which careful precautions have been taken to arrive at a correct conclusion.

'In every series the statistics show a lower mortality than that obtained by other methods of treatment. In many instances the decrease in mortality has been remarkable, sinking to half that which it was before.

'In no instance has a physician recorded a large series of hospital patients under his own care in which a careful comparison of the statistics has been unfavourable to the antitoxic treatment.

'Great care should be taken to perform the injection with strict aseptic precautions. The skin should be carefully washed with soap and water, and subsequently with 1 in 20 carbolic lotion. The syringe should be boiled immediately before use. In the choice of a syringe, it is necessary to select one which can be boiled without damage. The piston should be made of asbestos or india-rubber, and all the joints made tight by washers of the same substances; no cement of any kind should be used in the joints. If care is not taken, septic troubles may arise.

'In a few instances abscesses have been recorded after injection, and these may be due to two causes: either the injection has not been performed with proper precaution, or the serum has been previously contaminated. The latter

can only be avoided by using serum from a thoroughly reliable source, and by taking care not to use serum from a bottle that has been left open and exposed to the air.

'The most suitable place for injection is the subcutaneous tissue of the flank. The injection should be made as soon as the disease is diagnosed, for the earlier the treatment is commenced, the better the chance of recovery. The quantity used must depend upon the severity of the case, the strength of the antitoxin, and the age of the patient. A severe case requires a dose larger and more frequently repeated than a mild case. Probably an adult requires a larger dose than a child, but this point does not appear to me to be definitely settled.

'As far as the strength of antitoxin is concerned, we are met with the difficulty that a uniform method of standardising is not always adopted. The testing of the serum is not an easy matter, and can only be performed by a skilled bacteriologist; but until a uniform system is adopted, it will be impossible for clinical observers to agree upon the proper dose to be employed in any individual case.'

Practical Disinfection.—The saliva and discharges of the nose and mouth should be regarded as virulently infectious, and should, as far as possible, be received into rags and burned before they have a chance to become dry; the excreta also should be disinfected. Any polluted linen that cannot be conveniently burned should be soaked for one hour in a solution of mercuric chloride (1 in 500) or in bleaching-powder solution (6 ounces to the gallon), and then well rinsed in fresh water before going to the wash.

CHOLERA.

Discovery and morphology of the organism—Growth on media—Distinction from the Finkler-Prior bacillus—Bacteriological diagnosis of cholera—Indol reaction—Variations in organisms—Occurrence and distribution—Transmission of the disease—Pathogenesis—Haffkine's antitoxin treatment.

The *Spirillum choleræ Asiaticæ*, the organism producing true Asiatic cholera, is generally known as Koch's 'comma' bacillus. This organism was discovered by Koch in 1884, in the excreta of persons suffering from cholera. The researches of Koch in Egypt and India during 1884 showed that this spirillum is constantly present in the contents of the intestine of cholera patients, but is not found in the healthy subject. Koch's 'comma' bacillus does not form spores; it is killed by drying; its thermal death-point is about 50° C., and it is very rapidly killed by sunlight.

Method of Staining.—The cholera spirillum stains best with an aqueous solution of fuchsine or gentian violet. It is not stained by Gram's method.

The bacillus grows readily on almost all media, whether oxygen is admitted or not, and after it has developed a saprophytic habit is much less easily killed by disinfectants than when fresh from the stool.

The Finkler-Prior spirillum is the only one which is at all likely to be confounded with Koch's 'comma.' It is found in large numbers in the stools in English cholera—cholera nostras, cholera infantum.

The general behaviour of the two organisms on different media is distinctive, as is seen in the following table:

	Potato.	Gelatine.	Indol Reaction.*
Koch's 'Comma' Bacillus	Grows only at blood-heat	Slowly liquefied	Obtainable in twelve hours.
'Finkler-Prior' Bacillus	Grows readily at room-temperature	Rapidly liquefied	Obtainable only after three days.

* Care must be taken that the broth is faintly alkaline.

The 'comma' bacillus produces sulphuretted hydrogen in broth cultures. In examining a sample of stool suspected to be choleraic, the microscopic appearance alone is often sufficient to establish its true character, while in other cases the culture test may yield positive results when the microscopic appearance is doubtful or negative. In true cholera the ileum presents a characteristic appearance, the mucous and serous coats being congested.

To establish the identity of an organism with Koch's 'comma,' it should be found to resemble it (1) morphologically; (2) culturally; (3) in its chemical products.

'Comma' bacilli straight from the stool are short, thick, curved rods, about 3·0 mm. long and 0·3 mm. thick. They also possess three or four flagella, while after subculture they are found to have only one. It is also a remarkable fact that the flagella of organisms fresh from the stool stain readily with the ordinary aniline dyes, while the flagella in the organisms taken from a culture require mordanting before they will take the stain. On repeated subculture, the organism grows out into longer and thinner rods, bearing very little resemblance to bacilli straight from the stool; hence it materially follows that cultures met with in bacteriological laboratories are not always typical.

In examining the body of a patient who had died from supposed Asiatic cholera, the condition of the ileum should first be noted, and a portion preserved for examination by being ligatured at both ends, and then placed in a tightly-corked bottle, if the examination cannot be made at once. In acute cases the mucous and serous coats are found greatly congested, and the epithelium is usually to a great extent detached in the shape of flakes.

The flakes may contain large numbers of the 'commas,' and it is frequently possible to report positively at once as

to the nature of the disease on the immediate microscopic examination of one of these flakes, whether from the contents of the ileum or in a living patient from the stool, a minute fragment of one of the flakes being suspended in salt-and-water, and examined by the hanging-drop culture, when practice will enable the trained observer to recognise the spirillum of Koch by its characteristic screw-like movement. Again, if a portion of a flake be crushed carefully between two cover-glasses, which are then drawn apart and stained, it will be found that in cases of cholera the organisms present what is known as the 'fish-in-stream' appearance; and when this is seen, the case may be reported as true cholera without awaiting the result of cultural experiments, which of necessity involves some delay, which in the face of a possible epidemic it is of the utmost importance to reduce as far as possible.

Such characteristic appearances are, however, only to be found in a minority of cases, and it is generally necessary to perform the following cultural experiments: Two or three gelatine tubes are melted, cooled to 35° C., and then inoculated and poured into plates, while at the same time some tubes containing sterilised Dunham solution are inoculated. (This solution consists of peptone 1 per cent., salt 5 per cent., in distilled water.) The gelatine plates are examined after forty-eight hours at 22° C., and the colonies produced by true cholera are distinguishable by the appearance of small funnel-shaped depressions in the gelatine, having yellowish points at their apex, while the gelatine begins to liquefy. Fragments of colonies having these characters are picked out with a platinum needle for microscopic examination, both in the hanging-drop culture and in cover-glass specimens. The Dunham solution tubes are incubated *for twelve hours only*, and are then probably cloudy from the rapid growth of the organisms,

and the production of indol and nitrites has proceeded sufficiently far to cause the appearance of the indol reactions (a distinct rose-madder tint) on the addition of a drop of pure sulphuric acid. The indol reaction was introduced in the year 1884; previous to this chief reliance was placed on the appearance of the young colonies.

Many other organisms besides Koch's 'comma' also produce indol and nitrates in sufficient quantities to yield the indol reaction, but not in this time (twelve hours), so that if the indol reaction is obtained, and the organisms are microscopically similar to Koch's 'comma,' we may report positively without delay. It is always advisable to adopt this method of inoculation into Dunham solution, because not only do we get the indol reaction, but a plentiful crop of organisms, probably nearly a pure culture, on which to do further work. In cases of true cholera the organism frequently cannot be demonstrated in the stool when the patient is on the way to recovery, so that the inability to demonstrate the organism in cases three or four days from commencement of the attack must not be taken as evidence that the disease was not true cholera. A detailed account of the cholera vibrio is contained in Dr. Klein's Appendix to the Local Government Board Report, 1893, from which most of the above details are taken.

There is no doubt that the term Koch's 'comma' should be taken to include a group of organisms in varying stages, or more probably a group of organisms all capable of producing cholera, but not all exactly similar in their cultural aspects.

Sheridan Delépine and James Richmond, in a paper on the 'Bacteriological Diagnosis of Cholera' (the *Journal of Pathology and Bacteriology*, April, 1895), call attention to the danger of placing over-much confidence in the bacteriological examination alone, and neglecting the clinical

characters, when determining whether a particular death is due to true cholera. They also draw attention to the fact that the organism in different years and in different places exhibits somewhat varying characters, and point out that these differences may have led observers to ignore the presence of true cholera, because such spirilla as they found did not possess precisely the characters that are supposed to distinguish the true cholera spirillum. They also draw attention to records where several varieties of 'commas' were found in cases of cholera, and that these variations were observed, not merely after repeated sub-culture, but when the organisms were taken direct from the ileum or stool.

For example, there are organisms which have produced disease clinically identical with cholera which have exhibited unusual variations from the time generally required to give the indol reaction or to liquefy gelatine, and these observers found that on growing a variety of the cholera organism repeatedly in broth which had been made alkaline, the indol reaction became less and less marked, and the organism liquefied gelatine more slowly; but on transferring them to ordinary peptone salt solution, the indol reaction was again obtained in a few hours.

As an instance of an outbreak possessing many of the characters of cholera, we may recall the Greenwich epidemic diarrhœa outbreak, which began on October 4, 1894, and lasted some twenty days, during which there were as many as 245 cases; the mortality, however, was comparatively low, as there were only eleven deaths. On bacteriological examination, Koch's 'comma' could not be found, but an organism of the *Proteus vulgaris* type was isolated.

Occurrence and Distribution.—The disease is endemic in the Delta of the Ganges and in Asia; in countries in which

it is not endemic, its course may be traced along the ordinary lines of traffic, showing that it is imported by travellers. The recently-issued report of the Medical Officer of the Local Government Board ('Cholera in England,' 1893) shows how perfectly the disease may be excluded from a country by the careful execution of stringent regulations to prevent the landing of persons suffering from the disease, or coming from infected ports without undergoing due quarantine and disinfection. The season of the year has great influence on the spread of cholera, and if infection does not reach us till the cold weather is about to set in, it is improbable that very much harm will be done, though isolated sporadic cases may occur during winter.

Cholera spreads most rapidly when the earth temperature is high; this happens chiefly when the ground-water is low, and this is in accord with the observations of Pettenkofer that increase in cholera is often preceded by a fall in the ground-water.

Transmission of the disease may take place by means of water (as at Hamburg), by milk, uncooked vegetables, or by fomites. Like enteric fever, the infection is confined to the bowel and stomach discharges; so that if reasonable care be taken, there is but little fear of the disease being transmitted from the patient to nurses or attendants.

The 'comma' is readily capable of a saprophytic existence; and thus, if cholera-stools were thrown out on to a rubbish-heap without being properly disinfected, the organism might live in such a position for a considerable length of time. If this happens, it is probable that by the action of rain the organisms will find their way into any well near which has its supply from the surface-water; or should the pollution occur to a stream near the intake

of a waterworks, the results may be very disastrous and far-reaching. In some waters cholera vibrio will live for considerable periods. Charcoal filters once infected have been repeatedly known to pollute water otherwise pure for many weeks, and cause grave epidemics.

When, therefore, any town is attacked with or threatened by cholera, special care must be taken to prevent, at all costs, pollution of the public water-supply, and arrangements should be made by the sanitary authority for the gratuitous supply of disinfectants, medicine, and food to those in need, and, if possible, to provide due isolation and treatment for persons attacked. All water should be boiled or passed through a Pasteur-Chamberland filter.

Cholera has been termed a filth-disease, and this title may fairly be applied to it, if we bear in mind that we mean, not that the disease can be generated by filth, but that in filthy surroundings there is the more reason to fear its ravages, and when once it has appeared, it will be expelled with greater difficulty.

Accumulations of night-soil, rubbish, etc., should be removed, and the places they occupied well cleansed. Uncleanly premises should be thoroughly scrubbed and limewashed, and proper ventilation insisted on.

Pathogenesis. — The symptoms of Asiatic cholera are intense and sudden fever and collapse, the face being drawn and pinched, and the tongue cold. The urine is suppressed, and the stools have the characteristic rice-water appearance. Death may occur in so short a period as twelve hours after taking the infection, or three hours after the first symptoms are noticed. The incubation period rarely exceeds two or three days. The symptoms differ from those that occur in English cholera only in intensity; hence we are at present compelled to rely largely on the bacteriological examination to decide whether a given

case is one of true Asiatic cholera or not. As a matter of fact, those cases of supposed cholera in which the 'comma' could not be found have very rarely proved infective.

The experiments of Marshall Ward and of Pettenkofer, who swallowed pure cultures of Koch's 'comma' in support of their contention that cholera was not caused by this organism, are held by some to throw doubt on the 'specificity' of the organism; but it is more probable that they escaped from ill effects by being in good health, and having a normal acidity in their gastric juice. It is probable that, had a larger number of people been experimented on, the conclusions arrived at would have been reversed.

Experiments on animals by injection of pure cultures are not productive of cholera unless some special means are adopted to neutralise the acidity of the gastric juice. This immunity of animals is no evidence whatever against the pathogenicity of the organism for man, as there are many other organisms which produce disease in man to which the lower animals are immune.

Haffkine's Antitoxin Treatment.—This treatment has been tried on upwards of five thousand persons in India, and it may fairly claim to have passed the experimental stage successfully. The results reported are much more successful than those that have hitherto attended the antitoxin treatment for diphtheria.

Professor Haffkine administers two injections—the first of weak vaccine, and a second of stronger vaccine at the end of five days. This second dose requires five days to act, before the full power of immunisation that it exerts is effected.

With reference to the employment of this remedy and its effects, as observed during an outbreak of cholera in a certain district in Calcutta, Dr. Simpson, Medical Officer of Health of Calcutta, says that 'after eight days—in fact,

after five days—the difference in liability to attack is very marked, the inoculated living in the same houses in Calcutta being twenty times safer from attack and eighteen times securer from death should cholera enter the house. This is protection of a very decided character.' No cases of cholera occurred among those who subjected themselves to both inoculations. We must, however, bear in mind that any person who has been persuaded to adopt this prophylactic measure is probably well informed on the subject of cholera, and fully alive to the wisdom of boiling drinking-water and avoiding uncooked vegetables, and may, therefore, be in the habit of using precautions neglected by the majority.

Preparation of Anticholeraic Vaccine.—The object of this treatment is the acclimatisation of the system to a greater amount of the cholera poison than it would be likely to be exposed to under ordinary circumstances. The treatment is prophylactic, not remedial, as is the case in the serum treatment of diphtheria. Haffkine uses two vaccines, the first being an 'attenuated' culture, produced by growing the cholera spirillum in broth at a temperature of 39° C., in flat-bottomed flasks, supplied with a current of sterilised air. Grown in this way, the bacilli become 'attenuated,' and may be grown repeatedly on nutrient media without regaining their virulence.

This vaccine is used first, 1 cubic centimetre being injected into each person, and five days are then allowed to elapse for it to exert its full effect. This prepares the subject for the second dose of an 'exalted' virus—that is to say, a culture which, so far from being 'attenuated,' has had its virulence intensified by being passed through a series of animals.

The cultures of both the 'attenuated' and the 'exalted' virus are not either sterilised or filtered, but both the

living bacilli and their products are injected together. There is no danger of imparting cholera by this procedure, as the cholera bacilli cannot live in the blood. Both vaccines may, if desired, be carbolised, in which case they will be sterile; but their power is unimpaired, and they may be preserved indefinitely in sealed tubes.

CHAPTER VII.

PYOGENIC ORGANISMS.

Pus formation is not necessarily due to bacteria—Organisms of pus—*Staphylococcus pyogenes aureus*—Methods of staining and growth on media—*Staphylococcus pyogenes albus*—Other staphylococci—*Bacillus pyocyaneus*.

In dealing with the organisms of pus, we shall describe only those of common occurrence, and which are supposed to give rise to pus formation, omitting those whose presence is probably accidental. The formation of pus is not of necessity dependent on the action of any micro-organism at all, for pus may be entirely sterile; the term *aseptic pus* has been applied to such purulent discharges. It has been shown experimentally that pus may be produced by the introduction into the tissues of sterilised bacteria of several kinds, the organisms alone being introduced without the soluble products of their growth, so that the exciting cause must be either the intracellular contents of the bacilli, or possibly the mechanical effect combined with the positive 'chemiotaxis' that most bacteria exhibit to the leucocytes. Certain other substances have been proved on injection to cause the formation of pus, such as solutions of nitrate of silver, strong ammonia, turpentine, etc.

A large number of micro-organisms give rise to the formation of pus, among which may be mentioned the following: *Staphylococcus pyogenes aureus*, *Staphylococcus*

pyogenes albus, the *Streptococcus pyogenes*, the *gonococcus*, the *pneumococci*, the *Bacillus tuberculosis*, the *bacillus of typhoid*, the *Bacillus coli communis*, etc.

Pus may also be formed by the actinomyces (ray-fungus) and by certain aspergilli; all these may give rise to pus-formation either alone or associated with other organisms. The organisms most frequently found in pus are the *Staphylococcus pyogenes aureus* and the *Staphylococcus pyogenes albus* and the *Streptococcus pyogenes;* besides these, there are others of the same group closely allied to the foregoing, but they are of somewhat less frequent occurrence.

Staphylococcus Pyogenes Aureus.—This organism was isolated and described by Rosenbach in the year 1884. It is a spherical coccus, about 1 μ in diameter, which sometimes occurs as a diplococcus, but more commonly in grape-like masses, from which it derives its name.

Method of Staining.—The organism stains with all the usual aniline stains; it can also be stained by Gram's method.

Growth on Media.—The coccus grows well on all the ordinary media, both at room-temperature and at blood-heat, and the cultures so made retain their vitality for many months.

Inoculated into broth, the organism produces a perceptible turbidity in about eighteen hours, while the gelatine begins to liquefy as soon as there is any visible growth, the liquefaction occurring in stab culture all along the stab. On agar and blood serum a thick streak develops, which is at first pale in colour, but later on develops the golden-yellow colour; exposure to diffused daylight is essential to the formation of the colour. The organism is found chiefly on the surface of the body, which appears to be its normal habitat; it has been found

by various observers in dust, earth and water, but its presence in these is probably accidental. The thermal death-point of the organism is given at 58° C. by Sternberg, provided the organism is in a moist condition; if desiccated, he finds that much greater heat is essential to ensure its destruction.

Pathogenesis.—The result of an injection of the organism into animals seems to be largely governed by the size of the dose, large doses being fatal, while small ones are without result.

Experiments on the human subject by various observers show that the inoculation of the organism is always followed by the production of a local lesion in which the staphylococcus may be recognised, and which heals up after a few weeks. The organism has been found in ulcerative endocarditis and in infective osteo-myelitis, and in a great number of inflammatory lesions and abscesses in every part of the body.

The *Streptococcus pyogenes* is dealt with under Erysipelas.

Staphylococcus Pyogenes Albus.—This organism occurs less frequently than the foregoing, but in all respects, with the exception of its colour, is not to be distinguished from it. It liquefies gelatine, and behaves in a similar manner to the *Staphylococcus pyogenes aureus* in every respect. Welch (quoted by Sternberg) is of opinion that, as its pathogenic properties are more feeble than those of the foregoing, the name *Staphylococcus epidermidis albus* is preferable, as he considers it hardly deserves the qualification of 'pyogenic.' He finds it to be the most frequent inhabitant of the skin, and to be so deeply buried in the epidermis as to render it impossible to be attacked by any of our present means of disinfection. It is the most frequent cause of stitch-abscess.

Other organisms belonging to this group are the *Staphy-*

lococcus pyogenes citreus, the *Staphylococcus cereus aureus*, *Staphylococcus cereus citreus* and the *Staphylococcus cereus albus*, all of which are so named from the waxen appearance of their cultures.

Bacillus Pyocyaneus.—It appears that there are two organisms found in blue and green pus, only one of which possesses true pathogenic properties.

They are separately described by Sternberg as the 'bacillus of Gessard' and the 'bacillus of Ernst'; the former of them seems to be pathogenic, while the other may be a harmless chromogenic saprophyte. It is the latter which produces the 'chameleon phenomenon.'

In a paper by E. P. Williams and Kenneth Cameron (*Journal of Pathology and Bacteriology*, January, 1896), the authors give an account of some cases of fatal disease in children in which bacilli presenting the characters of both Gessard's and Ernst's bacilli were isolated and examined by them; they further state that from the facts observed by them it seems probable that these bacilli are capable of many variations in form and colour-production, according to their environment, and that further experiments will prove Gessard to be correct in his opinion that they are but varieties of races of the same bacillus.

ERYSIPELAS.

Fehleisen's streptococcus—Growth on media—Media must contain peptone—Virulence is more rapidly lost in broth than in solid media —Occurrence and distribution—Pathogenesis—Exhibits varying degrees of infectivity—The possible identity of the organism with the *Streptococcus pyogenes*—Practical disinfection.

The *Streptococcus erysipelatis* was first described by Fehleisen in the year 1883; it is found in great numbers in the lymph channels of the skin in persons suffering from

erysipelas. By some observers it is believed to be identical with the *Streptococcus pyogenes*, but it is more probable that there is a group of streptococci which are exceedingly alike in their microscopical and cultural characters.

The streptococcus, whether in pure culture or in section, can be stained by the ordinary aniline dyes.

Growth on Media.—The organism grows in peptone-broth, on gelatine, agar or blood serum; on potato the growth, if any, is imperceptible. The organism grows equally well in the presence or absence of oxygen. When grown in beef-broth at 37° C., the medium becomes turbid in twenty-four hours, and after some three or four days multiplication ceases. Living organisms have, however, been found in the sediment that collects at the bottom of the liquid after so long a period as ninety days, and by recultivating a considerable quantity of this sediment on to fresh media, new growths have been obtained. The cessation of growth in broth after three or four days is due more to the exhaustion of the medium than to the formation of a metabolic product injurious to the growth of the organism. This point is proved by Louis Corbett and W. S. Melsom in a valuable paper in the *Journal of Pathology and Bacteriology*, vol. iii., November, 1894. Little or no growth takes place in meat-broth that has not been peptonised. In broth cultures the organisms grow out lengthwise into chains of 30 or 40 elements, of which the individual cocci may vary very much in size, both large and small cocci being found in one chain. It also sometimes happens that a new chain starts away from one of the cocci in a chain, thus producing branching. The variation in the size of the cocci is also noticed in cultures on other media.

The growth on gelatine is slow; the colonies are generally small and discrete, while on agar at 37° C. the colonies are often larger, and sometimes spread into a connected mass,

particularly if the agar is moist. When grown in broth the virulence is rapidly reduced, but may be restored by passing through an animal. In stab or shake culture in gelatine, the colonies appear as small whitish spheres, but the gelatine is never liquefied. Sternberg finds the thermal death-point of the organism to lie between 52° and 54° C.

Occurrence and Distribution.—The disease is less frequent in the tropics than in temperate latitudes, and is found in cold climates, such as Iceland and Greenland. The greatest number of deaths in this country occurs in the months of November and January, and the least in the summer months.

Women are more susceptible than men, but the mortality among them is less, while it is very high in children up to the first year of life. Traumatic erysipelas is most common, and probably the cases that are termed 'idiopathic' are really due to a slight traumatic injury or abrasion so small as to escape notice. This streptococcus is probably the most frequent cause of puerperal fever. Predisposing causes are wounds, injuries, overcrowding in surgical cases, intemperance, want of proper nourishment, unhealthy and dirty surroundings, and bad ventilation. The disease sometimes becomes endemic in a ward, and is expelled with difficulty. It appears at times to exhibit very much more marked powers of infectivity; it is probably conveyed by air as well as by contagion and by fomites. Artificial immunity has been produced in rabbits, but the period of protection is short; when unprotected, the disease is fatal to them in about half the number of cases.

Dr. Bokenham is inclined to the opinion that the *Streptococcus erysipelatis* and the *Streptococcus pyogenes* are the same organism under different conditions. He has also studied the *Streptococcus pyogenes* with a view of preparing antistreptococcic serum, and considers it probable that a

combination of this with antidiphtheritic serum might produce more satisfactory effects than we have hitherto been able to obtain from the use of antidiphtheritic serum alone. An antistreptococcic serum prepared by Marmorek is also used. The probable identity of the *Streptococcus erysipelatis* and the *Streptococcus pyogenes* is supported by Jordan, Frankel, and Von Eiselberg (Schenk), and the two organisms are described as one by R. Würtz (*Précis de Bactériologie Clinique*, 1896).

Practical Disinfection.—Care should be taken to ensure thorough cleanliness and proper sanitary conditions of the surroundings, and the attendants should be isolated with their patients. The affected portions should be washed with an antiseptic, as in the case of scarlet fever.

GONORRHŒA.

Specific organism first discovered by Neisser—Cultivated by Bumm—Method of staining—Special media necessary for culture—Pathogenicity demonstrated by Bumm—In gonorrhœa the gonococcus is associated with other micro-organisms—Researches of Bosc—Bacteriological diagnosis—Practical precautions.

The diplococcus which is the cause of this disease was first discovered by Neisser in the year 1879, and six years later was cultivated by Bumm on blood serum. The gonococcus is a facultative aerobe, but grows best when oxygen is excluded; it is a very strict parasite, and can only be cultivated on special media, and must be recultured at frequent intervals, or its vitality is lost.

It grows only at blood-heat. The organisms occur in pairs, and appear somewhat biscuit-shaped when seen by a high power. The thermal death-point is shown by Sternberg to be about 60° C.

Method of Staining.—The gonococcus is stained best by

Löffler's methylene blue, and is decolourised by Gram's method, which serves to distinguish it from certain other diplococci that occur in gonorrhœal pus, but not from all.

Growth on Media.—Bumm succeeded in growing the gonococcus on human blood serum, while other observers report good results on blood serum agar, blood serum gelatine, plover egg albumin, etc. The best results seem to have been obtained by Wertheim, who prefers a mixture of two parts of glycerine agar and one part of human blood serum; on this medium he obtained well-defined growths in so short a time as twenty-four hours after inoculation. A pure culture of the gonococcus assumes a raised appearance similar to a mulberry, and is of a yellowish-white colour. It is necessary to subculture every three days, or the vitality is lost.

Occurrence and Pathogenesis. — Gonorrhœa is known throughout the whole of the globe, and the specificity of the diplococcus of Neisser is fully admitted; its specificity has been fully demonstrated by Bumm, who, after obtaining pure cultures from gonorrhœal pus, cultivated the organism through twenty successive generations, and then introduced it into the urethra of healthy men with positive results. In gonorrhœa the pus may contain gonococci in pure culture during the first few days, but later on staphylococci and streptococci will probably be found. The gonococci themselves are peculiar in being most frequently found *in* the pus-cells. Bosc mentions as many as fourteen other organisms besides the commoner staphylococci (nine of them being diplococci) which occur in the pus, and has published a table by the aid of which their identity may be established. After the acute stage of gonorrhœa has passed, and there is no longer any considerable flow of pus, the gleet that follows may still contain the gonococcus, and so long as there are any floating pus filaments to be

seen in the urine, it is possible that the gonococcus is present, and might produce gonorrhœa on coitus with a healthy female. Würtz recommends that, before making search for the organism in such filaments, an artificial irritation should be excited by the injection of nitrate of silver solution, so as to cause a more copious discharge of pus, in which the gonococcus, if present, can be demonstrated. This view has also been discussed favourably in certain American journals, and a short article has appeared on it in the *Medical Press* (November 20, 1895).

The gonococcus has also been found in cystitis, in chronic urethritis, and in bubos, though the latter are chiefly caused by streptococci.

Practical Precautions.—The gonorrhœal pus is infective on any mucous surface, and if accidentally introduced into the eye may cause its loss, unless treated within two days. Patients should therefore be warned of this danger.

GLANDERS.

The bacillus of glanders was first described by Löffler and Schutz—Proof of its specificity—Morphology—Method of growth in culture—Attenuation occurs rapidly in culture—Susceptible animals—Farcy—Diagnosis of glanders—Malleïn—Preventive measures.

The *Bacillus mallei* was first described in the year 1882 by Löffler and Schutz, and was proved by them to be the specific cause of the disease by the successful inoculation of horses and asses with pure cultures of the bacillus.

The organism is a short thick rod about 2 μ long by 0·5 μ thick; that is to say, it is somewhat shorter and thicker than the tubercle bacillus.

Growth on Media.—The bacillus grows on potato at blood-heat, but only slightly at room-temperature; it grows slightly on gelatine, and readily on glycerine agar, but the

growth on the latter does not produce the characteristic appearances seen when the organism is grown on potato.

On potato the growth is apparent in three to four days; at first it has the appearance of honey, but later on becomes yellower, and eventually darker, till it approaches a chocolate colour.

With the exception of the pyogenic organisms, glanders is almost the only coloured pathogenic organism. Cultures on all media rapidly become attenuated, and die easily unless kept at blood-heat. Schenk states 'that the infective power of the virus' (probably meaning the specific discharge) is not destroyed by drying for three months. This is explainable if the organisms are protected by being surrounded by a dried coating of albuminous matter.

By some observers it is believed that the organism forms spores, but no method of staining has yet been published by which they can be demonstrated.

Method of Staining.—The glanders bacillus stains with difficulty with the ordinary aniline dyes, Löffler's methylene blue being the best. The bacillus will not stain with Gram's stain. When it is desired to stain it in sections, the following procedure may be adopted:

1. *Wash the section in water.*

2. *Stain in carbol-fuschine for twenty minutes, heated to* 50° *C.*

3. *Transfer to slide; blot with filter-paper; heat with* 1 *per cent. acetic acid for thirty seconds to one minute; wash with water; blot; dehydrate with alcohol; blot and mount in balsam.*

Pathogenesis.—The disease is communicable to many horses, mules, asses, field-mice, and guinea-pigs. Cattle are entirely immune, and white mice and rabbits partly so.

In man, glanders occurs after infection from a diseased horse, generally through the infective discharge coming

into contact with some slight traumatic injury. In the horse, when the disease affects the skin, it is termed 'farcy.' The discharge either from the nostrils or from ulcers contains comparatively few bacilli, and these are accompanied by large numbers of pyogenic organisms, so that it is not easy to demonstrate the bacillus either by staining or by culture.

It is said to be easy to obtain a pure culture by Strauss's method (quoted by Sternberg). He recommends the injection of the suspected discharge into the abdominal cavity of a male guinea-pig. If the *Bacillus mallei* is present, the scrotum will be red and shining after three days; and when suppuration takes place later, the pus will be found to contain the glanders bacillus in pure culture.

The Diagnosis of Glanders.—In 1890 Helman and Kalning, working independently of each other, with the view of providing a curative and immunising material, discovered certain effects of extracts of the bacillus on animals affected with glanders, which subsequent experimentation has proved to be of the utmost service in diagnosis. In this country a liquid glycerine extract, prepared after the manner of Koch's 'tuberculin,' is sold under the name of 'malleïn.' The London County Council has recognised its value, and encouraged its use by their veterinary inspectors. It is stated that in the case of one large horse-owning company, in which glanders has been known to exist, the Council has entered into an arrangement to pay full value compensation for any horse which has reacted to malleïn, and which, on being killed, yields no evidence of glanders.

When an affected animal is tested with this reagent, a decided rise of temperature takes place, and a swelling follows at the seat of inoculation. The swelling is of more importance diagnostically than the rise of temperature,

while no effect is produced in a healthy animal except a trifling rise of temperature.

There is no record of any successful attempts to *treat* the disease in this way, mallein being employed for diagnosis only. Quite independently many horse-owners have adopted mallein as a test, and have isolated the reacting. This procedure on the part of horse-owners has, however, received a rude shock by a case in which it is stated that a practitioner had used mallein in a stable with a view of isolating those animals which had reacted, though yielding no clinical evidence of glanders. Ten animals gave typical reaction. One of the Council's inspectors visited the stable subsequently, and at once placed the official label on them. This mode of proceeding, though possibly technically correct, will, it is to be feared, have a deterrent effect on horse-owners in London, which affords nearly 80 per cent. of the cases recorded in Great Britain.

Preventive Measures.—All horses suffering from suspicious discharges should be examined, and, if found to be suffering from glanders, should be forthwith slaughtered and the carcase burned, special care being exercised in handling it.

The stables and all clothing which may be contaminated should be carefully disinfected with mercuric chloride.

SYPHILIS.

Syphilis appears to belong to a group, the other members of which are tuberculosis, leprosy and glanders—Lustgarten's bacillus is probably the specific organism—Some observers have described streptococci—Methods of staining—Growth on media—Bacillus of Eve and Lingard—Capsulated diplococcus of Disse and Taguchi—V-shaped bacillus of Dr. Van Neissen—The Contagious Diseases Act.

Several observers have described various organisms as the cause of syphilis, but, with the exception of the bacillus

of Lustgarten, their discoveries have received very scant confirmation.

The disease bears such a close family resemblance to tuberculosis, glanders and leprosy that we cannot but expect that it will be ultimately established that it is due to a specific bacillus. Lustgarten described his bacillus in the year 1884; it is a slightly curved rod, somewhat smaller than the tubercle bacillus. He found it in the primary sore in syphilis. He does not appear to have succeeded in growing it in artificial culture.

The organism has been found by other observers in the syphilitic gummata of the intestine and in mucous membrane of the mouth.

Method of Staining.—The bacillus of Lustgarten stains with the usual basic aniline dyes, and also by Gram's method.

Lustgarten's method of staining is as follows: Sections are placed in gentian-violet aniline-water for twelve to twenty-four hours at the ordinary temperature of the room, then for two hours at 40° C. The sections are transferred to absolute alcohol for a few minutes, then placed for ten seconds in 1·5 per cent. solution of permanganate of potassium, and washed in sulphurous acid. If the ground-substance of the sections is not completely decolourised, the second part of the process must be repeated. After this the sections are dehydrated, cleared, and mounted in balsam. These bacilli, after staining by the Ziehl-Neelsen method (unlike the tubercle bacilli), are easily decolourised by mineral acids.

In searching for the bacillus of Lustgarten in the syphilitic lesions in congenital syphilis, several observers have failed to find it, but have reported streptococci as well as the capsulated diplococci of Disse and Tagucchi.

Growth on Media.—Eve and Lingard reported in the year

1886 that they had succeeded in cultivating from the blood of syphilitic patients a bacillus much resembling the bacillus of tubercle, but which grew readily on blood serum, forming a thin, yellowish-brown layer.

In the same year Disse and Tagucchi claimed to have discovered a diplococcus which they were able to grow on artificial media, and with which they were able to produce a disease in animals which they considered analogous to syphilis.

The discovery of the bacillus of syphilis is also claimed by Dr. Van Neissen,* of Wiesbaden, who has described a micro-organism in the blood of syphilitic patients, which he finds most frequently as a diplo-bacillus. The two rods are not in a straight line, but inclined to one another at an acute angle, presenting a V-shaped appearance. The remarkable feature about this reported discovery is that he finds the bacillus when submitted to subculture exhibits other forms and produces mycelial threads and spores. The organism is said to liquefy gelatine, but to grow best on blood serum. The entire account of the discovery seems highly improbable.

It is stated that in the reign of Radama VI., King of Madagascar, a French physician, Dr. Mailloux, subjected the slaves of the king, some 2,000 in number, to a preventive inoculation. Unfortunately the process communicated the disease to the entire number.

An admirable article, entitled 'The Social Evil and the Propagation of Venereal Disease,' appeared in the *Lancet* of February 1, 1896, in which attention is called to the great benefits that are derived from the systematic inspection of prostitutes, and the evils that must arise from want of regulations or supervision of any kind. To be convinced

* *Lancet*, January 4, 1896.

of this, it is only necessary to examine the statistics of the venereal disease among our soldiers before and after the repeal of the 'C. D.' Acts, and it is very greatly to be regretted that these valuable measures are not now in operation.

CHAPTER VIII.

INFLUENZA.

The specific organism was discovered in 1892—Method of staining—Growth on media—Epidemics of note—The disease has produced different clinical effects in different years—An attack is not protective—Prophylaxis.

The organism causing this disease was first described by Pfeiffer in the year 1892. It is found in the sputum and blood of influenza patients during the febrile period; it is a very small rod with rounded ends, and is generally found in pairs, but on cultivation grows out into strings in a similar way to anthrax.

Method of Staining.—The bacillus stains with some difficulty; it is best stained with warm carbo-fuchsine, or Löffler's methylene blue. It is not stained by Gram's method.

Growth on Media.—The influenza bacillus grows in stab culture in grape-sugar agar at 37° C., in a thin whitish streak, exhibiting no distinctive characters. On the surface of glycerine agar on which a few drops of blood have been smeared it forms small transparent colonies which are perceptible with difficulty. It grows in broth containing grape-sugar and glycerine, and in the case of all media it must be subcultured every eight days at least, or its vitality will be lost.

Occurrence, Distribution, and Pathogenesis.—The claim of Pfeiffer's bacillus to be recognised as the specific cause of influenza is admitted and confirmed by many observers, the bacillus being found in the blood and sputum in influenza and in no other disease. Influenza is a contagious disease, characterized by a short period of incubation, namely, from twelve to twenty-four hours, and a sudden onset, with rapid rise of temperature, sometimes preceded by a rigor. It is often accompanied by serious complications, and usually followed by extensive and prolonged loss of strength. The disease is reported to have been epidemic in England in the years 1729, 1732-33, 1737, 1742, 1758, 1762, 1767, 1775, 1782, 1803, 1833, 1837, 1847. A good account of the epidemic of 1847 is to be found in the medical journals of that date by the late Dr. Peacock. From 1847 till the December of 1889 England has been free from its ravages. In the May of that year the disease broke out in Asia, spreading to St. Petersburg by October, and reaching London by November, though subsequent reports show that there were some isolated cases as early as October. After Christmas the disease spread with such rapidity that it is shown by statistics that one-third of the male population suffered from the disease. Since the spring of 1890 there have been sporadic cases, and a regular epidemic between January and April in the years 1891, 1892, 1893, 1894.

The disease may occur in a simple or uncomplicated form, or may be accompanied or followed by respiratory or gastro-intestinal lesions or neuroses. The latter may follow even a simple case, and may be accompanied by respiratory lesions; but no case involving both respiratory and gastro-intestinal lesions has been recorded. In 1889-90 the respiratory lesion was by far the most common, in 1890-91 the gastro-intestinal, and it was not till the third year of the

epidemic that the cases affecting the nervous system were seen. In half the cases there is enlargement of the spleen, and many are accompanied by rashes, whilst a few present patches of purpura; and there are some cases on record where there was hæmoptysis, hæmatemesis, epistaxis, and other hæmorrhages, during the acute attack. The simple form lasts from three to five days, and the complicated from eight to ten, except those affecting the nervous system, when the patient is often months, or even years, in shaking off the effects, and there are a few cases where insanity or paralysis has resulted. It is not uncommon for the same patient to have two attacks in one year, and in each fresh epidemic those who have had the disease once are far more liable to be attacked than those who have previously escaped. Half the cases are accompanied by violent pains in the back, reminding one of small-pox, whilst darting, screwing pains at the back of the eyes are almost pathognomonic of the disease; there is also intolerance of light, and frontal headache.

The disease is very fatal to the weak and aged.

Prophylaxis. — Care should be taken to keep up the general health, and no reliance whatever should be placed on any attempts at 'aerial disinfection.'

TETANUS.

First obtained in pure culture by Kitasato—Morphology—Characters of growth—Method of staining—Method of obtaining pure cultures—Resistance of spores to heat—Production of artificial immunity in animals—Tetanus antitoxin—Researches of Dr. Sidney Martin on the metabolic products of the tetanus bacillus in the human body.

Kitasato was the first to obtain pure cultures of the tetanus bacillus, in the year 1889; but it had been shown several years previously by Sternberg, and later by Nicolaier, that tetanus could be produced in animals by subcutaneous inoculation of garden-earth.

The bacillus is a slender rod, and generally shows a spore at one end; this spore, being larger than the bacillus, gives it the appearance of a drum-stick. The bacillus sometimes grows out into long filaments, in which no spores can be seen.

Method of Staining.—The tetanus bacillus can be stained by all the usual aniline stains, and by Gram's method. The spores may be demonstrated by the method of double staining given on p. 89 *et seq.*

Growth on Media.—The bacillus is a strict anaerobe, and must therefore be grown either in stab culture or in an atmosphere containing no oxygen. In glucose-gelatine or glucose-agar stab culture, a feathery radiated appearance is seen, together with a certain amount of gas-formation, and, in the case of the gelatine, of liquefaction. The organism can only be got to grow with difficulty in gelatine, even when glucose is added, but grows satisfactorily on glucose agar. All cultures possess a peculiar and characteristic smell. The organism liquefies solid blood serum, and will not grow on potato. The spores are very resistant to heat and to chemical reagents; in fact, the organism

has been obtained in pure culture by heating ordinary garden-earth to 80° C. on two or three successive days, and then preparing agar shake cultures from the earth so treated, in which all bacteria—except possibly a few of the thermophilic organisms—will have been killed by the heat to which they have been exposed. To destroy the vitality of the spores, they must be boiled at least twenty minutes, and may require a still higher temperature.

Pathogenesis.—Cases of tetanus used to be described as 'traumatic' and 'idiopathic'; but, viewed in the light of bacterial knowledge, it seems probable that idiopathic cases are merely those in which the traumatic injury was so small as to escape notice. The organism is pathogenic to man, guinea-pigs, mice, and rabbits, while birds are but slightly susceptible. Immunity has been produced in mice, guinea-pigs, and rabbits by inoculation with attenuated cultures. A tetanus antitoxin is now prepared at several bacteriological institutions, and cases of its successful employment are frequently reported in the medical journals.

Dr. Sidney Martin has recently devoted attention to the isolation of the poisonous bodies produced in acute traumatic tetanus. He recognised the danger of attempting the extraction of such easily-decomposable bodies as these products may be expected to be by the aid of chemicals, and hence he confined himself to the use of alcohol, ether, and water alone as a means of separating them from the tissues. He worked on materials from seven fatal cases of tetanus, employing the blood and spleen. After having made and purified his extracts, he experimented as to their physiological action on mice and rabbits, and proved that he had succeeded in separating two distinct bodies, one of which produced the fever of tetanus, while the other produced the spasmodic muscular effects. He has arrived at the following general conclusions:

'1. That in all cases of traumatic tetanus there are present in the blood and in the spleen the products of bacterial action—viz., albumose and certain acid organic bodies.

'2. That to the albumoses must be ascribed the production of the fever of tetanus. They produce none of the tetanic symptoms.

'3. That the other extract contains the substances which are the direct excitants of the muscular spasms of tetanus.'

MALIGNANT ŒDEMA.

Discovered by Coze and Feltz—Forms spores—Method of staining—Must be grown on special media under anaerobic conditions—Occurrence of the disease—Method of obtaining a pure culture—The bacillus is the cause of surgical gangrene.

The *Bacillus œdematis maligni*—also known as the *Bacillus septicus*—was first described by Coze and Feltz in the year 1872, and afterwards studied by Koch and by Pasteur. The organism is a motile rod with rounded ends, about 4 μ long and 0·1 broad. It forms spores both at room-temperature and at blood-heat. No development takes place below 16° C. (Schenk), and the most favourable temperature is about 38° C. The spores are mostly situate at the end of the rod, and are stated by Sternberg to be very resistant, but neither their death-point nor that of the bacillus is given.

Method of Staining.—The bacillus stains readily with all the basic aniline dyes, and is decolourized by Gram's method of staining. The flagella may be stained by Löffler's method (Sternberg).

Growth on Media.—The distinction between malignant œdema and anthrax, which is not easy by the microscope alone, is readily seen by their behaviour on culture. The

organism is a strict anaerobe, and must therefore be grown either in stab or shake culture, or in a vacuum, or in an indifferent gas. As growth occurs at room-temperature, cultures in gelatine are possible. Development is accelerated by the addition of 2 per cent. of glucose.

The gelatine is liquefied, and gas is formed at the same time. The gas consists chiefly of hydrogen and carbonic acid (Würtz), and has a peculiar and disagreeable odour, due to minute traces of other gases. The same gas-generation occurs in agar stab cultures. Blood serum is liquefied; there is no visible growth on potato.

Distribution and Pathogenesis.—The bacillus is frequently present in the soil and in dust; in the intestine of man and certain mammals; and has been found by Van Cott in musk-sacs, thus affording an explanation why an injection of tincture of musk has occasionally been followed by an attack of malignant œdema.

The bacillus cannot easily be obtained by culture from earth or dust, and so the readiest plan is to inoculate subcutaneously either a rabbit or a guinea-pig with garden-earth.

On the death of the animal, which may occur in twenty-four to forty-eight hours, the bacillus will be found in plenty in the œdematous fluid, but not, like anthrax, in the blood, except later, when it has multiplied after death.

Sternberg points out that the gas manifested in the frothy exudation when an animal is inoculated with garden-earth is absent, or nearly so, when the inoculation is made from a pure culture, and is therefore probably due to other organisms.

The bacillus is the exciting cause in surgical gangrene, and is pathogenic for horses, pigs, sheep, rats, mice, and some birds, while cattle are immune.

The result of an injection into an animal is to some

extent dependent on the size of the dose, and the larger animals often recover. When this is so, they are said to possess a subsequent immunity, which may also, according to Roux and Chamberlain, be induced by the injection of filtered cultures, or of serum from animals that have died of the disease.

SYMPTOMATIC ANTHRAX.

The specific organism was discovered by Bollinger and Feser—Morphology—Method of staining—Growth on media—Pathogenesis—Production of artificial immunity.

The bacillus of symptomatic anthrax was first described by Bollinger and Feser in the year 1878, who obtained it from the affected tissues in animals suffering from 'quarter-evil.'

The bacillus is a rod about 4 μ long and 0·5 μ thick. It is motile, and forms spores, which are situated at different positions in the rod, and, from their large size, cause its distortion. The thermal death-point of the bacillus is 80° C., while that of the spores may be very considerably higher, especially when dried.

Method of Staining.—The bacillus may be stained by the ordinary aniline dyes, and the flagella may be demonstrated by staining them first with Ziehl's stain, and then staining the bacilli with methylene blue; the flagella may be stained by Löffler's method.

Growth on Media.—The organism is strictly anaerobic, and grows best on media containing a small addition of glucose; it can either be grown in stab culture or in an atmosphere of hydrogen. Spores are most quickly formed in agar cultures incubated at blood-heat. In both agar and gelatine gas is formed, and the cultures have a peculiar odour, while gelatine is slowly liquefied.

Cattle and sheep are almost the only animals subject to this disease; guinea-pigs are susceptible, and die within thirty-six hours after inoculation. It is stated that cultures on solid media preserve their virulence better than those in broth. The production of immunity in cattle has been successfully attempted by injecting them with attenuated cultures.

CHAPTER IX.

PNEUMONIA.

Organisms most frequently found in pneumonia—A large group of cocci have been isolated by Kruse and Panzini — *Micrococcus pneumoniæ crouposæ*—Diplo-bacillus of Friedlander—Methods of staining—Cultural differences—Method of obtaining pure cultures—The organisms may exist in the healthy throat—Value of bacteriological diagnosis—Practical disinfection.

The *Micrococcus pneumoniæ crouposæ* is the most frequently occurring organism in pneumonia; it was found by Sternberg in healthy sputum in the year 1880, and was independently discovered and described by Pasteur a month or two later, and more completely studied by Fränkel at a later date. The *diplo-bacillus of Friedlander*, also known as the *diplococcus of Friedlander*, was described by him in the year 1883, and is another of the more commonly occurring organisms associated with pneumonia. It is not so frequently present in pneumonia as the micrococcus of Sternberg. In addition to these two organisms (which in all probability are really two classes or groups of the more frequently-occurring diplococci in pneumonia, the one represented by the micrococcus of Sternberg, and only growing at blood-heat, while the other, represented by the diplo-bacillus of Friedlander, grows at room-temperature), a group of no fewer than nineteen cocci have been isolated from pneumonic sputum by Kruse and Panzini (in the year

1892), who worked on pneumonic material from various sources; they found these organisms, which were remarkable for their virulence, to be chiefly diplococci. The *Pseudo-diplococcus pneumoniæ*, described by Sternberg as discovered by Bonome in 1889, is probably a member of this group. J. Washbourn (in a paper read before the Pathological Society of London, and printed in the *Journal of Pathology and Bacteriology*) does not appear to have met with organisms of varying virulence, but in other respects his experiences confirm those of other observers, particularly as regards growing the organism (Sternberg's micrococcus) for any length of time on artificial media.

Both organisms may be found in the blood, and are surrounded by a capsule the substance of which is soluble in water. When found in the blood or cultivated in broth, both organisms are apt to be lanceolate in form, instead of spherical, while on solid media they are generally round. They sometimes occur three or four together, and on agar may grow out into long chains.

Method of Staining.—The micrococcus of pneumonia stains with Gram's method of staining, the diplo-bacillus of Friedlander is decolourised, while they both stain with all the ordinary basic aniline dyes. The two organisms are very similar in microscopic appearance, but are readily distinguished by their cultural differences.

Growth on Media.—The following table shows the chief points of difference:

	Agar and Blood Serum.	Growth occurs.	Vitality.
Diplococcus of Sternberg	Minute transparent drops	Only at 37° C.	Soon lost in culture.
Diplococcus of Friedlander	Abundant grayish masses	Readily at room-temperature	Vigorously retained in culture.

The micrococcus of Sternberg does not grow on potato, and but slightly on gelatine, while the organism of Friedlander produces a thick yellowish growth on potato, and grows vigorously on gelatine, while in stab gelatine it produces what is known as the 'nail' culture. The death-point of Sternberg's organism is given by him as 52° C., while that of Friedlander's organism he finds to be 56° C.

The *Micrococcus pneumoniæ crouposæ* so soon loses its vitality in culture that special means have to be adopted to keep it alive, whereas the diplo-bacillus of Friedlander retains its vitality vigorously, and it has been found that fresh growths could be obtained from cultures a year old. Pure cultures of both organisms are most easily obtained by inoculating a guinea-pig with pneumonic sputum, when one of the above-mentioned organisms will probably be found in the heart's blood some days later. It was, indeed, by inoculating a guinea-pig with his own sputum to serve as a control that Sternberg first met with the organism of pneumonia. Many other observers confirm the finding of the organism in healthy sputum, and, like the Klebs-Löffler, it may frequently exist in the throat of healthy persons without causing any injurious effects. J. Washbourn, after trying various methods for the preservation of the life of the organism, finds the best plan is that which is also found satisfactory in the case of influenza, namely, to grow the organism on a few drops of blood smeared on the surface of glycerine agar.

Bacteriological Diagnosis. — This is recommended by Würtz, who suggests the examination of liquid drawn from the hepatized portion of the lung, the blood and the sputum. If the micrococci are found in the blood, he regards the prognosis as extremely grave, whereas, on the other hand, he cites cases, where the organisms were not found in the blood, which terminated favourably. With

regard to the sputum, if a bacteriological examination is made, it must be remembered that it was in healthy sputum that the micrococcus was first found, and that its presence in *small* numbers would not therefore be conclusive. The diplococci may be found in the blood a day or two before the appearance of the 'rusty sputum.'

Pathogenesis.—Both of these organisms have been found to be the cause of pleurisy, endocarditis, pericarditis, meningitis, etc. In its clinical features pneumonia presents strong resemblances to the specific fevers, and though isolated cases are most common, epidemics do occasionally occur. Great alternations of heat and cold, chronic alcoholism, syphilis, and plumbism, all predispose to pneumonia by lowering the general health. The micrococcus of Sternberg is very fatal to mice on inoculation, less so to rabbits, while pigeons and fowls are immune.

Practical Disinfection. — The pneumococci have been found in the dust of a room occupied by pneumonic patients (Emmerich). In experiments by Bordoni Uffreduzzi, quoted by Sternberg, pneumonic sputum retained its virulence when exposed on a cloth to direct sunlight for twelve hours, and when exposed to diffused daylight only, an exposure of eight weeks failed to kill the organisms; this resistance was probably due to the protection afforded by the dried coating of albuminous matter. (It also seems probable that the organism referred to must have been the diplo-bacillus of Friedlander, seeing that the diplococcus of Sternberg loses its vitality so readily.)

Sternberg found that his diplococcus was killed by two hours' exposure to a very weak solution of mercuric chloride (1 in 20,000). This experiment was probably made on a pure culture, not on pneumonic sputum.

Patients should expectorate into a disinfectant solution, while all soiled linen should be immediately disinfected.

RELAPSING FEVER.

Obermeier's spirillum is the specific cause of the disease—Morphology—Methods of staining—Attempts at culture hitherto unsuccessful—Experiments on monkeys—Pathogenesis—The disease is not common at the present day—Practical disinfection.

In the year 1873 Obermeier described the spirillum that bears his name, which he found in the blood of patients suffering from relapsing fever during the febrile period. The organism is motile, and some observers believe it to form spores. The spirillum is very much longer and thinner than most other organisms, being about 8 μ long and about 0·1 μ thick. The organisms are pointed at the ends, and are peculiar in having no sheath, so that if treated with caustic potash they entirely dissolve.

Method of Staining.—It is stated that the organism can be stained by all the ordinary basic aniline dyes, but both Schenk and Würtz recommend Günther's method, who places the air-dried cover-slips in 5 per cent. acetic acid for ten seconds to bleach the blood corpuscles. The acid is then removed by blowing, and the last traces neutralised by exposure to the vapour of strong ammonia. The cover-slips are then stained by an aniline-water solution of gentian violet, rinsed in water, and put up in Canada balsam.

Growth on Media.—No attempt at artificial culture has as yet been successful, though it has been found possible to keep the organisms alive for some time in a saline solution. It has also been found possible to communicate the disease to monkeys by inoculating them with the blood of relapsing fever patients, and it has further been shown that the spleen plays an important part in the recovery of these

animals from relapsing fever, as healthy monkeys usually recover from the disease, whereas if the spleen is removed, the spirilla multiply enormously in their blood, and cause the death of the animal.

Pathogenesis.—The ages at which the chief number of attacks occur are between fifteen and twenty. The incubation period is about twelve days, and an attack affords little or no protection. In some respects relapsing fever exhibits resemblances to typhus fever, but is admitted on all hands to be a distinct specific disease, as the *Spirillum Obermeieri* is found in all cases of relapsing fever, and in no other cases of disease whatever.

Relapsing fever has not recently occurred in this country to any appreciable extent, and is essentially a disease likely to affect persons exposed to unhealthy surroundings and want of food.

Practical Disinfection.—The excreta and secretions should be disinfected in the same manner as recommended in enteric fever.

SCARLET FEVER.

The streptococcus described by Klein in 1882 is probably the specific cause of scarlet fever—Distribution of the disease—Mortality greatest in young children—Difficulty of exercising sanitary control owing to the nature of the infective material—Pathogenesis—Epidemics—Successful treatment of cases with Marmorek's antistreptococcic serum.

Dr. Klein, in the year 1882, described a streptococcus occurring in cases of this disease. He found it in the desquamating particles of skin, in the blood and the sputa of patients. It is non-motile, aerobic or anaerobic; does not form spores; is killed by drying and by exposure to sunlight in the presence of oxygen.

Occurrence and Distribution.—The disease is scattered throughout Europe, but is more prevalent in the northern portions, and rarer in Asia. Epidemics occur at intervals, exhibiting a remarkably regular quinquennial recurrence. In epidemics the mortality is generally low—namely, about 3·0 per cent.—but it has on some occasions risen to 30·0 per cent. The mortality is greatest in October and November, and least in March, while almost the precise reverse is experienced in New York.

One attack is usually protective. The mortality is greatest at the age of three, and above this age rapidly diminishes. It is therefore wise to keep children as far as possible from infection in their earlier years, as later in life they are far less likely to be attacked, and the case-mortality is very much less. The disease requires very strict sanitary control, on account of the long period of infectiveness, and the readiness with which the infective material (desquamating epidermis) may adhere to clothing, etc.

No definite relation has yet been traced between the prevalence of the disease and the rise and fall of the ground-water, or any other meteorological condition.

The disease is probably conveyed chiefly by fomites, and the breath, sputa, and excreta of patients should be considered as infective. No doubt the chief danger lies in the dissemination of the disease by the desquamating particles of skin. In the case of the Hendon outbreak, the disease appears to have been conveyed by milk; but the matter is one on which authorities differ. There is no evidence of the disease having been at any time water-borne, nor is it air-borne to any considerable extent, so that hospitals need not be considered as a source of danger to the surrounding neighbourhood.

Pathogenesis.—The incubation period is from about two

to seven days, and a rash is not always found. Epidemics are frequently ushered in by the occurrence of numerous cases of 'sore throat,' and, as frequently happens in most epidemics, the cases are less severe at the end of the epidemic. The disease is highly infective throughout. Women when in a weak condition—as in pregnancy, for example—are very prone to the disease, and it has been found to manifest itself after the occurrence of some local traumatic injury, when the patient has been exposed to infection which in good health would probably not have taken effect.

Marmorek has treated several cases successfully with his antistreptococcic serum.

SMALL-POX AND VACCINIA.

Evidence of the probability of a bacterial agency in vaccine lymph—Researches of Dr. Copeman—Glycerised lymph—Successful culture of the small-pox bacillus in an egg—Experiments by Dr. Copeman as to the production of vaccine with the culture so obtained—Distribution and pathogenesis of small-pox—The infection is air-borne for very considerable distances—Preventive measures.

A considerable number of different organisms have been described as occurring in vaccine lymph and in variolous crusts; but it appears that all of them are of accidental occurrence, with the exception of the very small, short bacillus recently described by Drs. Klein and Copeman. Two pieces of evidence seem to point strongly to a bacterial agency in vaccine lymph: first, the fact that if vaccine lymph is heated up to 60° C. its efficiency is destroyed; and second, and more important, is the fact that filtration of the lymph through a Pasteur-Chamberland filter removes the active principle to which the lymph owes its efficiency.

Certain observers have described monads and psorosperms as the active agents in small-pox; but it appears that Dr. Copeman has conclusively established the pathogenicity of the minute bacilli described by him. He finds that the bacilli in both small-pox and cow-pox are similar so far as morphology and cultural characters are concerned; so that we must assume that vaccine owes its action to the presence in it of what was originally the small-pox bacillus, which has become modified or attenuated in such a way as to render it capable of conferring an immunity against small-pox almost as complete as is produced by an attack of that disease. An important point proved by Dr. Copeman is that the extraneous organisms that may be accidentally present in vaccine lymph are destroyed by the addition of pure glycerine, which is allowed to act for a certain time before the lymph is used. This addition of glycerine is in no way prejudicial to the activity of the vaccine, which is, if anything, more active than before. The advantages of the addition of glycerine are confirmed by Dr. Klein and by several Continental observers. The minute bacilli which are the active agents in vaccine lymph can be demonstrated in large numbers in the early stages of the vesicles, but they are not found when the vesicles have reached maturity, which may very possibly be due to the fact that they have formed spores.

Growth on Media.—It has been found impossible to obtain growths on any of the ordinary media, such as gelatine, agar, potato, blood serum, or any of the modifications of these; but Dr. Copeman has succeeded in obtaining abundant growths without difficulty, when inoculation was made into an egg, from a suspension of variolous crusts in salt-and-water. He found that growth proceeded best when the egg so treated was incubated at blood-heat for one month, and that then a pure culture was obtained of a

bacillus which, we must admit, is in all probability the bacillus of small-pox. From this culture in egg he proceeded to inoculate a calf, and from it a second calf, and in turn a third. From this last a child was vaccinated, and the vesicles had the normal appearances. Other vaccinations were made from different 'removes' of the same vaccine, all of which were satisfactory. Unfortunately, the calves used were already employed in the production of vaccine lymph, and hence the experiments cannot be regarded as so absolutely convincing as they would have been had the animals been used for the purposes of these experiments alone. Dr. Copeman's experiments are, however, in process of repetition, and when fully confirmed cannot fail to be of the greatest value.

The Distribution and Pathogenesis of Small-pox.—Small-pox has been recognised and dreaded for fully the last two thousand years, and was probably the best-known to the ancients of all the 'ills that flesh is heir to.' While at intervals it spreads widely over the world, it can still be said to be endemic in India and certain parts of Egypt. The 'pandemics,' which are now becoming less frequent, usually have their origin from these parts.

It has been noticed that the mortality from small-pox is greater in England, India, and America during the winter and spring than during the summer and autumn. Soil does not, so far as is at present ascertained, appear to have any influence on its spread.

A heavier mortality is found among males than females; both susceptibility and mortality are heavier among the coloured races than among the whites.

The infection of small-pox, unlike that of most of the other specific fevers, is air-borne for considerable distances, while at the same time we know too little about the specific poison to be able to easily destroy it, so that the disease is

one of the most infectious of the specific fevers. The virus exists in the blood, in the contents of the eruptions, in the dry scabs, and in the excretions and secretions of the patient. The poison clings tenaciously to all articles of clothing, particularly woollen goods.

The usual incubation period is twelve days, but it may be delayed to the seventeenth or eighteenth day; on the other hand, it may, if inoculated, be as short as seven days. After the incubation period the well-known and characteristic symptoms appear, the rash usually appearing on the third day after the onset, though cases are recorded where it did not appear till the fourth day.

Preventive Measures.—Vaccination, while it does not give in every case an absolute immunity, very greatly diminishes the susceptibility, and, if an attack does take place, modifies its violence very considerably.

As the infection of small-pox is undoubtedly carried by the air for considerable distances, it follows that all small-pox patients should be treated in a special hospital, situated as far as possible from crowded centres, and that the ambulances used for the conveyance of small-pox patients should be reserved for them alone. Much more energetic action is taken in Australia than in this country in the carrying out of preventive measures against the spread of small-pox, with the result that the disease is practically unknown there. The following are some of the chief points on which stress is laid: All who have recently come into contact with a small-pox patient must be re-vaccinated, unless this has recently been successfully done, or they have previously had an attack of small-pox. All members of the infected household must be detained at a quarantine station for a period of eighteen days after the last exposure to infection. All furniture, books, bedding, and clothing in the infected house must be thoroughly disinfected, as far as

possible, by a steam-disinfector. Books and manuscripts which might be injured by exposure to steam are to be treated with an alcoholic solution of mercuric chloride. All property which is of little value is to be burnt, while the house itself must be well fumigated with burning sulphur, 1 pound being used for every 1,000 cubic feet of air space; the walls are to be stripped and the ceilings limewashed.

HYDROPHOBIA.

The specific organism is not yet discovered—Incubation period—Examination of suspected animals — Post-mortem appearances — Pasteur's method for the production of immunity—Treatment must be instituted as early as possible after the infection has been received—Report of the English Commission on Pasteur's work—Statistics of the Pasteur Institute.

This disease, which is commonly termed rabies in animals, is capable of affecting man, dogs, wolves, cats, and rabbits. It is, in all probability, due to a specific organism, but no organism has yet been proved to exist, by the usual methods of staining or culture.

In man the incubation period varies very greatly, namely, from a few days to several months, while in the case of inoculated animals it is usually about nine or ten days.

Examination of a Suspected Animal.—On examining an animal dead of rabies, the following appearances will generally be seen: The brain and spinal cord are found to be congested, and contain an excessive number of leucocytes. The cells of the salivary glands are in a degenerating condition, the alveoli themselves being filled with degenerate cells.

The appearances on post-mortem examination are not absolutely decisive, and so it is preferable to use Pasteur's method of inoculation of a portion of the spinal cord into the dura mater of a rabbit or a dog, which will exhibit

symptoms of rabies within twelve days if the suspected animal really suffered from the disease. The test animal, in the case of the dog, may exhibit either the furious state, in which it barks in a peculiar manner and is very aggressive, or it may exhibit the dumb or paralytic condition; both of these conditions end in death. Pasteur discovered, by experimenting on dogs and rabbits, that whereas inoculation with a fresh cord from a rabid animal never failed to produce typical rabies, yet when a cord that had been dried for some days was employed this did not happen; and that by starting with one that had been dried for fourteen days, and then following up with one that had been dried for a less time, it was possible to produce a 'protection' against rabies, and that animals so treated might be bitten with impunity by rabid animals. This success led him to attempt to use this process as a remedial measure in the case of persons already bitten, with the intention of conferring immunity before the infection from the bite has had time to take effect. It is obvious that success must be to some extent governed by the length of time allowed to elapse between the infection and the inception of the treatment, and that the sooner the patient is treated the greater will be his chance of escaping from the disease, the best results being obtained when the treatment is commenced within three days after the infection. The treatment was thoroughly examined and reported on favourably by the English Commission on Pasteur's Researches.

In Australia, where all dogs are quarantined for a period of six months on importation, rabies is practically unknown.

The following are the returns of the inoculations made, as a preventative against rabies, at the Pasteur Institute for the past ten years:*

* *Annales de l'Institut.*

Year.	Number of Persons inoculated.	Number of Deaths.	Rate of Mortality.
1886	2,671	25	0·94
1887	1,770	14	0·79
1888	1,622	9	0·55
1889	1,830	7	0·38
1890	1,540	5	0·32
1891	1,559	4	0·25
1892	1,790	4	0·22
1893	1,648	6	0·36
1894	1,387	7	0·50
1895	1,520	2	0·13

If these figures do not prove that the danger of hydrophobia is greatly exaggerated in the popular mind, we do not know what can do so. Here are 1,520 persons not only bitten, but bitten so badly that they thought it worth while to travel long journeys to seek the aid of the Pasteur Institute. All these persons were bitten under such circumstances that great danger was recognised, and they represented probably the worst cases out of many thousands, the great majority of whom did not resort to the Institute for treatment. Out of the 1,520 persons treated, 156 were bitten on the head or face, 829 upon the hands, and 535 upon other parts of the body; while 122 were bitten by animals experimentally proved to be mad, 949 by animals declared by veterinary certificate to be so, and 449 by animals only suspected of being mad. The patients came from the following countries: France (1,263), England (193, including 20 from British India), Switzerland (35), Spain (11), Belgium and Holland (6 each), Egypt, Greece, and Turkey (2 each), Italy and Russia. Last year, of the 1,520 patients who were thought worthy of inoculation, only 2 died.

The explanation is forthcoming, however, when we are told that, out of the 1,520 patients, only 122 were bitten by dogs proved to be rabic; in every other case the animal

was only suspected. In the case, therefore, of persons bitten by dogs positively proved to be mad, the mortality, after the patients had been subjected to Pasteur's inoculation, was only ·016 per cent. How much of this immunity from hydrophobia was due to the inoculation, and how much to Nature, cannot be judged until we can compare with these cases a sufficient number of other cases bitten by unquestionably rabid dogs, but never inoculated by Pasteur's method of treatment.

CHAPTER X.

MALARIA.

First recognised as a parasitic disease by Laveran—Varieties of the organism — Manson's mosquito theory — Tertian, quartan and summer-autumn fevers — Sporulation — Crescents — Surgeon-Major Ross on the value of the microscope as an aid to diagnosis—Examination of the blood for the parasite—Permanent preparations—Staining—References to recent literature.

It was first shown by Laveran, in the year 1880, that certain amœboid organisms were to be found in the blood of malarial patients; and as the organisms described by him were crescent-shaped, they received the name 'Laveran's sickles.' It has since been found that the organisms described by him occur chiefly after an attack of malarial fever, and are rarely present in the blood during the febrile period. Later on it began to be recognised that the varying forms of malarial fever are each caused by distinct varieties of parasites.

As our knowledge of these remarkable organisms is as yet very far from complete, and as there are considerable differences of opinion on several important points, we shall do no more than touch on the life-history of the parasite, and give references to the original papers of recent date.

It is not yet *thoroughly* established whether the varieties of fever known as *tertian*, *quartan*, and *quotidian*, or *summer-autumn*, are caused by entirely distinct parasites,

though the evidence seems to point to this. Golgi and Canalis admit the existence of five distinct varieties of parasites; Grassi and Felleti only two; while Laveran himself was of opinion that there was only one parasite in varying forms.

According to Surgeon-Major Ross, it may be taken as established that Dr. Manson's theory is substantially correct respecting the part played by the mosquito as host to the parasite; so that the life-history of the organism may be considered as consisting of the human or fever cycle and the mosquito or flagellum cycle.

In the human or fever cycle, the parasites invade the blood corpuscles themselves, and develop within them, finally sporulating after having demolished their host. In the mosquito or flagellum cycle, the parasites are free, or are contained in a species of sheath, from which they soon free themselves, and then, by virtue of their motility, propel themselves into the tissues of the mosquito, where they remain quiescent. Up to the present, we believe the parasite has not been actually demonstrated in the mosquito in its quiescent stage, but this will probably be done ere long. The mosquito dies soon after depositing its eggs, and its body will probably fall into water, to which it goes to lay its eggs; and thus the quiescent parasites may enter the intermediate host, man, in drinking-water; or, again, the body of the mosquito may dry up, and, becoming dust, may be inspired. In connection with this latter possibility, it is to be remembered that it is said that malaria is apt to manifest itself if the surface of the soil is disturbed.

In the quartan type of the spring fevers, the parasite is found in the blood in all forms during the entire length of the attack. The sporulating forms consist of seven to ten spores arranged in rosette form, while in the spring tertian all forms, with the exception of the youngest, are profusely

pigmented; and when sporulation takes place there are a much larger number of spores grouped together — namely, from fifteen to twenty, arranged round the central pigment. No crescents are to be seen in either of these fevers.

In the summer-autumn tertian and quotidian fevers the parasites are always smaller than the intermittent fever parasites, and only the youngest forms are found in the blood, and then only during the first hours after the exacerbation, and crescents set in after the fever subsides.

In the mosquito cycle crescent bodies are found plentifully. On spreading a drop of blood from the mosquito on a slide, and observing without staining, it will be found that in five minutes or so one-third of the crescents become spherical, and a short time afterwards about 5 per cent. of the spheres are seen to develop flagella.

Surgeon-Major Ross is of the opinion that when it becomes more general to use the microscope as an aid to diagnosis in all cases of fever (speaking of countries where malaria is prevalent), it will be found that a very much larger number of cases are due to the parasite than have hitherto been supposed. Another point of importance, in his opinion, is that while crescents are swarming in the blood (after an attack of fever) while the patient is convalescent, and possibly to all appearance fit for duty, he should on no account be allowed to return to work till he has had rest and good feeding for at least a fortnight.

Examination of the Blood for the Parasite.—Surgeon-Major Ross recommends the examination of fresh blood without drying or staining, and warns against trying to obtain too thin a film by pressing the cover-slip on the slide, as there is then a danger of causing some of the corpuscles containing parasites to burst, so that they then appear as free; this is particularly likely to happen in the summer-autumns.

In squeezing the blood from the finger, the same effect may be produced by the use of undue violence.

Surgeon-Lieutenant Rogers speaks in favour of permanent preparations, and finds Maynard's suggestion to fix unstained specimens in the vapour of osmic acid (2 to 4 per cent.) yields good results. He advises the spreading of a drop of blood over the cover-glass with the edge of another, and fixes the cover-glass down on the slide with four small drops of paraffin. Würtz recommends staining the parasite with a concentrated aqueous solution of methylene blue, or with gentian violet or hæmatoxyline. He states that double staining may be effected by acting successively on the dried blood, first with a strong aqueous solution of eosin, which stains the corpuscles pink, and then with a concentrated aqueous solution of methyl blue, which colours the leucocytes and the parasites blue. He considers the dark colour of the blood in malarial fever to be due to the pigment from the parasites being accumulated in the leucocytes.

References to some Recent Literature.—A discussion on the malarial parasite, before the Royal Medical and Chirurgical Society, published in the *British Medical Journal* of February 15, 1896, gives a synopsis of the views held by various workers as to the life-history of the parasite.

A paper by Dr. Maynard was published in the *Indian Medical Gazette* of October, 1895, and papers by Surgeon-Major Crombie and by Surgeon-Lieutenant Rogers appeared in the same journal in February, 1896.

The Goulstonian Lectures, by Dr. Patrick Manson, were published in both the *British Medical Journal* and the *Lancet* during March, 1896.

ACTINOMYCOSIS.

First described by Bollinger—Commonly known as 'wooden tongue' —Appearance of the fungus in discharges—Method of staining—Growths on artificial media do not form 'clubs'—Pathogenesis —'Madura disease' probably identical.

The *actinomyces*, or ray-fungus, was first described by Bollinger in the year 1876, though its manifestation in cattle, commonly known as 'wooden tongue,' was recognised many years previously, and described by M. Laber. The natural habitat of the fungus seems to be on the ears of cereals, and the invasion of an animal by the fungus is generally due to the piercing of a mucous surface by a portion of a cereal to which the fungus was attached; possibly the fungus may also gain access to the system by inspiration. If the pus from one of the abscesses is examined, small yellow granules will be found, which consist of clumps of the fungus. On squeezing one of these clumps between two cover-glasses, and then staining with aniline water methyl-blue, it will be seen that the fungi are arranged in groups radiating out from the centre, and club-shaped.

Method of Staining.—To stain the actinomyces in section in tissue, we may either employ Gram's method or we may use carbol-fuchsine and picric acid, thus staining the fungus red and the tissue yellow.

Growth on Media.—In artificial media the club shapes are not found. The fungus grows well, and for almost unlimited times, on artificial media, glycerine agar and bread being the best. The cultures on bread, when fully developed, have a very peculiar appearance, showing a dull-gray raised and wrinkled growth, of considerable thickness, somewhat similar to the lichenous growth commonly seen on apple-trees.

The ray-fungus, when grown on artificial media, develops mycelial threads and spores. Spores are readily formed, which are, according to Würtz, very resistant to heat, requiring fourteen minutes' boiling to destroy their vitality.

Pathogenesis.—The fungus may occur in nearly every part of the body. In man it generally gains access by some slight traumatic injury, as only two cases are on record where the disease was supposed to have been contracted from affected animals, and these are doubted by some authorities. Cattle affected by the disease are not uncommon abroad, where but little importance is attached to the disease. It is comparatively rare in this country, being chiefly confined to Norfolk. Owing to the great resistance of the spores to heat, it is obvious that the flesh of animals suffering from the disease ought not to be considered fit for human food.

The disease in man corresponds pretty closely to that observed in animals, but there is less tendency to localisation, by the abundant formation of connective tissue and the frequency of calcification. The tendency of the disease in man is to become chronic, and it is only by the implication of some vital organ, or by the exhaustion following prolonged suppuration, that the patient succumbs. The disease spreads by continuity, and no tissue seems able to resist its invasion. Besides this, second embolic foci may occur, perhaps the commonest seat being the liver. No doubt many cases have gone unrecognised in days gone by, and have been certified as due to pyæmia; but the actinomyces is not greatly inclined to suppuration, and where it is kept free from contamination by other organisms, as in the case of an actinomycosis lesion occurring in the cranium, it may remain almost dormant for long periods. The disease does not extend by the lymphatic system, and is characterized by the appearance of a chronic

swelling, which gradually enlarges, softens, and inclines to approach the surface, when fluctuation is sometimes to be felt. The skin becomes bluish-red, as over a chronic abscess, and eventually a small yellow point forms, and a yellow serous or pus-like fluid escapes; and in this discharge the yellow granules will be visible. If a little of this fluid be allowed to run gently down the side of a test-tube, which is then held up to the light, the small yellow grains will be visible, which may be picked out, placed on a slide, and pressed down with a cover-glass. It will transmit to the finger a sensation similar to that of squeezing a drop of solid fat, if the granule was taken from man; while if from an animal, the granule is more gritty, from calcareous degeneration. On examining the slide with a low power, a number of ovoid, kidney-shaped masses are seen, which with a higher power show the characteristic club-shaped structure. The periphery of the swelling always feels hard, and a chronic fistulous opening into the cavity may persist for months. The general tendency of the disease is to spread continuously, the older portions of the cavity sometimes showing a tendency to form scar tissue, as in animals. There is rarely any pain, fever, or constitutional symptoms.

Bostrom considers the softening process an index to the life and activity of the organism, and says that when a centre is formed by granulation, the fungus is either inactive or dead.

The club forms are regarded by Bostrom as degeneration forms of the terminal filaments of the fungus. Crookshank says that each filament is enclosed in a sheath, and it is owing to this undergoing mucilaginous degeneration that the club forms are produced; and if a little water is run under the cover-glass, the club form disappears, leaving the mycelium exposed to view. The active fungus appears

in the form of cocci arranged in chains or threads, which freely interlace to form a network in the centre of the colony. Bostrom has proved that these filaments are the active portion, and could be cultivated on blood serum or agar, while from the club-shaped portions he did not obtain any growths. Crookshank has found that the organism is more easily cultivated from man than from animals, and that the most satisfactory medium is glycerine agar. After repeated subculture the growths exhibit very peculiar forms, a good collection of which is to be seen at King's College. The pathogenicity of the organism has been shown experimentally by injecting a pure culture into the peritoneum of rabbits and into the subcutaneous tissues of calves, and in each case a typical actinomycosis has resulted. Kanthack is of the opinion that 'madura disease' is produced by the same or a similar organism.

YELLOW FEVER.

Specific organism at present unknown—Dr. Carmona's method of securing immunity by the use of urine.

This disease is endemic in the West Indies, Mexico and the West African coast. Various organisms have been described by different observers as the specific cause of this disease, but up to the present it is uncertain which is the specific organism. The method of conveyance of the disease is also unknown. It was formerly regarded as akin to malaria, but it has far more points of resemblance to cholera in its etiology. In the year 1892 Sternberg suggested the employment of the blood serum of convalescent patients as a means of conferring immunity on persons about to proceed to districts where the disease is prevalent.

Some years previous to this, however, Dr. Manuel Carmona Y Valle, of Mexico (*Journal of American Public*

Health Association, April, 1896), devised an ingenious and apparently very successful method of treatment founded on the well-known general principles on which all sero-therapeutic treatments are based. His theory is that the immunising properties present in the blood of convalescent patients are to be found in the urine, which he uses by preference as it is comparatively easily obtained, while it would be rather a questionable proceeding to draw anything like large quantities of blood from a patient just recovering from yellow fever. This theory receives confirmation from the work of M. Bouchard, who states (in a paper read at the International Medical Congress at Berlin, 1890) that he has found the urine of immunised animals to possess immunising properties similar to those found in their blood serum. Dr. Carmona injects from 2 to 4 c.c. of the urine of a convalescent patient taken between the fourth and fourteenth day of the disease. For convenience the urine may be evaporated on a water-bath, and the residue dissolved in water as required. Dr. Carmona cites over a thousand cases of the employment of his method, in none of which did any evil consequences follow the injection, while in only very few cases have inoculated persons been subsequently attacked by yellow fever. As Dr. Carmona points out, an *absolute* immunity could hardly be expected: he gives vaccination as a parallel, in which the protection against small-pox, although great, is not absolute. Though he does not claim at present that the technique he employs is necessarily perfect, he has certainly justified his contention that physicians practising in districts where yellow fever is rife should give this process a trial.

OTHER PATHOGENIC ORGANISMS.

A large number of organisms have been described as the cause of other diseases in man, among which may be

mentioned bronchitis, Bright's disease, beri-beri, carcinoma, cholera infantum, cystitis, dengue, dysentery, eczema, endocarditis, gangrene, 'green diarrhœa,' measles, meningitis, mumps, nephritis, ophthalmia, peritonitis, rötheln, sarcoma, septicæmia, whooping-cough, etc.; but the information on all these appears at present to require further confirmation before they should be included in a work intended to be of an introductory nature only.

These remarks also apply to many of the diseases of the lower animals, of which may be mentioned: Eczema epizoötica ('foot and mouth' disease), fowl cholera, grouse disease, hog cholera, pneumonia in horses, pleuro-pneumonia of cattle, 'rinderseuche,' septicæmia in cattle, swine plague, silkworm disease, etc. These diseases are of no great consequence, so far as this country is concerned.

The following diseases, however, which are due to parasitic fungi, are of practical importance, viz., pityriasis versicolor (*Microsporon furfur*), thrush (*Oidium albicans*), favus (*Achorion Schönleinii*), and ringworm (*Trichophyton tonsurans*).

Microsporon Furfur.

This organism, which is found in pityriasis versicolor, belongs to the same family as the *Tricophyton tonsurans* and resembles it in microscopic appearance; it has not yet been artificially cultivated.

Thrush.

In the white patches sometimes found in the mouths of infants fed on milk, the spores and filaments of an organism can be distinguished; this thrush fungus is by some considered to be identical with the *Oidium albicans;* it can be grown on milk, bread, gelatine, or agar, and on potato. On all these media it produces a very copious growth, the

growth on potato being a remarkably thick, raised patch. If an intravenous injection is administered to rabbits, the

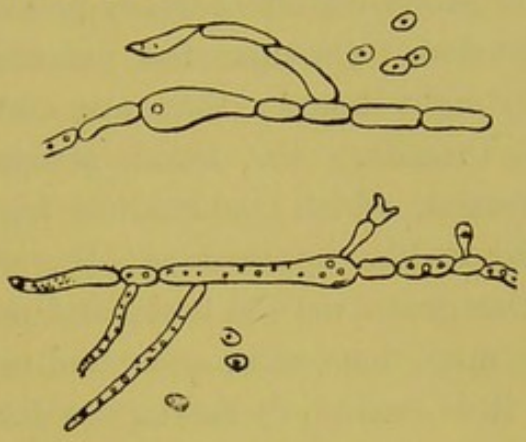


Fig. 19.—Mycoderma Albicans (Thrush).

animals die in about thirty-six hours, when their viscera will be found full of the mycelia. The patches are always found to give an acid reaction.

Favus (Achorion Schönleinii).

Favus was first recognised by Bateman, but it was not until the year 1839 that Schönlein published the fact that the

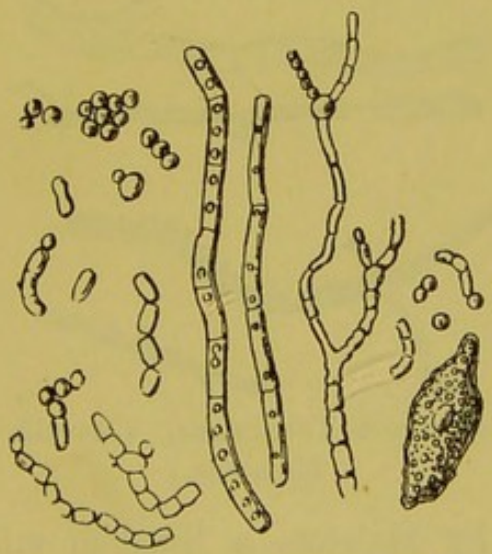


Fig. 20.—Achorion Schönleinii. (Growth from a favus patch.)

yellow patches were composed of the mycelia and spores of a parasitic fungus. The fungus was first cultivated by

Bazin. In its earlier stages it is indistinguishable from the *Tinia tonsurans*, but soon assumes the honeycomb appearance. It grows on all ordinary media except milk. Gelatine is liquefied. On agar the colonies appear distinctly in forty-eight hours; they are surrounded by a fine fringe of threads. On blood serum star-shaped colonies are formed, which radiate out from the centre, producing a flower-like appearance; the gelatine is not liquefied. It also grows well on bread and potato.

Favus affects man, dogs, cats, mice, and rats; to the two latter animals it is commonly fatal; the disease is readily transmissible from animals to man. The favus patches are distinguished by their yellow colour, their peculiar smell, and their slightly cup-shaped appearance.

Tricophyton Tonsurans.

This fungus, which is the cause of herpes tonsurans, ringworm, onychomycosis, and certain other affections, was

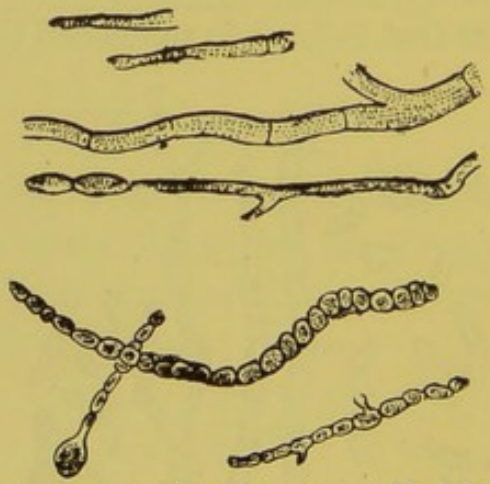


Fig. 21.—Trichophyton Tonsurans. (Mycelia and segmented filaments.)

first described by Malsten, a Swedish microscopist, and about the same time by Gruby.

The organism, which belongs to the oidium group, was first cultivated by Leslie Roberts, who obtained growths in

broth to which malt extract and sugar had been added, growth occurring in twenty-four hours. A pure culture may be obtained from an affected hair by making an agar plate and incubating it for three days at blood-heat, when the colonies will make their appearance as whitish spots. When grown on gelatine, the medium is liquefied. To diagnose a case of ringworm, it is generally sufficient to examine one of the suspected hairs under a low power (a quarter-inch), when it will be found to be covered with spores. To facilitate examination, the hair may first be soaked in 40·0 per cent. caustic potash, and then in alcohol and ether. If the patch itself is examined, spores will be found on the surface, while a little below will be seen a matted mass of mycelial branching. The organism may be stained with eosin, and permanent preparations may be put up in glycerine.

The disease affects man, dogs, cats, cattle, and many other animals.

CHAPTER XI.

MICRO-ORGANISMS OTHER THAN BACTERIA—FERMENTATION, ETC.

The yeasts, moulds, algæ and protozoa: their method of growth, classification, mode of occurrence, chief species, etc.—The examination of yeasts—Fermentation and ferments—Fermentation by yeasts—High and low fermentation—Fermentation by moulds and bacteria—The acetic fermentation of alcohol, the nitrification of ammonia, the ammoniacal fermentation of urea, the lactic and butyric acid ferments—Mixed fermentations—The unorganised ferments, or enzymes—The diastatic, peptic, rennet and pancreatic ferments.

In addition to the bacteria proper are a large number of micro-organisms which, although more highly organised, are very closely related to the bacteria. They are known as yeasts (or saccharomycetes), moulds, algæ, and protozoa. There are a great number of varieties of the above organisms, and we cannot attempt to describe even all the most important ones; but it will answer our purpose to detail a few of the more common kinds, and give the principal features of the different orders. The yeasts are the most important, as they play an important part in our daily life, being the basis of such great industries as brewing, wine and vinegar making, etc. Most of the above organisms are harmless saprophytes, while others are of pathological importance, as being associated with disease.

YEASTS, OR SACCHAROMYCETES.

The yeasts, or *Saccharomycetes*, are a group of organisms of the greatest importance on account of their connection with the great process of fermentation. They are round or oval cells, which generally multiply by *gemmation* or budding. Reproduction by gemmation consists of the budding out of daughter-cells in different places from the gradually enlarging parent-cell. The buds formed become divided from the parent-cell by a diaphragm, but sometimes they remain adherent, forming a chain. The cells containing granular protoplasm are surrounded by a thin membranous wall, and often exhibit in their interior one or more colourless lacunæ, known as *vacuoles*, which probably consist of fat globules. So long as the conditions remain suitable, the saccharomycetes invariably multiply by gemmation, but in presence of lack of nourishment, such as, for instance, if the cells are washed free from nutrient material, or are placed on a moist porcelain or plaster of Paris surface, a most remarkable change in the constitution of the cells is seen to take place. In about twelve hours or so, the time varying with different species, the cells will be seen to have increased in size, their contents to have become homogeneous, and in the course of a few more hours such cells are found to contain two to four shining spots, which become more spherical and surround themselves with a thick membrane. In the course of time these new cells or *ascospores*, as they are called, become liberated by dissolution of the mother-cell. On introducing these spores, which are 4 to 5 μ in diameter, into a saccharine liquid, they germinate and multiply, as usual, by gemmation. Sometimes the growth of the saccharomycetes, especially on solid media, by the growth of the cells in the form of chains, gives rise to a misleading appearance re-

sembling the mycelial growth of a mould. The yeasts, however, never give rise to a true mycelium nor to a typical fruit-bearing hyphæ.

The following are the principal varieties of yeasts:

Saccharomyces Cerevisiæ.—This is the typical English

FIG. 22.—SACCHAROMYCES CEREVISIÆ.

brewery yeast. It grows as rounded or slightly ellipsoidal cells, which give off small cells by budding. The cells are from 8 to 9 μ in diameter, and occur both singly and in short chains. Spores occur three or four together in a mother-cell 4 to 5 μ in diameter.

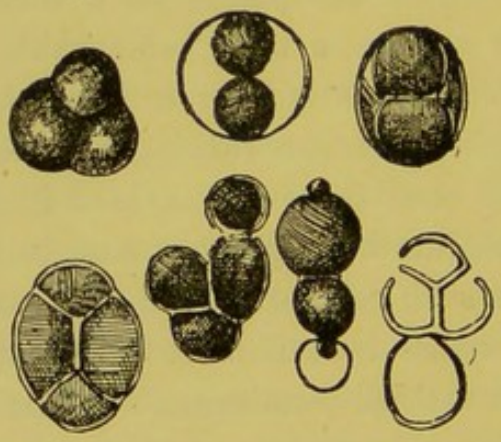


FIG. 23.—SACCHAROMYCES CEREVISIÆ. (Stages in the development of ascospores.)

Saccharomyces Ellipsoideus.—This yeast takes the most important part in the fermentation of grape-juice and other spontaneous fermentations. It is usually rounded or ellipsoidal in shape, and it sometimes assumes a sausage

form. The cells average about 6 μ long, singly or united in little branching chains. Two to four spores are found in a mother-cell 3 to 3·5 μ in diameter.

Saccharomyces Apiculatus.—This yeast can hardly be said to be a true saccharomyces, as it has not yet been ascertained to have any spore formation. It is a very common variety, however, and occurs in ferment-wine and spontaneously fermented beer; on sweet succulent fruits, such as grapes, cherries, plums, gooseberries, etc. The cells of this yeast have a most characteristic citron shape (hence the name), from the prominences at the end of which the budding takes place. The cells are 6 to 8 μ long, and 2 to 3 μ broad. This yeast invariably appears at the onset of the vinous fermentation of grape-juice, but it soon gives way to the *Sacch. ellipsoideus* and *Sacch. pastorianus*. It only gives rise to a very feeble alcoholic fermentation.

Saccharomyces Pastorianus.—This yeast—of which three varieties, known as I., II. and III., have been isolated by

Fig. 24.—Saccharomyces Pastorianus I.

Hansen—is very polymorphic in shape. The cells are oval or club-shaped, and also occur as elongated, ellipsoidal, pear-shaped cells. Two to four spores are found in a mother-cell. It takes a part in many spontaneous fermenta-

tions, and in the vinous fermentation it usually succeeds the *Sacch. apiculatus*. It is classed as a 'wild' yeast, the spores of which frequently occur in the atmosphere of breweries. The *Sacch. pastorianus* is one of the yeasts causing 'disease' of beer, to which it gives an unpleasant, bitter taste.

Saccharomyces Mycoderma.—The cells of this yeast are oval, elliptical, or cylindrical, 6 to 7 μ long and 2 to 3 μ thick, united in freely-branching chains. Spore-forming cells may reach 20 μ long. One to four spores in each mother-cell. This yeast forms the skin or 'mould' on the surface of fermented liquids, without, however, exciting fermentation. When forced to grow submerged in a saccharine liquid, it gives rise to a small quantity of alcohol, but no growth takes place.

Saccharomyces Conglomeratus.—Forms cells, which are round and united in clusters, consisting of numerous cells produced by budding from one or more mother-cells. There are two to four spores in each mother-cell. This yeast occurs on rotting grapes and in wine at the commencement of the fermentation.

Saccharomyces Minor.—Occurs in oval or spherical cells 6 μ in diameter, arranged in chains of 6 to 9 elements. The spore-forming cells are larger (7 to 8·5 μ), and contain from two to four spores 3·5 μ in diameter. This yeast is stated by Engel to be identical with that employed by bakers to ferment bread.

Saccharomyces Exiguus.—Conical or top-shaped cells 5 μ long, and reaching 2·5 μ in thickness, in slightly branching colonies. There are two to three spores in a row in each mother-cell. This yeast is often present in the after-fermentation of beer.

Torulæ.—The term *torula* has been used both by Pasteur and Hansen to denote a number of organisms closely related to the saccharomycetes in their form and mode of

growth, but differing from them in not giving rise to spores even when cultivated under the most diverse conditions. The *torulæ* produce little or no alcohol when grown in saccharine liquids. To this group belong the *Saccharomyces rosaceus*, *niger* and *albus*, the pink, black and white torula respectively that are frequently met with in the air.

Examination of Yeasts.—Hansen, to whom the present scientific aspect of the fermentation industries is due, elaborated a scheme for the examination of the saccharomycetes, which depends upon the isolation of a pure culture, and the observation of the temperature and time they take to form 'ascospores' and 'films.'

The importance of this method of investigation will be apparent when applied to the examination of brewery yeast to determine the presence of disease or 'wild' yeasts, which are the cause of such diseases as muddiness, ropiness, bitter or acid taste, which render the beer undrinkable, or injure its keeping properties. Starting with pure yeast, thorough cleanliness, and means of keeping the beer free from 'wild' yeasts and other organisms, beer may be preserved both bright and clear, even though the temperature be comparatively high. To obtain pure yeasts, it is first necessary to take a single cell, and from this to grow a series of cells in sterilised beer-wort, to break this up into different portions of seed-material, from which other crops are produced, and so on, until a sufficient quantity of pure yeast is produced to bring about the necessary fermentation in a large bulk of wort.

The characteristic appearance which at one time was thought to belong to the yeast-plant has been shown by Hansen to have no existence, except in a very limited sense. Throughout the entire series of Hansen's researches the leading idea obtains—namely, that the shape, relative sizes, and the appearances of the cells, taken by themselves, are not sufficient to characterise a species. The

following is a brief account of the methods employed by Hansen to determine the characteristics of a species:

(*a*) *The Microscopic Appearance of a Yeast.*—The growths, after growing in sterilised wort for twenty-four hours, are examined under the microscope. The general characteristics are then noted; for instance, whether the cells are round or oval, as is the case with the *S. cerevisiæ*, or elongated sausage-shaped cells, as in the case of the *Sacch. pastorianus* varieties. It is a very different matter, however, when these two species are mixed, or other varieties are present. In this case but little is to be learnt from a direct microscopic examination.

(*b*) *The Formation of Ascospores.*—By the determination of the temperature and time necessary for the various species to form ascospores, Hansen made the first step in devising an analytical method for the examination of yeasts. After making a large number of experiments, Hansen was able to determine the following conditions which regulate the formation of spores in the saccharomycetes:

(1) The cells must be placed on a moist surface, and have plenty of air.

(2) Only young and vigorous cells can exercise this function.

(3) The most favourable temperature for most of the species is about 25° C.

(4) A few saccharomycetes form spores when present in fermenting nutrient liquids.

A small portion of a young and vigorous growth is transferred to a moist gypsum block, prepared as follows: To well-baked plaster of Paris add distilled water until the plaster is nearly liquid; pour this on to a sterilised glass plate on which rests a small mould of metal or paper. The blocks are dried and sterilised. They are then laid in a shallow tray containing a little sterile water, the whole arrangement being kept well covered by a bell-jar.

The apparatus can be placed in an incubator if any special temperature is required, or may be kept at the ordinary temperature of the room.

Hansen found that the formation of spores takes place slowly at low temperatures, more rapidly as the temperature is raised to a certain point; when this point is passed their development is again retarded, until finally a temperature is reached at which it ceases altogether. The mass on the plaster plate is carefully examined from time to time. After a certain lapse of time, which varies with the different species, roundish plasma particles appear in the cells, and these are the first indications of spores. In their further development they become surrounded by a wall, which is seen more or less distinctly in the different species. The spores may expand to such an extent that the pressure which they exert on each other whilst they are still enclosed in the mother-cell brings about the formation of the so-called partition-walls. During the further development a complete union of the walls may take place, so that a true partition-wall results; the cell then becomes a compound spore divided into several chambers. During germination the spores swell, and the wall of the mother-cell, which was originally thick and elastic, becomes stretched thinner, and finally becomes ruptured, and then remains as a loose shrivelled skin partially covering the spores, or it becomes gradually dissolved during germination. The importance of this method of examination is seen from the following fact: The *Sacch. cerevisiæ* does not form spores until a period of ten days has elapsed; whilst, on the other hand, the *Sacch. pastorianus* II. (the most common 'wild' yeast), when kept under exactly the same conditions, gives evidence of the commencement of spore-formation after seventy-seven hours.

(*c*) *The Formation of Films.*—Hansen subjected the films which appear on the surface of fermenting liquids to a

thorough examination. To produce these films, Hansen proceeded as follows: Having obtained his pure cultivation, drop cultures were made into four-ounce flasks, half filled with sterilised wort, and protected from falling particles by being covered by a well-fitting cap. The films first appear as small opaque points, which gradually increase in size and then run together, forming irregular patches floating on the upper surface of the liquid. As soon as the film becomes apparent to the naked eye, it is examined. The film at length overspreads the whole surface of the liquid, and becomes adherent to the walls of the flask. A very necessary condition for the formation of films is perfect rest of the liquid in which they are being formed.

The following tables, compiled by G. H. Morris (*J. S. C. I.*, 1887, p. 119), show the differences exhibited by a number of varieties of yeasts examined by Hansen's methods, with respect to the formation of ascospores and films respectively:

ASCOSPORE FORMATION.

Temperature.	*S. cerev.* I.	*S. past.* I.	*S. past.* II.	*S. past.* III.	*S. ellips.* I.	*S. ellips.* II.
37·5° C.	None					
36-37° -	29 hours					None
35° -	25 hours	—	—	—	—	31 hours
33·5° -	23 hours	—	—	—	None	23 hours
31·5° -	—	None	—	—	36 hours	
30° -	20 hours	30 hours				22 hours
29° -	—	27 hours	None	None	23 hours	
27·5° -	—	24 hours	34 hours	35 hours		
26·5° -	—	—	—	30 hours		
25° -	23 hours	—	25 hours	28 hours	21 hours	27 hours
23° -	27 hours	26 hours	27 hours			
22° -	—	—	—	29 hours		
18° -	50 hours	35 hours	36 hours	44 hours	33 hours	42 hours
16·5° -	65 hours	—	—	53 hours		
15° -	—	50 hours	48 hours	—	45 hours	
11-12° -	10 days	—	77 hours	—	—	5·5 days
10° -	—	89 hours	—	7 days	4·5 days	
8·5° -	None	5 days	—	9 days	—	9 days
7° -	—	7 days	7 days	—	11 days	
3-4° -	—	14 days	17 days	None	None	None
0·5° -	—	None	None			

FILM FORMATION.

Temperature.	*S. cerev.* I.	*S. past.* I.	*S. past.* II.	*S. past.* III.	*S. ellips.* I.	*S. ellips.* II.
40° C. -	—	—	—	—	—	None
36-38° -	None	—	—	—	None	8-12 days
33-34° -	9-18 days	None	None	None	8-12 days	3-4 days
26-28° -	7-11 days	7-10 days	7-10 days	7-10 days	9-16 days	4-5 days
20-22° -	7-10 days	8-15 days	8-15 days	9-12 days	10-17 days	4-6 days
13-15° -	15-30 days	15-30 days	10-25 days	10-20 days	15-30 days	8-10 days
6-7° -	2-3 months	1-2 months	1-2 months	1-2 months	2-3 months	1-2 months
3-5° -	None	5-6 months	5-6 months	5-6 months	None	5-6 months
2-3° -	—	None	None	None		None

MOULDS.

The moulds, mycelial fungi, or hypomycetes, as they are called, are frequently seen upon the surface of articles of food, fruit, etc., after keeping for some time in a damp place. They are, for the most part, harmless saprophytes, but several of them are of pathological interest, as being associated with, or the cause of, various morbid processes. In some instances this is seen only in animals. This circumstance does not strictly show that such moulds are pathogenic for man; but it is certainly difficult to establish strictly that they are not pathogenic, and as the conditions of moisture, etc., which usually favour their growth are insanitary, their presence should be taken to indicate insanitary conditions. The moulds form spores, and, like the bacteria proper, are remarkable for the great resistance they offer to external influences, and which under favourable conditions, such as moisture, warmth, and nourishment, develop into complete individuals.

The spores, or *conidia*, as they are called, shoot off little buds, which lengthen at the end by fission, giving rise to a long thread of cylindrical cells, which sometimes branch, forming a freely-growing network of fibres known as *mycelia*.

The heads of these mycelia then form spherical or oval cells, which are the seed-bearing organs known as the

hyphæ or *thallus*. It is from these organs that the moulds derive their name of hypomycetes. Some hyphæ form large round or cylindrical mother-cells, or *sporangia*, in the interior of which spores are formed by endogenous formation. According to the form of the seed-bearing organ, the moulds are divided into four divisions, viz., *Mucorineæ*, *Aspergillinæ*, *Penicilliaceæ*, *Oidiaceæ*.

1. **Mucorineæ.**—In the mucors, or headed moulds, the ends of the hyphæ swell into knobs known as columella, around which a seed-capsule or sporangium forms. When ripe, the spores burst the enclosing membrane, and thus become free.

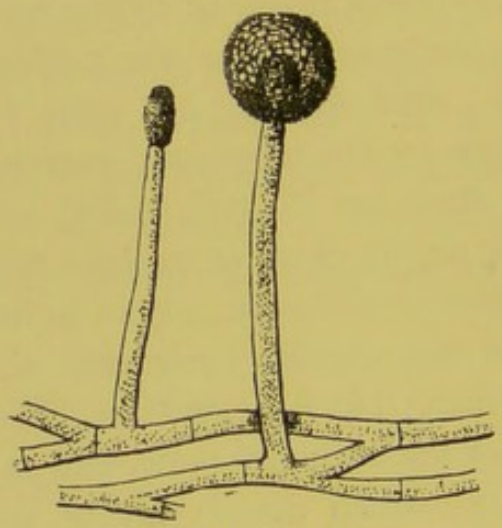


Fig. 25.—Mucor Mucedo.

2. **Aspergillinæ.**—The Aspergillinæ, or knob-moulds, have hyphæ, the heads of which are covered with a number of spores, carriers, or sterigmata, from the extremities of which the spores divide off into rows.

3. **Penicilliaceæ.**—The Penicilliaceæ, or pencil moulds, differ from the two former varieties by forming branched hyphæ, known as *basidia*, on the terminals of which are seen the sterigmata, from which the conidia, or spores, are separated in the form of chains.

4. **Oidiaceæ.**—The hyphæ of the Oidiaceæ form no special spore-bearing organs, but the hyphæ become

articulated at their extremities, and so divide off the spores in the form of segments. The most important members of these groups are the under-mentioned :

Mucor mucedo.—This is the commonest mould, and is frequently seen growing upon food-stuffs, particularly stale moist bread and upon animal excreta. It possesses a branching mycelium with hyphæ bearing the swollen sporangia, or spore-bearers. This mould grows well on an acid medium, forming a white fur, and bears black fructification heads. It is not pathogenic.

Mucor rhizopodiformis forms a similar growth to the above. A culture on bread gives rise to an aromatic odour.

Mucor corymbifer forms a dense white fur on bread, resembling cotton-wool.

Mucor ramosus grows upon bread and potato as a white fur which soon changes to grayish-brown.

These last three mucors are pathogenic. Intravenous injection of fluid containing their spores causes a fatal disease in rabbits.

Aspergillus niger, **A. albus**, and **A. glaucus** grow upon

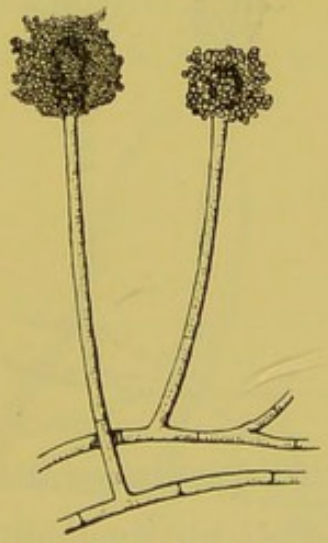


Fig. 26.—Aspergillus Glaucus.

bread, candied fruit, etc., on which are seen the stout swollen club-like fructifying hyphæ, upon which are

arranged the sterigmata. The latter two organisms grow best at blood-heat, when they soon overgrow the nutrient medium owing to their rapid growth.

Aspergillus Flavescens and **A. Fumigatus.**—The former is distinguished by its well-marked fructifications and greenish colour of its culture, the latter by its fine fructifications and ash-gray fur. On gelatine plates the filaments grow rapidly into the medium, causing its liquefaction. Both organisms grow at blood-heat. Both are pathogenic, growing in various parts of the body—particularly the ear, producing the disease known as otomycosis; they have been also found growing in the lungs and on the nasal mucous membrane. The spores cause the death of rabbits on intravenous injection.

Penicillium Glaucum.—The *Penicillium glaucum* is the very common green mould seen on the bark of trees, old walls, etc. It grows in the form of locks of cotton-wool, and during sporulation forms a green fur of a peculiar musty odour. The mycelium consists of horizontally

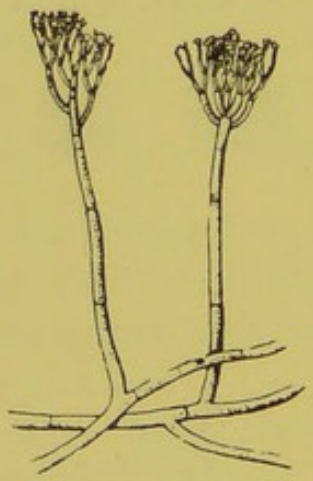


FIG. 27.—PENICILLIUM GLAUCUM.

arranged straight or slightly undulating jointed filaments, from which the spore-bearing hyphæ stand vertically up, dividing at their upper ends into forks (basidia), from which fine processes branch off (sterigmata) in the shape of a hair

pencil, and are segmented at their ends into rows of fine globular spores or conidia. The mould grows well on bread pap in the form of a fur which is white at first, but afterwards becomes of a fine green colour. The fungus grows on gelatine plates first in the form of fine threads diverging from a point, and not giving rise to sharply defined colonies, but radiating out over a considerable extent of surface. The spore-bearing hyphæ which rise above the level of the gelatine are put in motion by air currents, and when this occurs the shedding of the spores can be readily observed. The earliest formation of spores takes place in the centre of the colonies, and is indicated by a green colour. The gelatine is liquefied.

Brown Mould.—The fur formed by the ' brown ' mould is brownish-yellow in colour, and is distinguished from *Penicillium glaucum*, which it otherwise resembles, by its closely-felted mycelium, the hyphæ being scanty, ramified, and segmented. It grows on gelatine, which quickly becomes liquefied. According to Trelease, this mould is identical with an alga, the *Cladothrix dichotoma*, which is frequently found in dirty water.

Oidium Lactis.—This mould grows as a white fur, and is

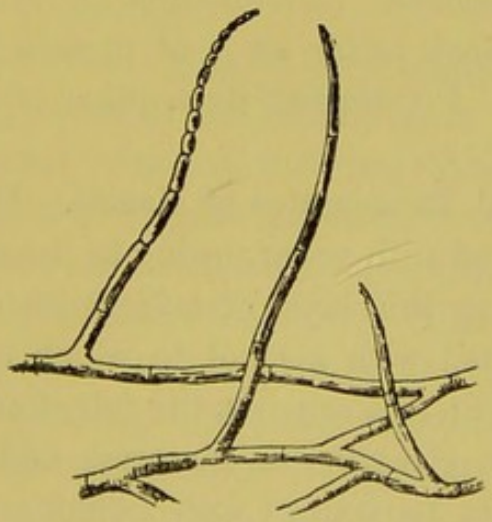


Fig. 28.—Oidium Lactis.

frequently found in sour milk and butter. The fibres of the mycelium grow upwards, become segmented, and support cylindrical conidia. The fungus grows on gelatine without liquefying it, diffusing at the same time an odour of sour milk. On agar it grows in the form of little stars, which then overgrow the medium. In a thrust culture the fibres of the mycelia are seen to permeate the medium. *Oidium lactis* grows very readily in milk, which it does not change in any special way. It is not pathogenic in man or animals.

Oidium Albicans.—The fungus causing the white patches occurring on the mucous membrane of the mouths of infants, known as 'thrush,' was formerly assigned to the group oidium, and described as *Oidium albicans*, but according to Rees and Kehrer it belongs to the yeast fungi, and must be spoken of as *Mycoderma albicans* (see p. 230).

Trichophyton tonsurans the fungus occurring in herpes tonsurans, which is also stated to be the exciting cause of impetigo contagiosa (an exanthem characterized by the formation of pustules), eczema marginatum, tinea carcinata (common ringworm), and onychomycosis (an affection of the nails); the fungus of favus (*Achorion Schönleinii*) and pityriasis versicolor (*Microsporon furfur*) are morphologically identical, as far as is at present known, with the *Oidium lactis*. For further description of these see p. 230 *et seq.*

Microscopical Examination of Moulds.—Moulds cannot be easily moistened with water, owing to the presence on their surface of a very thin layer of fat; hence a portion of the mould is treated with alcohol to which a little ammonia has been added; this removes the fat, after which they can be mounted in glycerine or glycerine and water. If preferred, they can be stained with Löffler's methylene blue, which stains the filaments of the mycelium and hyphæ, the

spores remaining unstained. For permanent preparations the moulds are best mounted in glycerine jelly, the cover-glass being ringed with varnish to preserve the specimen.

Culture of Moulds.—Hansen recommends the addition of 0·1 to 0·2 per cent. of hydrochloric acid to the culture medium, in order to restrain the growth of bacteria.

ALGÆ.

A number of the minute water-plants known as the algæ are included with the micro-organisms. They are classed in two main divisions, *Leptotricheæ* and *Cladotricheæ*.

The *Leptotricheæ* are divided into three genera, viz., *Crenothrix, Beggiatoa, Leptothrix;* the *Cladotricheæ* are included in a single genus, *Cladothrix*.

(*a*) **Crenothrix.**—These are very common in running or stagnant water. They form simple threads, the separate cells of which surround themselves with a distinct sheath, and then change themselves by segmentation at their ends into roundish spores. The threads are motionless, and, especially in their younger stages, group themselves into little patches. The most important member of this group is the *Crenothrix Kuhniana*.

Crenothrix Kuhniana.—This is very frequently found in water containing organic matter or iron. It sometimes occurs in such great numbers that the water is unusable owing to the unpleasant odour and taste it produces. The organism produces a thick vegetable mass in the water, either brown or greenish in colour, frequently imparting a reddish or greenish tint to the water, and is capable by its presence in reservoirs of deteriorating large quantities of water at a time.

In microscopical appearance it exhibits, according to Zopf, both cocci and rod forms, as well as filaments. The cocci become invested with a gelatinous material, and

multiply by division, giving rise to irregularly-shaped zooglœa masses, sometimes of enormous size. When cultivated in marsh-water the cocci grow into rods, which by continuous division form filaments which radiate out in all directions from the zooglœa. When this growth has attained a certain age, a sheath is produced which contains ferric hydrate. By a continual process of division which takes place within the sheath, such a pressure is exerted against its top end that it is forced open, and thus the rods and cocci escape. Sometimes the cocci and rods

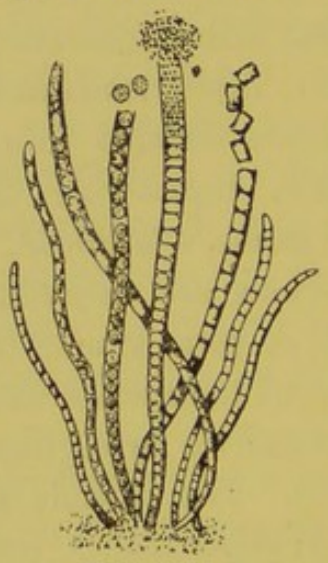


FIG. 29.—CRENOTHRIX KUHNIANA.

develop within the sheath into rods and threads, and push their way through the walls of the sheath, giving rise to a paint-brush appearance.

(*b*) **Beggiatoa.**—The Beggiatoa are distinguished by the presence of grains of sulphur in the cells, which are seen as highly refracting granules. The Beggiatoa are widely distributed, and are found both in fresh and salt water containing decomposing vegetable and animal matter. In the waters of sulphur springs they are especially abundant, and accumulate upon the muddy bottom, or upon the organic matter undergoing decomposition, as a white, gray,

pinkish or violet layer; the bottoms of ponds, springs, etc., are often coloured reddish by the abundant growth of this organism.

Mayer has shown that they are able to decompose sulphate of soda in organic solutions suitable for their growth. Like the previously described genus (Crenothrix), spherical, rod-like, filamentous and spiral forms are included in the life-history of the species. The filaments show a differentiation as to base and free-growing extremity; but, unlike the crenothrix, the segments into which the filaments divide are not included in an external sheath. The filaments are flexible, and exhibit a gliding movement; they are able to multiply abundantly in hot sulphur waters having a temperature of 55° C. and above.

(*c*) **Leptothrix.**—These are distinguished from Beggiatoa by the absence of sulphur grains, and from the Crenothrix by the fact that the segments are not enveloped in an exterior sheath, as well as by the comparative thinness of the cylindrical segments, otherwise they present the same variety of forms as has been ascribed to the Beggiatoa and Crenothrix. All the varieties of Leptothrix are common in the mouth and slime of the teeth. One of them, the *Leptothrix buccalis*, is believed to be intimately connected with dental caries. The threads penetrate the tissue of the teeth, after the enamel has been acted upon by the acids generated by the fermentation of the food.

Cladothrix Dichotoma.—This is the commonest microorganism occurring in both stagnant and running water in which organic matter is present. It is frequently found in the refuse-water of factories, especially sugar manufactories. In Russia it frequently occurs in the water-supplies of towns. It is to be obtained from the surface of putrefying vegetables or animal matter immersed in river or swamp water.

It consists of long, motionless filaments which sometimes grow to a millimetre in length, and which may possess pseudo-branches. According to Zopf, the cocci-like reproductive elements grow into rods, and these into fine filaments, from which latter the pseudo-branches are given off. This apparent branching of the filaments is the distinguishing generic character of the species. The sheaths of the filaments are often coloured yellow, red, olive-green or brown by oxide of iron. The *Cladothrix dichotoma* withdraws iron from water, and thus fixes it, often causing obstructions in iron pipes.

Cladothrix dichotoma can be readily cultivated on infusions of rotting vegetables and animal substances, forming small tufts and floating masses. On gelatine plates it forms small yellowish dots surrounded by a brownish halo which extends more and more over the gelatine. On reaching the surface it appears as a small brownish button surrounded by a very brown halo, and a depression due to the slow liquefaction of the gelatine. On agar it grows at 35° C., as a thick shining expansion, which adheres so closely to the medium that it is impossible to remove it without carrying away some of the agar. The growth has a tendency to form concentric rings. Sometimes the growth becomes covered with a grayish efflorescence, which is dry and very brittle. The agar becomes brown in colour. All the cultures have a very strong mouldy smell.

PROTOZOA.

A number of organisms have been noticed by various observers in the blood of both man and the higher animals; they occur associated with a number of diseases, but they often occur in the healthy subject. These organisms belong to the animal, and not the vegetable, kingdom.

The Protozoa are unicellular bodies which can only live in a moist or liquid medium, and in the absence of moist nutrient material they become converted into round resistant cysts. Protozoa may also possess a kind of larval condition, consisting of small roundish or irregular masses of protoplasm which move by means of projecting, limb-like processes (pseudopodia), or in some cases by flagella. They frequently lose their motility when they take up their residence in other cells. The contents of the cysts separate by division into particles known as *sporocysts*, the contents of which break up into a number of sickle or crescent like bodies.

The various divisions of the Protozoa are known as *Plasmodia*, *Coccidia*, *Psorospermi*, *Amœba*, etc.

The parasite occurring in the blood in malaria, known as the *Plasmodium malariæ*, has already been described (see p. 221 *et seq.*).

Protozoa have been described by many observers as occurring in dysentery, carcinoma, sarcoma, in a form of 'eczema' of the nipple known as Paget's disease; also in other pathological and morbid conditions of the human and animal subject.

A very fatal disease in young rabbits, due to a parasite known as the *Coccidium oviforme*, has recently been thoroughly studied by Pfeiffer, although its existence was known as far back as 1839. In India a fatal disease known as *surra* occurs in horses, mules, camels, etc. The cause of the disease, which is characterised by fever, jaundice, and great prostration, followed by death, is ascribed to the presence of a flagellated parasite which occurs in the blood of the affected animal in vast numbers.

Surgeon-Major Bruce, of Natal, has recently investigated the fatal disease which occurs in horses, etc., caused by the bite of the tsetse fly. Dr. Bruce finds

that the disease produced by the bite of the tsetse does not affect wild, but only domesticated animals, especially horses; also that it is not due to any inherent venom in the fly itself, but to the communication of certain flagellated germs from other diseased animals.

Flagellated monads have also been described by various investigators as occurring in the blood of apparently healthy rats, fish, etc.

Much work has yet to be done to determine the exact part played by the Protozoa in disease. For detailed information, we would refer the student to the original communications on this subject in the home and foreign medical journals.

FERMENTATION.

The term 'fermentation' is derived from *fervere*, to boil, and was formerly applied to all those cases where a liquid or semi-liquid mass was seen to become puffed up and to disengage gas without any apparent cause, among the earliest observed forms of this phenomenon being the fermentation of grape-juice and the leavening of bread. Owing to the mystery with which these well-known processes were surrounded, the term gradually came to be applied to all those chemical processes which were brought about by the presence of a body known as a *ferment*, the presence of which was indispensable, as the necessity for its presence was unintelligible. The meaning of the term 'fermentation' has now been much extended, until at the present day we mean those chemical changes which take place in a substance through the agency of a body derived from the animal or vegetable kingdom, termed a *ferment*. The ferment remains the same, qualitatively, both before and after the reaction. Hence we may class many bodies

as ferments to which the word 'ferment,' as meaning a 'boiling,' is misapplied.

All ferments possess three properties:

1. They are nitrogenous organic bodies.

2. They are unstable; that is to say, they are destroyed by reagents, such as heat, acids, etc.

3. A relatively small quantity of the ferment is capable of producing great changes in the body acted upon, especially if the products of the change be removed as they are formed.

Ferments can be divided into two classes, as follows: the *formed* or *organised ferments*, and the *unformed*, '*soluble*' *ferments*, or *enzymes*.

(*a*) **The Formed or Organised Ferments.**—These have a definite organized structure, and are capable of independent growth and multiplication. They include:

1. The yeasts, or saccharomycetes.
2. The moulds, or fungi.
3. The bacteria proper, or schizomycetes.

Fermentation by Yeasts.—From time immemorial brewers have been familiar with the art of preparing beer from an infusion, or 'wort,' of malted barley. This infusion, or 'wort,' if left alone, soon putrefies, becomes muddy and covered with a floating film, emits a disagreeable smell, and assumes an offensive flavour. Experience has, however, shown that the wort can be made into excellent beer by the addition of a little yeast, the remains of a previous operation, which the brewer can always find in the receptacles in which new beer is kept. Under the influence of this yeast an internal working in the mass occurs, gas is disengaged, producing effervescence, the sweet taste disappears, and is replaced by the characteristic flavour of beer, dear to man in all ages and places. If the practice of the operation of brewing is old, the science

is modern, and is the outcome of a series of discoveries principally due to Black, Lavoisier, Liebig, Schwann, and latterly to Pasteur and Hansen.

Sugar disappears during fermentation; but that is not all. Carbonic acid gas is given off, and alcohol is formed. Sugar contains carbon, hydrogen, and oxygen; so does alcohol, but in other proportions. In carbon dioxide or carbonic acid gas there is only carbon and oxygen. The decomposition of sugar is, therefore, accompanied by a complete breaking up. Fermentation consists, therefore, in the breaking up of chemical compounds, the molecules of which they are composed being torn apart from one another, and then allowed to form simpler and more stable compounds. Owing to the setting free of such energy as has been stored up in such a highly complex substance as sugar, which is no longer required to maintain the high level of combination, a certain proportion is released in the form of heat. This is why the temperature of a fermenting liquid always rises without the addition of any external heat.

Thus, it is seen that the act of fermentation is commonly the result of, or rather the accompaniment of, vital action. In the presence of the *Sacch. cerevisiæ* or other yeast, in saccharine liquids which contain small quantities of phosphates and albuminoid matter, *glucose* is converted into alcohol and carbonic acid gas, together with small quantities of glycerine, succinic acid, etc. Yeast contains also a soluble ferment which converts sucrose, or ordinary cane-sugar, into glucose. Therefore cane-sugar, or sucrose, may be converted into alcohol and carbonic acid gas, the soluble ferment first converting the sucrose into glucose, which then becomes decomposed by the action of the yeast. The conversion of the glucose may be represented by the following formula :

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2.$$
Glucose. Alcohol. Carbonic acid gas.

When the proportion of alcohol produced in a fermenting liquid amounts to 12 per cent., the further growth of the yeast is prevented, and with 14 per cent. fermentation ceases altogether. Temperature, again, is a most important factor in alcoholic fermentation, 25° to 30° C. being on the whole most favourable.

Hansen, of Copenhagen, has enormously extended our knowledge of the yeasts. He has shown that there are a number of distinct forms, differing, it is true, but little among themselves in shape, but possessing very distinct properties, more especially in respect of the nature of certain small quantities of secondary products to which they give rise, which are highly important as giving character to the beers produced. Hansen has shown how these varieties of yeast may be grown or cultivated in a state of purity even on the industrial scale, and has in this manner revolutionised the practice of brewing, particularly on the Continent. During recent years these pure yeasts, each endowed with its particular properties, have been grown with scrupulous care in laboratories equipped especially for the purpose, the pure growths then being despatched to different parts of the world, particular yeasts being employed for different beers. In this way scientific accuracy and the certainty of success are introduced into an industry in which much was left to chance, and in which everything was subordinated to tradition and empiricism.

Hansen carried on his researches on a most extensive scale in connection with the large brewing industry at Old Carlsberg in Copenhagen, where very naturally he gave early attention to the study of the saccharomycetes.

There are two kinds of fermentations employed in breweries, the 'low' and 'high.' Whether the *Saccharomyces cerevisiæ* producing these two kinds of fermentation are identical species or not is still a disputed point, but they undoubtedly retain their distinctive mode of action through many generations, although Rees states, in opposition to Pasteur, that the one kind may be transmuted into the other.

'Low' Yeasts.—These are employed in making the German and Austrian lager-beers, which differ from English beers, prepared by the 'high' fermentation process, by not containing so much alcohol or extractive; but they are of more delicate flavour, and they seem likely in time to entirely replace the heavier beers prepared from the 'high' yeasts. The 'low' yeasts consist of round or oval cells 8 to 9 μ in diameter. In sporulation there are three to four spores of 4 to 5 μ in each mother-cell. This fermentation takes place at a very low temperature, not higher than from 5 to 10° C., a temperature at which other forms of yeast are inert. This low temperature is maintained by passing currents of purified cooled air over the surface of the fermenting vessels, or by floating metal cones containing ice in the beer; the number used is regulated by the temperature of the external air. As would be expected, this fermentation proceeds more slowly than the 'high' process, taking on the average about fourteen days, the cells during the fermentation falling to the bottom of the fermenting vat.

'High' Yeasts.—These are especially used in the manufacture of English beers, whose bouquet and richness in alcohol render them more acceptable to English tastes than the somewhat milder German beers, prepared by the 'low' fermentation process. The 'high' fermentation yeast consists of cells which are rather larger and more globular, and have a greater tendency to form branched

chains than the 'low' yeasts. The temperature best suited for the carrying on of this fermentation is between 15° and 18° C. The reaction in the fermenting vats is much more violent than is the case with the 'low' yeasts. The rapid emission of carbonic acid brings the cells to the surface, where they form a frothy mass.

Fermentation by Moulds.—Many of the mould fungi are capable of setting up fermentation in saccharine liquids, and are able to act under certain circumstances as true alcoholic ferments. Some of the species of *Mucor*, when immersed in a fermentable saccharine liquid, such as wort, very quickly change their appearance: the submerged mycelium swells irregularly, and a large number of transverse septa appear, which divide it into barrel-shaped or irregular cells, filled with highly refractive plasma. These cells then multiply by budding, like true yeasts. If, then, the above-mentioned cells are brought to the surface of the liquid, or otherwise under aerobic conditions, they are again able to develop the typical mould form. The most active fermentative power is possessed by *Mucor erectus*, which in ordinary beer-wort can be made to yield up to 8 per cent. by volume of alcohol.

Fermentation by the Bacteria.—The bacteria proper are the cause of a very large number of fermentations. These bacterial fermentations have only very recently been studied from the purely biological standpoint, and it is only in a very few of the cases that the processes have been studied by the aid of pure cultures of the micro-organisms which are the cause of the particular fermentation.

As early as the year 1838 the view was expressed by Turpin and Kutzing that the acetic fermentation was caused by a micro-organism, which Kutzing described under the name of *Ulvina aceti*. Starting from this, Pasteur in 1864 furnished experimental proof of the correctness of

this view, and also published a method based on the results, for the manufacture of vinegar. He assumed, however, that the acetic fermentation was caused by one species of organism, which he called the *Mycoderma aceti*. Subsequent research, however, has shown that there are different species of acetic acid bacteria.

The fermentations which are known to be due to bacterial life can be conveniently classed under four headings as follows, according to the chemical change they induce in the fermentable substances:

1. Fermentation by oxidation.
2. Fermentation by hydration.
3. Fermentation by simple decomposition.
4. Fermentation by reduction.

We will now consider a few of the more important fermentations which fall under these respective headings.

1. **Fermentation by Oxidation.**—There are two very important fermentations belonging to this group—the acetic fermentation of alcohol, and the oxidation of ammonia into nitrates, which takes place in the soil.

Acetic Fermentation of Alcohol.—The conversion of wine and other alcoholic liquids into vinegar, on prolonged exposure to the air, is a phenomenon which has been known from the earliest times. As has already been stated, Pasteur first showed that the cause of this oxidation was the *Mycoderma aceti*. Hansen found, however, that this organism consisted of two species, which he named the *Bact. aceti* and *Bact. Pasteurianum* respectively. The *Bact. aceti* consists of short bacilli about 2 μ long, slightly contracted in the middle, so that they somewhat resemble the figure 8, and occur in chains of various lengths. Abnormal forms are frequently seen, particularly in old cultures, which frequently attain a length of 10 to 15 μ or more, and are often swollen into irregular shapes. The free

normal cells are motile. In order to develop vigorously, *Bact. aceti* not only requires a plentiful supply of oxygen, but also a fairly high temperature.

Vinegar has been defined to be 'the product of the alcoholic and acetous fermentation of a vegetable juice or infusion.' This definition includes all kinds of brewed vinegar, but excludes wood-vinegar. Brewed vinegar of whatever source will naturally be distinguished from wood-vinegar (acetic acid and water), by containing extractive matters which will remain when the sample is evaporated.

In the case of malt-vinegar, by which we understand vinegar brewed either entirely from malted barley or from a mixture of not less than one-third malt and two-thirds barley, we find the extractive matter to range about 2·5 per cent.

The process of vinegar-making is as follows: The malt or malt and barley (the latter finely ground) are 'mashed,' or soaked in successive quantities of hot water till all that is soluble is extracted. The clear liquor is then run off into another vessel, and yeast added. Fermentation then takes place, with evolution of carbonic acid. The 'wort,' or 'wash,' is then pumped over piles of birch-twigs placed in high vats, to which a regulated supply of air is supplied. The twigs become coated with *Mycoderma aceti*, 'vinegar plant,' and the alcohol produced by the fermentation is then converted into acetic acid.

Small quantities of other bodies—as acetic ether, aldehyde, etc.—are formed, which give malt-vinegar its pleasant taste and smell.

In good working all the alcohol is not converted into vinegar, as a little alcohol improves the flavour and assists the 'keeping' of the finished product, which is generally kept for a year in order that the flavour may fully develop.

Nitrification of Ammonia.—The well-known and important

process of oxidation which takes place in the soil, whereby the organic nitrogen and ammonia are converted into nitric and nitrous acid, is the result of the activity of certain micro-organisms. This process of 'nitrification,' as it is called, was first studied by Müntz and Schloesing in 1877, who found that nitrification did not take place in soil that had been sterilised by heat, or that had been treated with antiseptics. They also showed that it was only ammoniacal nitrogen that could undergo true nitrification, the organic nitrogen having to first be converted into ammonia, which change also takes place by the agency of micro-organisms. Warington, Heräus, Munro, and others, did further work on these organisms; but it was not until 1890 that the organisms were obtained in a pure condition by Winogradsky and Frankland, who worked independently.

The difficulty of obtaining these organisms in a state of purity was enhanced by their inability to grow upon any of the usual culture media, they requiring a medium entirely free from organic matter for their growth. This difficulty was overcome by Professor Kühne, who devised in a most ingenious manner a medium which was entirely free from organic matter and of jelly-like consistency; this he obtained by means of gelatinised silica. For method of preparing this silica jelly, see p. 65.

The discovery of these 'nitrifying' organisms in the soil was of the very greatest importance, as it disproved one of the most fundamental principles of vegetable physiology, which stated that green plants alone had the power of building up protoplasm from inorganic materials. The 'nitrifying' organisms of Winogradsky and Frankland consist of ellipsoidal cells, the young cells being nearly spherical. They are from 0·9 to 1 μ broad and from 1·1 to 1·8 μ long, occasionally seen in short chains, and do not form spores.

2. **Fermentation by Hydration.**—The most important fer-

mentation process which falls under this head is the conversion of urea into ammonium carbonate by the action of the *Micrococcus ureæ*.

Ammoniacal Fermentation of Urea.—Freshly-passed urine is faintly acid in reaction, and contains about 3 per cent. of urea, but is free from ammoniacal salts. On standing, however, the urea disappears and ammonia is formed, and the urine becomes strongly alkaline in reaction. Liebig, who attempted to find the cause of this change, came to the conclusion that it was due to the presence in the urine of decomposing particles of the mucous membrane of the bladder. Pasteur and Van Tieghem found, however, that all ammoniacal urine contained an organism, which brought about this change, which they called the *Torula urinæ*, but which is now generally known as the *Micrococcus ureæ*. The change this organism brings about in the urine may be represented by the following equation:

$$\underset{\text{Urea.}}{CO(NH_2)_2} + 2H_2O = \underset{\substack{\text{Ammon.}\\ \text{carbonate.}}}{CO(NH_4O)_2}.$$

The *Micrococcus ureæ* is generally seen in pairs, tetrads, and in chains which are often of considerable length. The cocci vary from 0·8 to 1·0 μ in diameter; but, according to Jaksch, it sometimes exhibits a more or less bacillary form. On gelatine plates, the *M. ureæ* appears after twenty-four hours as white pearl-like colonies, which after some time become like drops of tallow. The gelatine is not liquefied. The alkaline reaction resulting from the ammoniacal fermentation of urea does not seriously interfere with the growth of the organism, which will grow in the presence of up to 13 per cent. of ammonium carbonate.

3. **Fermentation by Simple Decomposition.** — As is well known, milk on standing, especially in warm weather, becomes acid and coagulates. The change which takes place

is one of simple decomposition, one molecule of milk-sugar (lactose) becoming converted into four molecules of lactic acid, which latter coagulates the milk by precipitating the albuminoids. This change is shown by the following equation:

$$\underset{\text{Lactose.}}{C_{12}H_{22}O_{11}} + H_2O = 4\underset{\text{Lactic acid.}}{C_3H_6O_3}$$

The fermentation ceases after a certain amount of lactic acid has been formed, but it will recommence, however, if the liquid be neutralised with carbonate of lime.

The *Bacillus acidi lactici* consists of short, somewhat thick non-motile bacilli 1 to 1·7 μ long by about 0·3 to 0·4 μ broad, generally occurring in pairs and in strings of 4 elements. The bacilli form spores, each situated at one extremity of the bacillus. On the surface of gelatine, in 'streak' cultures, a thin delicate growth is formed along the whole tract of the needle. The gelatine is not liquefied.

The *Bacillus acidi lactici* sets up the lactic fermentation in solutions of milk-sugar, cane-sugar, dextrose and mannite. In the case of the first two sugars, the ferment appears to first exert an 'inverting' action, whereby one molecule of these two sugars is respectively converted into two molecules of dextrose or glucose, which in turn is broken up by the action of the ferment into two molecules of lactic acid. These changes are shown by the two following equations:

(*a*) $$\underset{\text{Milk-sugar.}}{C_{12}H_{22}O_{11}} + H_2O = 2\underset{\text{Dextrose.}}{C_6H_{12}O_6}.$$

(*b*) $$\underset{\text{Dextrose.}}{C_6H_{12}O_6} = 2\underset{\text{Lactic acid.}}{C_3H_6O_3}$$

The most favourable temperature for the fermentation appears to be from 35° to 42° C., while at 45° C. it ceases.

4. **Fermentation by Reduction.**—It is to this class that the various butyric acid ferments belong, which are the cause

of 'rankness' in butter; they also take a great part in the 'ripening,' and help to impart the characteristic taste and aroma to the different varieties of cheese. When milk which has undergone the lactic acid fermentation is neutralised with carbonate of lime, so that calcium lactate is formed, it will as a rule enter into a butyric acid fermentation. This spontaneous butyric fermentation takes place most vigorously at 35° to 40° C. Starch, dextrine, cane-sugar, glucose, and cellulose, are among the large number of substances which are fermentable by these butyric acid ferments, which are very widely distributed in Nature. The two chief butyric acid ferments are the *Bacillus butyricus* and *Bacillus amylobacter*.

Bacillus butyricus.—This forms short and long thin rods with rounded ends, seldom forming threads. Large oval spores are formed, which are very resistant to external influences. The bacilli are very motile; they liquefy gelatine very rapidly, giving rise to a strong butyric acid smell. In milk it coagulates the albumen and decomposes it, forming peptones and ammonium butyrate.

Bacillus amylobacter, or *Clostridium butyricum*.—This, which is always found in putrefying plant infusions, forms large thick motile rods, which are often associated in the form of chains. A large spore forms in one end of the rod, thus causing the bacillus to become spindle or club shaped—hence the name *clostridium*. In solutions of sugars, lactates and in cellulose-containing plants, it gives rise to decompositions in which butyric acid is formed. The bacilli are strongly anaerobic, and have not yet been satisfactorily cultivated.

In the bacterial fermentations of this class many carbohydrates and fatty acids undergo decomposition: a part of the carbon is oxidised to carbon dioxide, whilst the remainder, having lost the oxygen taken up in the forma-

tion of the carbon dioxide, is left as a reduced product of the reaction. In most cases a part of the hydrogen is removed as water, and in some cases as free hydrogen.

Mixed Fermentations.—To this class belong a large number of fermentations which form the foundations of such important industries as butter, cheese, and wine making, the curing of tobacco, the fermentation of bread, the tanning of leather, the manufacture of koumiss (fermented milk), ginger-beer, indigo, etc. All these and many other important processes in which fermentation plays an important part have yet to be thoroughly investigated.

It is of interest in this connection to mention that much work is now being done on the Continent in the way of improving low-grade wines by the employment of pure cultures of micro-organisms obtained from high-class vintages, whereby much of the characteristic aroma and bouquet of the best vintages are communicated to a considerable extent to the poorer qualities of wine. In the same way butter, cream, and cheese are being improved and kept of standard quality by the same means. The extension of these scientific processes will no doubt do away to a large extent with the somewhat empirical and uncertain methods of dairying now in vogue.

(*b*) **The Unorganised Ferments, or Enzymes.**—These have no organised structure or power of multiplication, but are highly complex bodies of an albuminoid nature, which possess the power of bringing about chemical changes on a scale altogether out of proportion to their own mass. The mode of action of these ferments is not quite understood, but appears to be similar to the process of *hydrolysis*, which takes place when sulphuric acid converts alcohol into ether, when theoretically a given quantity of acid is capable of converting an unlimited amount of alcohol. These 'soluble' ferments are divisible into a number of

groups, the four most important of which are the following:

(1) The Diastatic Ferment.
(2) The Peptic Ferment.
(3) The Rennet Ferment.
(4) The Pancreatic Ferments.

The action of the first class appears to be due to the presence of one or more living cells in a body; the action of the second may be traced to the molecules of which they are composed, and which are the carriers of the chemical force that causes the changes. The second class of ferments will only be dealt with here.

1. Diastatic Ferment.—The best-known instance of this occurs in extract of malt. If a solution of extract of malt be heated to about 30° C. with its own weight of starch in solution, the starch is completely converted in a few minutes into maltose and dextrine, and ceases to answer the iodine reaction. The diastatic fermentation occurs to a small extent in the saliva. The diastatic body may be obtained by filtering saliva, and mixing with five or six times its weight of alcohol. The very slight precipitate which falls is dried at the temperature of the air. It has a strong diastatic action.

The diastase of malt is capable of converting no less than 2,000 times its weight of starch. The change can be illustrated by the following equation:

$$\underset{\text{Starch.}}{6C_6H_{10}O_5} + 4H_2O = \underset{\text{Dextrin.}}{2C_6H_{10}O_5} + 2(\underset{\text{Maltose.}}{C_{12}H_{22}O_{11}} + H_2O).$$

2. Peptic Ferment.—This fermentation takes place in the stomachs of animals when food is digested, and the stomachs are the sole source of pepsin.

Various theories of digestion have been advanced from

time to time, that the changes were due to heat only, to grinding only, or to the action of strong acids, etc.

Gastric juice exudes when the surface of the stomach is touched. It is usually colourless, and always acid in reaction. Human gastric juice has a specific gravity from 1·001 to 1·010, and contains less than 1 per cent. of solid matter. On boiling, it is not coagulated, but its power is totally destroyed. It may be kept for months unchanged.

The power of gastric juice in dissolving proteids may be traced to the pepsin it contains, which can only act in the presence of an acid, preferably hydrochloric.

These peptonising ferments convert coagulable albuminoids into soluble or diffusible albuminoids or peptones, which are not precipitated on boiling.

The Preparation of Pepsin.—The British Pharmacopœia directs that the mucous membranes of the stomachs be washed, and then scraped off and dried. Pepsin may also be obtained by digesting the membranes in glycerine, in which the pepsin is soluble, and precipitating with alcohol.

Peptonising ferments are often secreted by many bacteria, a large number of which cause liquefaction of the gelatine medium on which they grow. These ferments appear to be more allied to trypsin than to papain.

3. The Rennet Ferment.—The fourth stomach of the calf has been long known for its milk-curdling properties, due to a ferment termed chymosin. This ferment is invariably present in the healthy human stomach, and is present in many animals. The chymosin may be extracted from the calf's stomach with water, but a better way is to digest with weak acid for twenty-four hours and carefully neutralise. Aqueous solutions of salicylic acid extract the ferment well, and will keep. Alcohol precipitates the ferment in an impure form. Prolonged contact with alcohol, especially if strong, is said to destroy the ferment. Fixed caustic

alkalies have a powerful destructive action upon it, even in very small quantities, and carbonate of sodium, calcined magnesia, etc., act the same, though in a lesser degree. Heat readily destroys chymosin, especially if acid.

4. The Ferments of the Pancreas.—Pancreatic juice is a more or less viscid liquid, invariably alkaline and readily putrefying. Alcohol causes an abundant precipitate, which carries down the ferments it contains. These are at least three:

1. A proteolytic ferment acting in neutral or alkaline solutions.

2. A diastatic ferment, similar to that in the saliva.

3. A fat-decomposing ferment which emulsifies and decomposes fats into glycerine and fat acids.

The proteolytic ferment (trypsin) does not exist in the perfectly fresh pancreas, but is formed in an hour or two's time. It may be extracted by digesting with water, precipitating with alcohol, re-dissolving, re-precipitating, and digesting in absolute alcohol. The precipitate is treated with water, acetic acid added, filtered, sodium hydrate added to slight alkalinity, filtered, concentrated at 40° C., filtered, and precipitated with alcohol. If necessary, it is purified by dialysis.

Trypsin acts most readily in a solution containing about 1 per cent. of Na_2CO_3, that being the strength of the juice. It acts in neutral and in very slightly acid solutions, but contact with warm acid fluids gradually decomposes it.

The diastatic ferment is obtained by treating the pancreas by extracting with glycerine, chloroform-water, solution of borax with boracic acid, brine, etc. By these methods solutions of the two ferments are obtained, which may be precipitated by alcohol. The ferment acts most readily on starch at from 30° to 40° C. One part of the diastase is said to be able to convert 40,000 parts of starch into sugar and dextrin.

The fat-decomposing ferment cannot be extracted or kept so readily as the others. If two parts of juice be agitated with one of olive-oil, or of some fat melting below 40° C., a perfect and persistent emulsion is formed immediately. The fat-globules are said to be finer than those in milk. Both the pancreas and its juice possess the power of decomposing fats. If a particle of the pancreas be dehydrated by means of alcohol, well teased out in an ethereal solution of butter-fat, and afterwards transferred to a drop of tincture of litmus, a cover-glass being placed above it, the litmus is seen to be reddened all round the particle, due to the action of the fatty acids separated from the butter.

CHAPTER XII.

PRODUCTS OF THE METABOLISM OF ORGANISMS—EXAMINATION OF DISINFECTANTS.

The products of the vital activity of micro-organisms—The ptomaines or cadaveric alkaloids: their characters and constitution — The principal ptomaines and their properties—Brieger's method for the isolation of ptomaines—The albumoses or toxalbumens: their characters, etc.—The albumoses of diphtheria, anthrax, cholera, typhoid, *Staph. pyogenes aureus*, etc.—'Tuberculin' and 'malleïn'—Tests for albumoses—'Intracellular' poisons—Chromogenic bacteria and colouring matters—The phosphorescent bacteria—Other products of the metabolism of micro-organisms—Antiseptics, germicides and disinfectants—The chemical agents used as disinfectants—The bacteriological examination of disinfectants.

As has already been stated, micro-organisms produce, as the result of their metabolism, or vital processes, a number of complicated bodies, known as ptomaines, cadaveric alkaloids, leucomaines, albumoses, toxalbumens, toxins, etc.

The pathogenic power of the bacteria which cause the various diseases in man and animals has been shown to result from the absorption into the body of these bodies, which are the result of bacterial activity.

The term *ptomaines*, or *cadaveric alkaloids*, was first applied to those bodies formed during putrefaction, but is now used for all alkaloids or bodies of a basic nature formed by the activity of micro-organisms. Many of these bodies, when introduced into the animal body, give rise to the same symptoms as do the particular organisms themselves;

so that we may say that bacteria affect the body chiefly through certain toxic principles which they elaborate.

Some of the ptomaines are non-poisonous, while others are excessively poisonous in even very minute doses. The toxic bodies are sometimes developed in such articles of food as milk, cheese, sausages, tinned fish, etc., whereby they contain organisms of putrefaction, which, giving rise to toxic ptomaines, cause disastrous effects upon being consumed.

The *albumoses*, or *toxalbumens*, like the ptomaines, are products of the vital activity of bacteria, and, like them, when separated from the bacteria from which they have been produced, and introduced into the animal body, give rise to symptoms similar to those produced by the bacteria themselves. They are amorphous, and have no basic properties; they have all the characters of albumin or a proteid.

The Ptomaines, or Cadaveric Alkaloids.—The ptomaines were first discovered in decomposing animal tissues, as their name 'cadaveric alkaloids' implies. It has long been known that the products of putrefaction, especially those formed in putrefying fish, are extremely poisonous. As early as 1814, Burrows in this country described a poisonous body as occurring in decomposing fish, and in 1820 Kerner described a poisonous alkaloid resulting from the decomposition of albumin. In 1856 Panum obtained a substance from putrid animal matter which he thought was derived from albuminoid matter by the agency of bacteria. This substance, to which he gave the name 'sepsin,' was found very fatal to dogs.

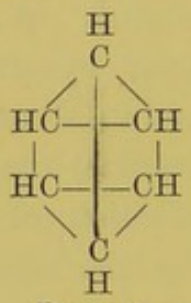
From this date many extended researches upon these bodies have been made by various investigators, among whom may be mentioned Bergmann, Schmeideberg, Zuelzer, Sonnenschein, Hager, Stas, Brieger, Gautier, Roux, Fränkel, Vaughan, Martin, and others.

The importance of the presence of these bodies in decomposing animal matter cannot be over-estimated. In various plants there occur a whole series of bodies, of which strychnine, quinine, atropine, nicotine, morphine, and others are well-known examples. All of these exert a specific action on the animal body, many of them causing very toxic effects.

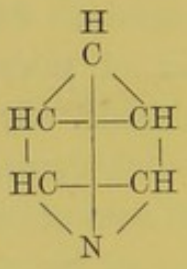
A number of the ptomaines have been built up artificially, without the aid of micro-organisms, by purely chemical synthetical methods. Trimethylamine, dimethylamine, and pentamethyline diamine (cadaverine) may be obtained from the products of the putrefaction of the animal body, and also may be prepared by the chemist artificially, the synthetically-prepared product giving rise, on inoculation into the animal body, to all the symptoms and post-mortem appearances of an attack of cholera. The ptomaines are nitrogenous bases having an alkaline reaction, and combining with acids to form salts. Their chemical constitution is very complex, and, like many of the vegetable alkaloids, they are derived from the base pyridine. The following are some of the characters of the vegetable alkaloids, which are the active principles of plants, and to which the ptomaines are strictly allied. They are very poisonous, having a bitter, acrid, and pungent taste. The alkaloids combine with acids to form salts, and are precipitated from their saline solutions by the addition of alkalies. They are mostly crystallised and colourless, insoluble in water, but soluble in alcohol, chloroform, benzine, and some in ether. The salts, on the other hand, are soluble in water, less so in alcohol, insoluble in chloroform, etc. Alkaloids are mostly precipitated by one or more of the following reagents: Potassio-mercuric iodide, gold chloride, tannic acid, phospho-molybdic acid, and picric acid. Alkaloids are either amides or amines.

If the former, they are composed of carbon, hydrogen, nitrogen, and oxygen; if the latter, the oxygen is wanting. Non-volatile amides are solid; on the other hand, volatile amines are liquid.

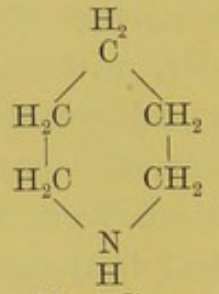
The chemical constitution of many of the alkaloids and ptomaines can be illustrated by the following constitutional formulæ:



Benzine.



Pyridine.



Piperidine.

As is seen from the above formulæ, pyridine is very nearly related to benzine, from which it is easily obtained by replacing one of the CH groups by nitrogen. Both benzine and pyridine have some of their carbon bonds unsatisfied, so that each C and the N having a bond free to combine with other atoms or groups of atoms, there is the possibility of a very large number of addition and substitution derivatives being formed. Perhaps the most simple case is the following. All the free bonds in pyridine may be satisfied by the addition of a single atom of hydrogen to each, when the result is the production of the alkaloid piperidine.

Leucomaines.—Gautier first pointed out the fact that certain bodies of an alkaloidal nature are produced within and by the living tissues of the animal body. These are without doubt the result of the metabolism of the protoplasm, or they may be the result of the decomposition of albuminoid substances within the body. The leucomaines exist in very small quantity in normal urine, but they very largely increase in quantity in certain diseases.

The following are some of the principal ptomaines :

Cadaverin, $C_5H_{14}N_2$.—This is a thick, syrupy, transparent, volatile liquid with a very unpleasant smell. It is produced in cultures of the *Spirillum choleræ Asiaticæ*, and the spirillum of Finkler and Prior, which have been kept for a month or more at a temperature of 37° C.

Neuridin, $C_5H_{14}N_2$.—This is the most common ptomaine of putrefaction, and was isolated by Brieger in 1884. It is to be obtained most abundantly from decomposing tissues containing gelatine. It has a very disagreeable smell, is very soluble in water, but insoluble in ether and absolute alcohol. It is isomeric with cadaverin, and is said to be non-poisonous.

Putrescin, $C_4H_{12}N_2$. — This base strongly resembles cadaverin, and is frequently found associated with it. This ptomaine was first obtained by Brieger from various sources, most abundantly from substances containing gelatine in a very advanced state of decomposition. It is obtained in the form of a hydrate, which is a transparent liquid having a boiling-point of about 135° C.

Saprin, $C_5H_{16}N_2$.—Resembles cadaverin, and is commonly associated with it in putrefying animal matter. Non-poisonous.

Methylamine, $CH_3.NH_2$.—Obtained from putrefying fish, and is present in old cholera cultures. Non-poisonous.

Dimethylamine, $(CH_3)_2.NH$.—Obtained by Brieger from putrefying gelatine, and by Bocklisch from decomposing fish. Non-poisonous.

Trimethylamine, $(CH_3)_3N$.—Found by Brieger in cultures of the cholera spirillum and the streptococcus of pus. Non-poisonous.

Neurin, $C_5H_{13}NO$.—Obtained by Liebreich as a decomposition product of protagon from the brain, and by Brieger from decomposing muscular tissue. Crystallises in the

form of plates and needles. This base is toxic in very small doses, producing total paralysis in frogs, etc.

Cholin, $C_5H_{15}NO_2$.—Obtained from hog's bile by Strecker in 1862, and later by Brieger from various sources, including cholera cultures. It is a syrupy liquid which combines with acids to form deliquescent salts.

Muscarin, $C_5H_{15}NO_3$.—This very toxic ptomaine is found in poisonous mushrooms, and can also be produced by the oxidation of cholin.

Methyl-guanidin, CHN.—Obtained by Brieger from decomposing horseflesh, which had been kept at a low temperature for several months. It is also to be obtained from cultures of the Finkler-Prior bacillus, and can be obtained artificially by the oxidation of creatin. This base is very poisonous in the case of guinea-pigs, causing total paralysis.

Tyrotoxicon.—This unstable body was first obtained by Vaughan from poisonous cheese, and subsequently by others in poisonous milk and ice-cream. It is decomposed at a temperature of 90° C. The symptoms produced by eating cheese or milk containing tyrotoxicon are vertigo, nausea, vomiting, cramps in the legs, griping pains in the bowels attended by purging, numbness, and great prostration.

Mytilotoxin.—The composition of this ptomaine is unknown. It was obtained by Brieger from poisonous mussels. The toxic effects produced are similar to that of curare (arrow-poison).

Typhotoxin, $C_7H_{17}NO_2$.—This was obtained by Brieger from broth cultures of the typhoid bacillus which had been kept for a week or more at a temperature of 37·5° C. The specific action of the typhoid bacillus is probably due to the production of this ptomaine. On inoculation of minute doses of this base into mice and guinea-pigs,

salivation, rapid respiration, diarrhœa, and death, is produced in about twenty-four hours.

Tetanin, $C_{13}H_{30}N_2O_4$.—This has been obtained from cultures of the tetanus bacillus, by Kitasato and others. It was obtained by Brieger from an amputated arm of a patient with tetanus. This base has been obtained by crystallisation from alcohol, in the form of yellow plates. When injected into guinea-pigs or mice, tetanin first causes the animal to fall into a lethargic condition, followed by increased rapidity of respiration and tetanic convulsions.

Cholera Ptomaines.—In addition to those that have been already described as obtained from cholera cultures, Brieger obtained two toxins which appear to be characteristic of this organism. One induces cramps and muscular tremors in all small animals, the other diarrhœa and symptoms of collapse.

Brieger's Method for the Separation of Ptomaines.—The putrefying mass is boiled with water and filtered; the filtrate is then treated with basic acetate of lead until no further precipitate falls on further addition of the lead salt. The liquid is then filtered, and the excess of lead removed from the filtrate by passing sulphuretted hydrogen, and the precipitated lead sulphide removed by filtration. The filtrate is evaporated to one-third of its bulk and mixed with fusel-oil (amylic alcohol); it is then thoroughly washed with water, and again reduced in bulk by evaporation; a little sulphuric acid and ether are added; the ether is evaporated, after which the remaining liquid is concentrated by careful evaporation to one-third of its bulk. The evaporation drives off most of the volatile fatty acid present; the liquid is then neutralised by the addition of baryta, filtered, and the excess of baryta removed from the filtrate by passing carbon dioxide; the barium carbonate which is precipitated is filtered off. The filtrate, after being

carefully warmed over a water-bath, is cooled, and mercuric bichloride added, when a somewhat heavy and dense precipitate is formed. This precipitate is carefully washed, and then suspended in water, and sulphuretted hydrogen passed; the white precipitate is decomposed, with the production of black sulphide of mercury; this is then filtered off. The filtrate is then carefully concentrated by very careful evaporation, until crystallisation begins to take place. All the inorganic salts crystallise out first; these are removed, and the mother-liquid further evaporated, when needle-like crystals are thrown out of solution. These may be dissolved in water, but they are insoluble in absolute alcohol, ether, benzine and chloroform. The substances so yielded, the ptomaines, may be precipitated by the salts, particularly the chlorides, of the heavy metals.

These crystals differ very considerably as to their solubility: hydrochloride of putrescin when obtained by the above method separates out in the form of acicular crystals, and on the addition of chloride of gold gives very insoluble crystals of octahedral form, while on the addition of chloride of platinum, octahedral crystals, which are much more soluble, are formed.

Albumoses, Toxalbumens, etc.—As has already been stated, many of the pathogenic bacteria produce a number of intensely poisonous bodies which are allied in their constitution and characters to albumins or proteids.

Löffler, in 1887, when examining the products of pure cultures of the diphtheria bacillus, found that if a broth culture was freed from the bacilli by filtration through a porcelain filter, and was then injected into a guinea-pig, it gave rise to the same local reaction and paralytic symptoms as when the bacilli themselves were inoculated.

Roux and Yersin isolated the pure albumose by filtering broth cultures of the Klebs-Löffler bacillus through a

Pasteur-Chamberland filter, and then precipitating the albumose from the filtrate by means of absolute alcohol. This was purified by redissolving in water and reprecipitating with alcohol. This body is obtained as a snow-like amorphous mass. It was found to be destroyed by a temperature of 50° C.

Hankin has isolated a similar soluble albumose from cultures of the anthrax bacillus. Roux, Fränkel, and Brieger and others have obtained similar bodies from cultures of the cholera, typhoid, and the tetanus bacilli, and from the pus-forming organisms, the pneumo-bacilli, etc.

Fränkel and Brieger divide these albumoses into two groups, one of which is characterised by its ready solubility in water, as in the case of that produced by the diphtheria bacillus; the other in which the albumose is insoluble, or nearly so, as in the case of those from cultures of the typhoid bacillus, the cholera spirillum and the *Staphylococcus pyogenes aureus*.

The toxalbumens from cholera cultures, when injected under the skin of a guinea-pig, caused its death in two days. It was not, however, toxic for rabbits, even when injected in considerable quantity. On the contrary, the toxalbumen from typhoid cultures was more poisonous for rabbits than for guinea-pigs. The toxalbumen from the *Staph. pyogenes aureus* killed both rabbits and guinea-pigs within a few days—in some cases at the end of twenty-four hours. The post-mortem appearances showed necrosis or purulent breaking-down of the tissues at the point of injection, with swelling and general inflammatory appearances.

Sidney Martin has very recently found, as the result of a prolonged investigation, that the albumoses in the case of diphtheria and anthrax are mixtures; and he has succeeded

in separating three and two well-defined albumoses respectively from cultures of these two organisms.

It has been pointed out elsewhere that Koch's 'tuberculin' and 'mallein' are the glycerine extracts of the toxins of the tubercle and glanders bacilli respectively, as has also their use as remedial and diagnostic agents in the diseases of which these two organisms are the specific cause.

All these albumoses, or toxalbumens, give with Millon's reagent* a white precipitate, which on warming becomes brick-red in colour, thus indicating their proteid or albumin-like character. They are precipitated, however, by a saturated solution of magnesium sulphate, which shows they are not ordinary albumins. On the addition of a drop of dilute sulphate of copper solution, followed by a slight excess of potassium hydrate solution (the biuret reaction), a rose-red, and not a violet, coloration is given, thus indicating that they belong to the albumin rather than the globulin group.

'Intracellular' Poisons.—Klein has recently shown† that, by intraperitoneal and by subcutaneous injection of guinea-pigs with small but definite doses of the protoplasm, living or dead, of various species of bacteria, these animals can be rendered tolerant of further injection in large amount of the protoplasm, whether the protoplasm secondarily injected be derived from the same or from some other species of organism. He found that the spirillum of cholera and of Finkler-Prior, the bacillus of typhoid, the colon bacillus, the *Proteus vulgaris* and the *Staph. pyogenes aureus*, when completely separated from their metabolic

* *Millon's Reagent.*—Mercury is dissolved in its own weight of strong nitric acid. The solution so obtained is diluted with twice its weight of water. The decanted clear liquid is then known as Millon's reagent.

† Local Government Board Report, 1893-94, p. 469.

products, all produce one and the same disease when their protoplasm is injected subcutaneously into guinea-pigs; and since they induced death under the same pathological and clinical symptoms, when injected intraperitoneally, Klein drew the inference that all these bacteria contain the same kind of poisonous body within their protoplasm. This body he termed the 'intracellular' poison, which must not be confused with the 'toxins' produced by the metabolism of the same organisms as the result of their growth in artificial media or the animal body. In the case of anthrax, Klein states that the pathogenic properties of this bacillus may be due to two poisons: the metabolic products, which pervade the system; on the other hand, the protoplasm of the bacilli, which contains the 'intracellular' poison. The tubercle bacillus he also cites as without question concerned with two kinds of poisons. Again, Klein states that neither tetanus nor diphtheria is due to the action of 'intracellular' poisons; in these diseases the specific organism remains limited to one locality of the body, where they produce their toxins, which when absorbed into the system give rise to the symptoms characteristic of one or the other disease.

Chromogenic Bacteria and Colouring Matters.—As before stated, many of the saprophytic bacteria, as the result of their vital action, give rise to many beautiful colouring matters, such as red, yellow, green, violet, etc. In some cases these colouring matters are contained in the substance of the bacteria themselves, or within the sheath; in others the organisms in themselves are quite colourless, and produce colouring matter as the result of the decomposition of the nitrogenous albuminoid matter contained in the nutrient media. The bacillus of the disease of milk, known as 'blue milk' (*B. cyanogenus*), when grown on gelatine, is of a pale-blue colour; but the organism soon colours the

whole of the medium of a dirty-green colour, which is soon replaced by a muddy-brownish tint. Many bacteria, to produce their characteristic colouring matter, require to be grown under certain conditions; many require light for the formation of the pigment; others, again, require a low temperature—for instance, the colouring matter of the *B. prodigiosus* will develop at blood-heat. Again, many bacteria lose their power of pigmentation after continued subculture on one kind of nutrient medium; this may be generally restored, as, in the case of the *B. prodigiosus*, the chromogenic power is restored by growth on potato.

The brilliant blood-like colouring matter of the *B. prodigiosus* was the cause of the phenomena known as the 'bleeding host' or 'bloody sweat.' The moist consecrated wafers, after being left on the altar in the church overnight, would be found the next day to be covered with little blood-like drops, which rapidly grew larger. What else, it was asked, could it be but blood, which could but mean some terrible portent of great calamity? It is needless to say that great capital was made out of this 'miracle' by the Church in the Middle Ages. It was a miracle which priest and layman could believe in with perfect honesty—one of which, owing to the want of apparent cause, the supernatural may have seemed the natural explanation.

Many of the bacterial colouring matters strongly resemble the aniline dyes in their behaviour to acids and alkalies, and in their appearances on media. Some cultures, after keeping, take on the peculiar metallic lustre so characteristic of the aniline dyes. Many of these bacterial pigments are soluble in water, while others are insoluble in water, but soluble in alcohol, ether, chloroform, etc.

The colouring matter of the *B. prodigiosus* can be extracted with ether; that of the *B. pyocyaneus* is soluble

in chloroform. On extracting the growth from a number of agar cultures of the *B. pyocyaneus* with chloroform, and filtering, a deep-blue solution is obtained, which on slow evaporation in the dark yields a crystalline residue of pyocyanine.

The cultural characters of a number of chromogenic bacteria will be found in the last chapter.

Phosphorescent Bacteria.—Many bacteria give rise to phosphorescence as a result of their vital activity. It is to these organisms that the beautiful phosphorescent phenomena sometimes seen in the sea, especially in the tropics, are due. They are also seen not infrequently in marshy places and on decaying wood; the luminescence occasionally exhibited by fish is also well known. The light given off from the gelatine cultures of some of these bacteria is sufficient to enable one to ascertain the time by a watch in a perfectly dark room, and even photographs have been taken by the light emitted by these organisms.

Beyerinck, who has carefully studied the light-giving bacteria, finds that the formation of light does not bear any direct relation to the growth of the organisms; but he finds that certain food substances are necessary for them to produce light. For instance, some require oxygen, although they will grow perfectly well under anaerobic conditions, without producing phosphorescence.

Other Products of the Metabolism of Micro-organisms.—In putrefactive fermentation, a number of substances are produced by the agency of bacteria. In addition to the very numerous and various bodies produced in the many fermentative processes—such as acetic, lactic, butyric, and other acids, alcohols, ammonia, albumoses, ptomaines, colouring matters, etc., of which many have been described in the previous pages—are a large number of other bodies, of which the following are a few which occur in the various

putrefactive processes: hydrogen, nitrogen, phosphuretted hydrogen, sulphuretted hydrogen, carbon dioxide, marsh-gas, formic acid, valerianic acid, many of the volatile and fixed fatty acids, free ammonia, ammonium sulphide, trimethylamine, propylamine, indol, scatol, etc.

The particular products yielded in putrefactive and fermentative processes vary necessarily with the composition of the decomposing material, the prevailing conditions, and with the species of organisms present. Many of the gaseous and volatile products of putrefaction are characterised by their very offensive smell. The anaerobic bacteria, generally speaking, give rise to the most malodorous products. In the case of a decomposing body of an animal, the odours evolved are worse in situations where oxygen has not free access. Anaerobic organisms are always present in the intestines, and after death they quickly invade the whole body, and grow under very favourable conditions as to temperature, the interior of the body providing in every way a suitable pabulum for their growth. The aerobic organisms on the surface of the dead body possibly assist the putrefaction in the interior by consuming the oxygen. The products of the putrefactive anaerobes are marsh-gas, sulphuretted hydrogen, and free hydrogen, with traces of such foul-smelling bodies as scatol, etc.

The products of decomposition evolved by the aerobic bacteria upon the surface are generally more simple in character, and consist mainly of carbon dioxide and ammonia.

ANTISEPTICS, GERMICIDES, AND DISINFECTANTS.

Certain substances prevent the growth of micro-organisms. If they do so in such a manner as to permit the organisms to grow again when removed from the restraining influence and sown on a suitable medium, they are called antiseptics; if they do not, and if they also remove their capacity to infect a susceptible living animal, they are called germicides, or disinfectants. In most cases, but not in all, an antiseptic is merely a germicide, or disinfectant, in a dilute condition. In the following pages we shall allude to both classes of substances as disinfectants.

Among the large number of chemical compounds that have well-marked germicidal or antiseptic powers, depending upon the strength and conditions under which they are used, are the following: The free acids and alkalies, chlorine, bromine, iodine, ozone, hydroxyl, sulphurous acid, hypochlorites, sulphites, the salts of mercury, zinc, and copper, boric acid, fluorides, the manganates and permanganates, carbolic and salicylic acids, chloroform, iodoform, formic aldehyde (formalin), chinosol, etc. In addition to these, many essential oils, and a vast number of organic compounds, particularly of the aromatic series, have been credited by various investigators as having more or less marked germicidal or antiseptic powers.

The examination of a substance for disinfectant capacity is an extremely complex matter. It has not always been recognised as such; and no department of bacteriological inquiry is more encumbered with inconclusive researches than the investigation of disinfectants. The conditions which determine the death of an organism in the presence of foreign substances, or its cessation of growth, vary not only for different species, but also for different races of

apparently the same species, according to their previous history, and for a single race at various ages. They are quite different for developed organisms and for spores ; and for each they may vary according as the organism is wet or dried. The capacity to grow on media after contact with inhibitory substances varies with the medium, and in particular may differ as between culture media and living animals. The number of organisms which can be disinfected by a given quantity of disinfectant is limited. The action of the disinfectant is influenced by the temperature. These sources of variation, if ignored, are naturally so many sources of error in any generalisations from individual experiments on antiseptic or disinfectant action. For any practical purpose, however, there are other complications. An organism occurs in various environments, which may sometimes be protective ; and substances capable of affecting the organism may or may not affect the bodies in its immediate neighbourhood, and produce results on the joint inoculation of the organism with its accidental envelope for the time being, which would not be produced if the envelope were removed. The inhibitory substance may adhere either to the organism itself or to its envelope, and on inoculation prevent growth, when in Nature the inhibitory substance might be ultimately removed, and the organism resume its vegetative capacity and its original virulence.

Almost every one of these factors exercises an influence on every disinfection experiment. But though we know that variations in them may produce varying results in the action of a disinfectant, we cannot at present say what extent of variations in the conditions will produce a particular variation of the result. Thus, if a substance be found, with all the conditions carefully noted, to exercise a disinfectant action in a certain strength, no general

inference can be directly made as to the result that it will give in altered conditions of experiment or in practical use. It is unfortunate that, in the majority of investigations which have hitherto been made, the conditions of experiment have varied to such an extent as to make it difficult to derive any conclusion from a comparison of their results. In consequence, the most various statements are made as to the disinfectant action of even ordinary substances; and there can be no doubt that many substances are used as disinfectants in strengths which exercise little or no action. It would, therefore, be of doubtful utility to describe the large amount of experiments which have been made up to now.

The collation of existing results would involve a minute examination of the differences between the conditions of each set of experiments, and absorb an amount of space and time disproportionate to the result; and it is probable that in the near future they will undergo substantial revision. It is extremely desirable that the student should realise for himself some method of at least elementary examination which can be applied to any substance which may be suggested as affording disinfectant action. For this purpose it is indispensable to treat separately the various factors which in practice combine to affect the action of the disinfectant. Thus, in practical experience organisms are seldom found without some particles to which they are attached, or by which they are surrounded. The presence of these particles is liable to exercise an important action upon the disinfectant, but their absence still leaves the organism undestroyed. The real question to be determined in the examination of a disinfectant is, therefore, the strength and time of exposure which will enable it to kill organisms in the presence of a relatively definite proportion of standard extraneous matter. Now,

the resistance of organisms varies in the way which we have indicated; and if the resistance of organisms from a particular culture—say of anthrax—were examined, the result would not alone fail to be necessarily true of other organisms, such as typhoid, but would probably not be correct for the other races of anthrax, or for the same culture at a later stage, or in altered conditions of dryness. There is at present no means available for defining an exact standard by which the resistance of organisms to disinfection can be measured. In order, therefore, to obtain some trustworthy datum as to the action of a disinfectant upon a given species of organisms, it is desirable, at the same time as observations are made upon the disinfectant under examination, to determine the strength and time of exposure required for the disinfection of the particular race on which the examination is conducted when subjected to other common disinfectants. For this purpose it is most convenient to expose the organisms to 1 in 1,000 solution of perchloride of mercury, and to a 5 per cent. solution of carbolic acid, both at a temperature of about 15° C., and to water or steam at 100° C. The times of exposure respectively necessary for disinfection by these three means will be serviceable data for estimating the degree of resistance offered by the particular specimens used. In the exposure of 100° C., care must be taken that that temperature is exactly reached, as a part of a mass of water may be at boiling-point while other parts are substantially below it. The method of exposure to the perchloride and carbolic solutions is the same as that next recommended for use with the disinfectant under examination, except that the strength of solution, and not the time of exposure, is kept constant.

It is more convenient, however, to determine the degree of concentration in which the disinfectant under examina-

tion will exercise disinfectant action within a standard time. It may sometimes be necessary also to determine the times in which disinfection can be effected by dilutions which are incapable of disinfecting within the standard time; this, however, is not usually of great importance for practical purposes, if the standard time be such as to be reasonably practicable in ordinary conditions of working. The exposure which represents most fairly and safely that which is practicable for the majority of purposes may be taken at ten minutes.

It is preferable in these determinations to use a measured quantity—say 4·5 c.c. of a twenty-four hours' culture at 37° C. of standard broth solution. In exact determinations it is desirable to count the number of organisms present; but serviceable results may be obtained without this precaution. The use of broth in this way unquestionably is liable to affect unfavourably those disinfectants which are liable to be decomposed by the substances which it contains. On the other hand, these substances are such as are extremely liable to occur in nature; and it is in most cases preferable that the action of the disinfectant on the mixture of organisms and organic matters should be jointly determined, rather than that any risk should be incurred of exposing the organism in a non-nutrient medium capable itself of exercising a detrimental action upon its resistance. It is certain that results so obtained must be for all practical purposes as safe as, and may be safer than, those which would be had by using emulsions of bacterial growths upon agar cultures in sterile distilled water.

In examining disinfectants soluble in water, standard solutions of the various strengths which it is proposed to apply, usually from 5 per cent. to ·005 per cent., are prepared, and 0·5 c.c. of each is added to the broth tubes, thus giving a disinfectant strength of one-tenth of the strength of the

respective standard solutions. These are allowed to remain at the temperature of the room, preferably as near 15° C. as possible. At the end of the ten minutes a loopful is inoculated into a flask containing 50 c.c. of broth, which is incubated at 37° C. and kept under observation for at least ten days. In each of these processes care must be taken to agitate the mixture so as to thoroughly mix the liquids, and any small bodies of growth or broth substance must either be completely rubbed down till they are not perceptible as solids, or else must be filtered off through sterile slag-wool. Those flasks which remain limpid are those in which the organisms have been killed, and the strengths of disinfectant necessary for use with the particular organisms examined is therefore determined. In the examination of the effect of a disinfectant on spore-bearing organisms, the experiments should be repeated with spores carefully dried *in vacuo* for some days and suspended in sterile water. In this case the number of organisms per c.c. in the suspension should be noted, and the process then conducted as before, 4·5 c.c. of suspension being substituted for the broth culture. The object of using so large a quantity of broth is to avoid the inhibition of growth of the organisms through the presence of that quantity of disinfectant which is carried over in the loopful. It has been shown that even in great dilutions some disinfectants will exercise this inhibitive action. Thus, for instance, a solution of 1 in 3,000,000 of perchloride of mercury is stated to have done so. It would not be conclusive to use a smaller quantity of broth instead of the 50 c.c., and to transfer to an equal quantity containing living organisms from the original culture a second loopful from the disinfectant solution as a control, because the organisms which have been in contact with the disinfectant solution may have been attenuated in their resistance without having been entirely disinfected;

and in that case the disinfectant contained in the loopful might fail to restrain the growth of the unattenuated organisms, and yet might be capable of doing so in the case of those which had been treated with disinfectant. In the process which we suggest, 50 c.c. of the original culture will contain only a loopful of disinfectant solution; and supposing, for example, that such solution was as strong as 5 per cent., there would therefore be a total proportion of 1 to 500,000 of disinfectant, an amount which on previous knowledge would be unlikely to arrest growth when composed of any disinfectant which requires as much as 5 per cent. for germicidal action. Similarly, if a dilution of 1 to 500 of a substance having the same disinfectant value as perchloride of mercury were employed, it would be present in the 50 c.c. of broth in the proportion of 1 to 12,500,000.

Instead of using broth flasks for observing the result previously obtained from the action of the disinfectant, we have found in practice that it is safe to use streak cultures on gelatine tubes, making three strokes on each tube in the same way as in the examination of membrane for the Klebs-Löffler bacillus. The probable reason why this method is satisfactory is that in the passage of the needle-point over the gelatine the organisms have the best possible chance of being at all events at some points deposited out of contact with the disinfectant; and in practice where the disinfection has failed, we usually obtain discontinuous growths.

In some cases a disinfectant has to be used under conditions where an exposure of ten minutes would be inconvenient or impracticable, as, for instance, in the disinfection of the hands of surgeons. In examining a disinfectant for such purpose, the standard time must be fixed at fifteen or thirty seconds, as the case may be.

An alternative method, which has been considerably used, is to impregnate silk threads in an emulsion of organisms,

either in broth or in sterile distilled water, and to inoculate these threads, usually after drying, into solutions of the disinfectant. This method is unsatisfactory, in that it affords no means of maintaining a standard relation between the number of organisms and the quantity of disinfectant, because the penetration of a disinfectant into the depths of the thread has been found to be notably irregular, and because traces of the disinfectant are extremely liable to adhere to the thread. Endeavours have been made to avoid the difficulty by washing the threads in sterile water until it is to be presumed that the disinfectant has disappeared. The presumption, however, is very apt to fail. With some disinfectants it is possible to form by the addition of a substance possessing no disinfectant capacity an inert insoluble compound; and attempts have been made to avoid the transfer of disinfectant with the organisms on the thread by applying such treatment. For example, it has been usual to wash the threads exposed to perchloride of mercury with ammonium sulphide. Irregular penetration, however, of the disinfectant makes it quite possible that some portions may not be reached by this process. The objections to this method are to a considerable degree removed when threads of slag-wool are used instead of silk.

It is found that the action of a disinfectant is enhanced in most cases by its application at a higher temperature than 15° C., even when that temperature is below such as would not itself injure the organism. It is also found in many cases that two separate disinfectants exercise a more powerful effect, and work in much weaker solutions than either of them separately. The action, however, of a disinfectant may be impeded by other substances in which it is conveyed. For instance, the presence of soap, which in itself possesses some disinfectant capacity, is liable to impede the action of mercuric perchloride; and most

ointments, with the exception of those in which 'lanolin' forms the base, reduce the disinfectant action of most substances which may be conveyed in them.

For certain purposes disinfection has been attempted by the use of solid substances possessing very slight solubility, or by inert solids steeped in disinfectant solution and dried. It is obviously a condition of disinfectant action that the substances should come in contact with every organism; and when the size of the organism is considered, it will be seen that the difficulties of disinfection are considerably increased by dispensing with the liquid form. In practice it is safe to say that nothing more than deodorant and mildly antiseptic effect can be obtained from such substances.

Many vapours have a well-marked disinfectant action. It may, however, be stated broadly that, until some means of obtaining an equal diffusion of a vapour in atmospheric air can be provided, the use of disinfectant in the form of vapour or gas must be largely illusory. A considerable number of experiments have been made demonstrating the action of vapours in glass bells and under laboratory conditions; but such observations avoid the difficulty of diffusion of vapours and gases in general into air to a much larger extent than is possible in practice.

CHAPTER XIII.

BACTERIOLOGICAL EXAMINATION OF WATER, FILTERS, MILK, AIR, SOIL, ETC.

The bacteriological examination of water—The nature and number of the organisms found in water—Determination of the number of micro-organisms in water—Examination for sewage bacteria—Isolation of the typhoid bacillus from water—Inhibition by phenol—Resistance of the typhoid and colon bacillus to phenol—Elsner's method—Other methods—Isolation of the cholera bacillus from water—Examination of filters—Examination of milk—Number of bacteria found in milk—Tuberculous milk—Milk diseases—Blue, red, yellow, bitter, stringy, soapy milk, etc.—The organisms producing these diseased conditions—Necessity for improved sanitary control of dairies—Examination of air—Number of bacteria in the air—Sewer air—Filtration of air—Examination of air by Hesse's and other methods—Examination of soil—Number of micro-organisms found in the soil—Methods of bacteriological examination of soil.

THE BACTERIOLOGICAL EXAMINATION OF WATER.

All natural waters necessarily contain micro-organisms, as they are constantly being carried into it by air-currents, and by the drainage from land-surfaces. It is only in water from deep artesian wells and deep-seated springs that organisms are very few in number, and it is very rare even in these to find them entirely absent. The number and variety of the bacteria in water depends upon several conditions, such as the amount of organic matter in the water, the temperature, depth, whether running or stagnant, pollution, source, etc.

Water forms the most natural vehicle for the distribution

of bacteria, but the number contained therein varies very much with the source of the water. In stagnant water, such as is found in brooks and small ponds, the number of micro-organisms present is always very great. The comparatively pure water of large lakes and upland streams often contains many bacteria, but these are always harmless saprophytes, which find their normal habitat in such waters. Thus, in the purest upland streams and lakes we frequently find that the number of bacteria in 1 c.c. is under 100, while in town sewage are many millions in the same volume. In ordinary rivers the number is generally between 1,000 and 100,000 per c.c. In the case of waters from deep-seated springs, the presence of more than 100 organisms per c.c. is conclusive evidence that the water has undergone some contamination with surface-water. Micro-organisms are also found, although not in great numbers, in rain-water, hail, snow, and even in the ice of glaciers. The water-supplies of large towns come for the most part either from rivers or lakes, with supplementary supplies from wells. Many of these water-supplies under ordinary circumstances may be satisfactory, but there is always the danger of sewage-pollution. It must not be forgotten that all polluted waters have a natural tendency to purify themselves if exposed to the air. As regards the nature of the organisms found in natural waters, they are for the most part bacilli, micrococci being somewhat rare, while spirilla are not unfrequently found. About 240 species have already been discovered in water, the majority being harmless saprophytes, although many pathogenic species are also found. As has already been pointed out, typhoid and cholera are essentially water-borne diseases.

In many of the recorded cases of water-borne typhoid and cholera, the amount of organic matter accompanying the specific pollution was so extremely small that the water-

supplies have been repeatedly proved by chemical analysis to be of high organic purity. Moreover, it has been shown that the organisms which are the cause of typhoid fever and cholera may, when introduced into potable water of good quality, not only retain their vitality for a considerable period of time, but may multiply almost indefinitely. Therefore the slightest contamination with the alvine discharges from a case of typhoid fever or cholera may serve to render dangerous millions of gallons of drinking-water. Thus it will be seen that the virulence of contaminated water is not necessarily dependent upon the organic impurity of the water, but upon the specific pollution.

A very important point to keep in view in the bacteriological examination of water is the great increase in the number of organisms which takes place on keeping the samples for a short time. Frankland states that a pure water containing, say, 5 organisms per c.c. when freshly drawn, may, even if kept in a sterile flask free from aërial contamination, contain after a few days perhaps 500,000 in the same volume—or, in other words, as many as are found in slightly-diluted sewage. In fact, it is precisely these purest waters, which initially contain only a very small number of bacteria, that exhibit this remarkable phenomenon of multiplication in the most pronounced manner. Less pure waters, such as those of ordinary rivers, for instance, and which contain initially a large number of bacteria (*e.g.*, 20,000 in 1 c.c.) exhibit, when similarly treated, a much less conspicuous increase in their bacterial population. He also points out, however, that whilst in sewage the number of organisms only gradually diminishes, in these pure waters 'after the rapid increase in numbers follows a correspondingly rapid decline, so that the numbers again fall below those found in impure surface-waters.' The above facts must be constantly before

one when interpreting the results yielded by the bacteriological examination of a sample of water.

A very large number of results showing the number of bacteria contained in the various water-supplies of large towns, both in England and abroad, have been published by various investigators, but they have but little practical importance. The following table is of interest. It contains some results recently obtained by Frankland on the water of the rivers Thames and Lea, both before and after filtration, during twelve months. The results are of interest, as showing the monthly variations during the year of the bacterial contents of the water supplied by the London water companies. They also show the great value a bacteriological examination of a water has in showing if the filter-beds are working efficiently, as it has been shown that all the bacteria can be removed from even a very impure water by proper filtration. The following table shows the number of organisms per c.c.

Name of Supply.	*Jan.*	*Feb.*	*March.*	*April.*	*May.*	*June.*
THAMES.						
Thames Water, unfiltered	92,000	40,000	66,000	13,000	1,900	3,500
Chelsea	127	152	54	38	43	63
West Middlesex	60	146	408	158	71	56
Southwark	177	766	742	47	47	24
Grand Junction	90	349	617	56	77	40
Lambeth	189	820	321	157	64	140
LEA.						
Lea Water, unfiltered	31,000	26,000	63,000	84,000	1,124	7,000
New River	27	90	169	77	37	60
East London	2,038	780	359	193	209	266
DEEP WELLS.						
Bath	6	47	6	33	7	17
Garden	5	19	8	4	27	71
New	12	4	5	7	8	20
Supply	55	81	15	69	139	219

Name of Supply.	*July.*	*August.*	*Sept.*	*Oct.*	*Nov.*	*Dec.*
THAMES.						
Thames Water, unfiltered -	1,070	3,000	1,740	1,130	11,700	10,600
Chelsea - -	37	32	36	14	82	71
West Middlesex	27	11	26	33	31	16
Southwark -	35	27	106	35	167	136
Grand Junction	15	4	20	16	25	208
Lambeth - -	55	33	92	27	126	151
LEA.						
Lea Water, unfiltered -	2,190	2,000	1,670	2,310	57,500	4,400
New River -	11	13	—	15	70	91
East London -	253	57	64	63	49	141
DEEP WELLS.						
Bath - -	8	—	8	4	34	
Garden - -	5	—	10	9	18	
New - -	4	3	—	96	19	
Supply - -	32	42	52	55	54	63

When examining water bacteriologically, especial care is necessary when taking a sample for analysis, in order to prevent outside contamination and to secure an average sample. About 50 c.c. should be collected in a sterile bottle or flask, which should be contained in sterile metal cases. Stoppered bottles should always be used, and all containing vessels should have been previously sterilised in the hot-air steriliser for three hours at 150° C. The most satisfactory receptacles for water intended for bacteriological examination are exhausted and sterile glass bulbs, the necks of which have been drawn out to a point. This fine point is broken with a pair of sterile forceps under the surface of the water, and after the latter has rushed in and filled the vacuum, the bulb is sealed up again with the aid of a spirit-lamp.

In collecting samples from rivers, lakes, tanks, or ponds, it is best to take the bottle out of the sterile metal case, take out the stopper, and hold the neck of the bottle

between a pair of sterile forceps or tongs. Wholly immerse the bottle under the surface of the water until filled; the bottle is then tightly stoppered, wiped with a clean duster, and returned to its case. In some cases it will be found more convenient to draw up a sample with a sterile pipette plugged with cotton wool, from which the sample bottle is filled. When collecting from a tap, the water should be allowed to run for at least ten minutes before taking a sample. In the case of well-waters it is sometimes necessary to draw off the water for some hours or more before proceeding to take a sample. If it is thought desirable to take a sample from definite depths, this can be done by the employment of small vacuum bulbs similar to those described above, which are let down to the necessary depth by means of a weighted wire or string; the drawn-out point of the bulb is then broken by a suitable mechanical arrangement.

All samples intended for bacteriological examination should be examined at once. If this is impossible, they should be kept in an ice-chamber, in order not to have the results vitiated by the multiplication of the contained organisms.

Determination of the Number of Bacteria.—From 0·02 to 0·5 c.c. of the sample, dependent upon its purity, is withdrawn by means of a small sterile graduated pipette, and added to a tube containing melted sterile nutrient gelatine at a temperature of about 27° C. The cotton-wool plug of the tube is then replaced, and the contents of the tube gently agitated, so as to thoroughly mix the contents. The plug is again withdrawn, and the contents of the tube poured on to a sterile glass plate, or into a Petri dish, as described on p. 70.

It is even more satisfactory to drop the proper quantity of water with due precautions into the molten gelatine (which should not have a temperature above 27° C.) con-

tained in a Petri dish, provided due care is taken to thoroughly mix the sample with the molten nutrient medium.

As attempting to measure a volume of less than 0·1 c.c. is not a satisfactory operation, it is best, in the case of a water suspected to contain a large number of bacteria, to dilute the water 50 or 100 or more times, as follows, before proceeding to the examination. Small sterile flasks containing about 49 c.c. of sterile distilled or, better, sterile natural water, receive 1 c.c. of the water under examination. This is well mixed, and again 1 c.c. of this first attenuation is taken and introduced into another flask until the degree of dilution is considered sufficient. Plates are then made from 1 c.c. of the various attenuations. The plates are then allowed to stand at a temperature of about 22° C., and examined daily. It is usual to count the colonies on the third or fourth day from starting the plate.

The accuracy of the results obtained depends to a very large extent upon the care with which the organisms are distributed through the nutrient medium. Care should also be taken that the original sample of water is well and thoroughly shaken, to evenly distribute the organisms contained therein, before withdrawing the quantity for the examination. A number of plates containing varying quantities of the sample should always be taken. Great care and practice are required, so as not to have too many organisms on a plate. A good plan is to aim at having about 100 colonies on each plate.

Counting the Colonies.—This is done by means of Wolffhügel's apparatus. This consists essentially of a glass plate divided into squares, each a centimetre square. Some of these squares are subdivided. The plate or dish is laid under this scale, and the number of organisms present is found by counting the number of colonies in a few of the

squares; an average is then taken, and the number of organisms present thus calculated. With a little practice very close approximations are to be obtained with this apparatus.

In the bacteriological examination of drinking-water it is very important to note the character of the species

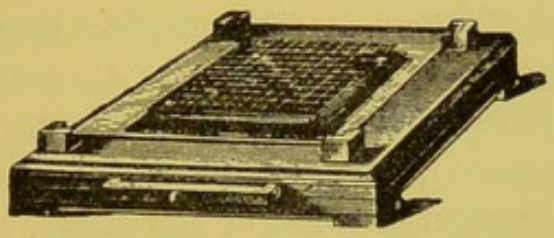


Fig. 30.—Wolffhügel's Apparatus.

present. It is as well to know approximately the number of organisms which liquefy the gelatine. These kinds are almost invariably putrefaction bacteria, and can only grow where there is plenty of organic matter, they being almost absent from pure waters.

The time and labour involved in ascertaining the characters and number of the species of micro-organisms by means of subcultures renders this operation prohibitive in the ordinary bacteriological examination of a drinking-water. This generally resolves itself into the enumeration of the bacteria present, and an examination for a specific pathogenic organism, typhoid or cholera, as the case may be.

With reference to the general question of the bacteriological examination of drinking-water, much information as to the character of a water is gained by incubating a small quantity of the sample at blood-heat for twenty-four hours. The number of organisms is then ascertained by an ordinary gelatine plate culture. The number of organisms so found is compared with the number of organisms

found by a direct gelatine plate culture, which is made on the water immediately upon the receipt of the sample. If a sample of water is polluted with sewage, a great increase in the number of the organisms will be found to have taken place as the result of the incubation. All the organisms normally present in fæces grow and multiply vigorously at blood-heat, whereas this temperature is fatal to the majority of the common water bacteria; therefore a corresponding decrease in the number of the organisms will be found to have taken place in a pure water.

A more convenient plan is to prepare an agar-agar plate culture with a fraction of a c.c. of the water. The resulting plate is incubated at blood-heat for thirty-six hours. This method is the most satisfactory, as it has the advantage that the actual number of the micro-organisms that will grow at blood-heat is ascertained.

The following examples show the value of these two methods:

	Polluted Surface-well Waters.			*Waters of Average Quality.*	
	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)
Approximate number of organisms per c.c. in the original water, as determined by a gelatine plate culture -	800	1,050	1,400	180	270
Number of organisms per c.c. appearing on an agar-agar plate colony, after incubating at blood-heat for twenty-four hours -	220	180	350	10	5
Approximate number of organisms per c.c., after incubating the water at blood-heat, the organisms then being determined by an agar-agar plate -	800,000	uncountable	uncountable		

The majority of the organisms from a polluted water which grow at blood-heat will be found on subculturing to be the colon bacillus. The presence of the *B. coli communis* in small numbers can hardly be considered as good evidence of sewage-pollution, but when it is found in large numbers it is fair to conclude this to be the case.

The colon bacillus is spoken of by Klein* 'as a certain index of fæcal pollution.' The recent researches of Dr. A. A. Kanthack, however, show that the colon bacillus is much more widely distributed than was formerly supposed, being found by him in pure water, saliva, dust, etc., so that the generally prevailing idea that its presence necessarily signifies excretal pollution is erroneous. The widespread distribution of the *B. coli communis* has, however, long been known to bacteriologists, and it is comparatively rare to find it absent from waters of high degree of purity that have been exposed to the air.

The Isolation of the Typhoid Bacillus from Water.—In waters that have been very copiously contaminated with sewage there is no great difficulty in detecting the typhoid or colon bacillus, if present; but it is necessary to bear in mind that usually, when drinking-water has suffered sewage-pollution, the amount of the pollution is relatively very minute when compared with the great bulk of the water-supply. The contamination of water by sewage is, moreover, in the majority of cases, of an intermittent nature.

When such waters are examined, as large a quantity as 1 c.c. or more of the water may be 'plate-cultured,' and even then it is easy to miss the colon bacillus, not to speak of the typhoid bacillus.

In order to isolate the *B. typhosus* suspected to be present in a sample of water, it is necessary to submit a large

* The Twenty-third Annual Report of the Local Government Board —Supplement containing the Medical Officer's Report, p. 67.

volume of the water to examination. This object is attained by concentrating the bacterial contents of the water by passing 1,000 to 3,000 c.c. or more of the sample through a small sterile Pasteur-Chamberland filter. By this treatment all the bacteria in the water are retained on the outer surface of the filter. The particulate matter thus retained is then brushed off the outer coating of the filter with a sterile brush or sponge into about 20 c.c. of sterile distilled water. One c.c. of this concentration, which contains the particulate matter representing from 50 to 150 c.c. of the original water, is then immediately submitted to plate culture by one of the undermentioned methods, to isolate the colon bacillus and also the *B. typhosus*, if present.

1. Inhibition by Means of Phenol.—The *B. typhosus* and the *B. coli communis* are among the limited number of micro-organisms which will grow in the presence of small quantities of phenol, which addition retards or inhibits the common water bacteria, such as the *B. fluorescens liquefaciens*, *Proteus vulgaris*, *B. mesentericus*, etc., the presence of which would liquefy the gelatine, and by their rapid growth would annihilate the *B. typhosus*, if present. The presence of a small quantity of phenol does not in any way interfere with the growth of the *B. typhosus* or the *B. coli communis*, but exhibits a marked inhibitory effect upon the common water bacteria, and, by the retardation and suppression of these, the colonies of the *B. typhosus* and the *B. coli communis* have a chance and leisure to appear.

The use of phenol for this purpose appears to be due, in the first instance, to Chantemesse and Widal,* who used nutrient gelatine containing 0·25 per cent. of phenol. Thoinot,† a little later, inhibited the growth of organisms other than the typhoid and colon bacilli, by adding 0·25 per cent. of phenol to the water under examination, which

* *Gazette des Hopitaux*, 1887, p. 202. † *Ibid.*, p. 384.

was then incubated at blood-heat and the water afterwards plate-cultured.

As pointed out by Holz, and confirmed by Dunbar, the above authors use a percentage of phenol which altogether prevents the growth of the *B. typhosus*. Dunbar states that 0·12 per cent. of phenol greatly interferes with the growth of the typhoid bacillus, while in the presence of 0·14 per cent. it will not develop at all. He further states that in the presence of small quantities of phenol the colon bacillus presents stronger resemblances to the typhoid bacillus than usual.

To ascertain if the resisting power of cultures of the *B. typhosus* to phenol differed, we tried the following series of experiments on different cultures of the organism, using varying percentages of phenol, with the following results:

	Percentage of Phenol.			
	0·05.	0·10.	0·20.	0·30.
B. typhosus (*a*) - - -	+	−	−	−
,, (*b*) - - -	+	+	−	−
,, (*c*) - - -	+	+	+	−
,, (*d*) - - -	+	+	−	−
B. coli communis - - -	+	+	+	+

Thus, it is seen that the resisting power of the *B. typhosus* to phenol varies with different cultures. The sample marked (*a*), which was freshly isolated from the dejecta from a typhoid case, had less resisting power than other samples which had been subcultured through many generations.

Parietti proposed the use of broth containing both phenol and hydrochloric acid to eliminate the common water organisms. He takes advantage of the fact that the typhoid and colon bacillus will grow in a slightly acid medium, whereas the majority of other organisms will not.

Parietti's method is as follows: The following solution is prepared: Five grammes of phenol and 4 grammes of pure

hydrochloric acid are added to 100 c.c. of distilled water. From 0·1 to 0·3 c.c. of this solution is added to a series of test-tubes containing 10 c.c. of sterile nutrient broth (=0·05 to 0·15 per cent. of phenol). The tubes are then incubated at blood-heat for twenty-four hours, to destroy any stray organisms that may have gained access to the tubes. From 0·1 to 0·5 of a c.c. of the water under examination is then added to the tubes, the contents well mixed, and the tubes again returned to the incubator. If, after twenty-four hours' incubating at blood-heat, any of the tubes appear to be turbid, they are submitted to ordinary plate-cultivation, and the resulting colonies carefully examined in sub-cultures. Frankland states that when only a few typhoid bacilli are present, the incubation must be prolonged for forty-eight or even seventy-two hours.

We have found the above method to be a very reliable one, although somewhat tedious. In practice, however, we prefer to use simple carbol-gelatine containing 0·05 per cent. of phenol. This quantity is quite sufficient to restrain the growth of liquefying organisms, and, moreover, with this quantity there is no danger of losing the typhoid bacillus if it is present.

2. Elsner's Method.—Dr. Elsner, of Berlin, has recently published* the results of an investigation made to ascertain the possibility of an early recognition of enteric fever by the bacteriological examination of the stools. He has been able to recognise the Eberth-Gaffky bacillus in some cases in as short a time as forty-eight hours. Dr. Elsner went over the existing methods for the separation of the *B. typhosus* and *coli*, with no better results than have previously been obtained. In all cases but one he found that either persistent organisms other than those sought to be isolated would grow to a sufficient extent to spoil the plate (*e.g.*,

* *Zeitschr. f. Hyg.*, xxi., 1.

B. proteus or *ramosus*), or else the *B. coli* would develop to an extent capable of preventing the recognition of the typhoid bacillus. The exception was slightly acid potato-gelatine, containing 1 per cent. of iodide of potassium. The process recommended is to boil potato-decoction (500 grammes to 1 litre of water) with 10 per cent. of gelatine. Sufficient of a 2 per cent. solution of sodium hydrate is added till only a faint acidity remains, litmus being used as indicator.

Elsner found that the *B. proteus* and *ramosus*, which always grow on carbolised gelatine, either never occurred on this medium, or were rapidly overgrown by the colon bacillus. The *B. coli* grew in twenty-four hours, presenting the usual appearance of that organism on acid media; the *B. typhosus* was scarcely visible in twenty-four hours, but in forty-eight hours appeared in small, shining, very finely-granulated colonies like little drops of water, which contrasted strongly with the larger coarsely-granulated, brownish colonies of the colon bacillus. The *B. coli* only acquired the appearance of the typhoid colonies when a great number of the organisms were present, and many, therefore, grew without finding room for their proper development. In plates made with weaker inoculations, it is impossible to mistake one bacillus for the other.

We have used this method with satisfactory results. The colonies of the *B. typhosus* appear more quickly on this medium than on carbol-gelatine, but otherwise this appears to be the only advantage it possesses. A number of other methods for the isolation of the *B. typhosus* and *coli* have been proposed by different investigators. Uffelmann has suggested the addition of 0·1 per cent. of citric acid to the nutrient gelatine, to restrain the growth of the liquefying organisms. Dunbar has found, however, that the amount of citric acid prescribed by Uffelmann is in excess of what the typhoid bacilli are capable of standing. He found that

in many cases, whilst the colon bacillus developed, the growth of the *B. typhosus* was restrained.

Holz has used faintly acid potato-juice, thickened with 10 per cent. of gelatine, with or without the addition of 0·05 per cent. of phenol, with satisfactory results. This method is practically the same as Elsner's, except that the potassium iodide is replaced by phenol. Gasser, Holz, Lyonnet, and others have suggested the use of various media tinted with fuchsine and other aniline dyes. The typhoid and colon bacilli are stated to decolourise, or to cause other changes, in such media where the growth of the colonies occur, whereas other organisms do not possess this property. Little reliance can, however, be placed on these appearances, as we have found other organisms give the same characteristics as the typhoid and colon bacilli.

After a considerable experience in the use of the above methods, we find that the best and most reliable processes to be employed for the isolation of the typhoid bacillus from water are the use of carbol-gelatine (0·05 per cent.), Elsner's method, and Parietti's acid carbolised broth. As soon as the colonies which develop on the carbolised or potato-gelatine become sufficiently advanced they are examined with a lens, and any suspicious colonies are carefully subcultured into faintly alkaline sterile milk-tubes, which are then incubated at 37° C. for thirty-six hours. The milk-tubes are then examined, and any that have become coagulated are rejected, as certainly not typhoid.

From the tubes that have not coagulated the following subcultures are prepared: (*a*) Gelatine 'streak' culture; (*b*) gelatine 'shake' culture; (*c*) broth culture.

The gelatine cultures are kept for three days at a temperature of from 18° to 20° C. The broth-tubes are incubated at blood-heat for the same length of time, and then tested by the indol reaction.

Messrs. Laws and Andrewes* failed, after a most prolonged investigation, to find the typhoid bacillus in the London sewage from the Barking and Crossness outfalls, but they found it present, as would be expected, in the sewage from the Homerton Fever Hospital.

With respect to the question of the detection of the typhoid bacillus in water, we are satisfied that the Eberth-Gaffky bacillus can be, and has actually been, detected and isolated from water, though some of the cases in which it has been reported may rest upon insufficient evidence. We would, however, consider that the discovery of any of the pseudo-typhoid organisms, such as have been already mentioned, should lead to as decided a condemnation of the water as though an organism possessing the precise morphological and cultural characters of the Eberth-Gaffky bacillus were isolated.

While we would not agree with those who would regard the bacteriological examination of water as useless, we still further dissent from the view—if, indeed, it is seriously held by any—that the biological examination can in the smallest degree supplant the chemical analysis of water, which, on account of the valuable data it yields, must always remain an integral part of the examination of potable water.

The most enthusiastic bacteriologist cannot deny that the specific organism may have been present in a given water-supply a week ago, and at the time of examination have disappeared. The incubation period of enteric fever is about fourteen days; so that if a sample of drinking-water were sent for examination when the disease declared itself, it might easily be three weeks since the conveyance of the infection, and during this time the Eberth-Gaffky

* 'Report on the Results of the Investigations on the Micro-Organisms of Sewage,' presented to the London County Council, December, 1894.

bacillus may have been annihilated by the common water bacteria.

Therefore, to say that a given water was safe because no specific organism was demonstrable, and to ignore the information that a chemical analysis might yield, would be entirely illogical.

The Isolation of the Cholera Bacillus from Water.—The detection of Koch's comma bacillus (*Spirillum choleræ Asiaticæ*) in water, as in the case of the typhoid bacillus, is a matter of some difficulty, as this organism is rapidly overgrown by the ordinary water bacteria. In the examination of suspected water-supplies, the best method to employ for the detection of this organism is to take advantage of the fact first noted by Dunham, that the cholera spirillum multiplies with great rapidity in alkaline saline peptone solution. The suspected water is examined as follows: To 100 c.c. of the water are added 1 gramme each of pure peptone and common salt; the mixture is made faintly alkaline with sodium carbonate, and then incubated at 37° C. At intervals of ten, fifteen, and twenty hours respectively, cover-glass preparations are prepared from the top of the liquid; these are then microscopically examined for spirilla. At the same time agar plates are prepared, and incubated at blood-heat. Any colonies that appear which resemble the cholera spirillum are examined microscopically; if the organisms are comma-shaped, they are at once subcultured into broth and other media. The broth-tubes after incubation are tested for the indol reaction, and if possible by animal inoculation.

It is well known that many impure, especially sewage-contaminated waters, contain spirilla and comma-shaped bacteria, many of which strongly resemble the cholera organism in many ways; care must therefore be taken that none of these are mistaken for the true cholera organism. None of these spirilla forms, however, give the indol

reaction, and Koch is of opinion that the presence of the cholera bacillus in the water is proved if comma-shaped organisms are found which exhibit the indol reaction, and which give rise to the characteristic symptoms on inoculation into the peritoneum of guinea-pigs.

BACTERIOLOGICAL EXAMINATION OF FILTERS.

When chemical analysis was the only means at command for examining water, it was found that in a majority of cases those waters which had been statistically convicted of spreading disease contained an excess of organic matter. Hence it was inferred that the organic matter was the cause of the disease; and filters were constructed of carbon, asbestos, natural stone, spongy iron, and similar materials, for the purpose of removing this excess of organic matter. It was found that they all did so in a greater or less degree, but that their efficiency in this respect decreased on use, and ultimately disappeared until the filtering medium had been renewed or cleansed. With precisely similar results, preparations of these materials, such as silicated carbon, manganous carbon, magnetic iron, and the like, were tried for the same purpose, and many filters composed of successive strata of several of these were constructed. It ultimately became known that the diseases caused by water were due to micro-organisms, and that the presence of excess of organic matter in most waters which were dangerous was due to the fact that the microbe was generally either conveyed through excreta containing soluble organic substances, or best nourished in waters of such composition. The filters already in use were thereupon assumed to act by arresting the microbes contained in the water. This assumption was after a time supported by experiment, in which a small quantity of infected water was

passed through the filter, and the filtrate was found to be sterile. Further investigation showed that this ceased to be the case when the filtration was continued for a few hours or less instead of a few minutes. It was found that in such case the filtrate contained the same organisms as the unfiltered water; and the sterility of the earlier filtrates was accordingly due to the circumstance that they had been examined before sufficient time had been allowed for the organisms to be washed through the filter. It was also found that the chemical matters arrested by the filter temporarily arrested a portion of the organisms, and served as a suitable culture-ground for such organisms, which survived and multiplied for considerable periods in the filter before being ultimately washed through. In consequence, the number of organisms became after a short time much larger in the filtrate than in the unfiltered water. Filters once polluted with the cholera or typhoid bacillus were also found to convey the bacillus to sterile water passed through them at considerable periods—up to six weeks or more—after pollution. This fact has been responsible for several epidemics, such as that of Lucknow in 1894, in which, out of 646 officers and men in the East Lancashire Regiment, 143 were attacked by cholera, and 92 died. This epidemic was conclusively traced to the infection of the barrack-room filter by the cholera microbe.

Sand-filtration.—The working of sand-filters on a large scale depends on the facts described above. A sand filter-bed consists of a layer of sand from 2 to 4 feet deep, supported on gravel. The fineness of the grains of sand, the depth of the filter, and the rate of filtration, all affect the working of the filter in the removal of organisms. The coefficients given for safe working are filtration through a sand-layer not less than 30 centimetres thick, at a rate not exceeding 100 millimetres per hour, and giving a filtrate

containing not more than 100 bacteria per c.c. These coefficients, however, take no account of the class of sand used or character of water filtered, and they are no longer regarded as trustworthy. When a filter-bed is freshly constructed, organisms are washed through it with great rapidity, but after a certain quantity of water has passed through, or the water has been allowed to stand upon it for a certain time, a slimy coating of detritus and bacteria is formed on the surface. If water is slowly passed through the filter when this coating has been formed to a sufficient extent, which will occur after a period varying mainly with the composition of the water, the majority of the bacteria will be retained by this surface, either by sticking to it or by being strained off. The increasing thickness of this coating will reduce the velocity with which the water passes, and at the same time some of the bacteria will tend to grow downwards into the lower strata of the filter, and, if the process were continued long enough, would be washed through into the filtrate, and ultimately become more numerous there than in the unfiltered water. The increasing resistance to the passage of water would also make it necessary for the pressure to be increased, which would in this class of filter assist the passage of organisms. It is therefore necessary in the working of sand-filters to run the filtrate of each bed to waste, or to permit a body of water to stand on the filter without filtration, until a sufficient coating has been formed to arrest organisms; to stop filtration when the deposit has increased to such extent as to threaten the renewed passage of organisms; and to remove the upper or filtering layer, and permit a fresh deposit to be formed. The indication for scraping usually adopted is that the filter-bed no longer passes the required quantity of water under the maximum permissible head. The sufficiency of this practice has not been clearly shown.

No general rule can be given for the depth to which the top layer must be removed, as it varies with the nature of the water and sand, temperature, etc.

The ordinary rules for the selection of the epochs for starting and arresting the filters, and the operation of removing the upper surface, require considerable experience and judgment; and it frequently happens that through carelessness or unavoidable mistake the filtration is imperfect. Thus, in 1894 the filters at Nietleben, Altona, Hamburg, and Stettin, being over 10 per cent. of the total sand-filters in use in Germany, where great attention has been given to the subject, passed the cholera organism, and permitted epidemics in their towns. In the same year the typhoid organism, of which the detection was difficult and uncertain with the means then at disposal, was, nevertheless, found beyond doubt in the Berlin water-mains.

Bacterial Filters.—It is obvious that for the purpose of bacteriological investigation such appliances as have been described are practically useless. Pasteur and Chamberland investigated a large number of earthen materials, beginning with ordinary biscuit porcelain. They found them to present very different degrees of resistance to the passage of bacteria. The difference did not appear to correspond to either the density of the material or the rate of filtration, in many cases a material of closer grain and less rapid output giving worse results than other materials more open in structure and more rapid in filtration. They ultimately found that the best results were obtained with a particular mixture of earths prepared with a special manipulation; and it is these substances which, when made in the well-known cylindrical form, constitute the Pasteur-Chamberland filter. This filter is found to be perfectly trustworthy in the removal of all organisms from liquids; it also retains any particulate matter, such as the

fatty globules from milk. The method of its action has not been determined, but it probably depends on some form of surface attraction, as many of the organisms which are arrested are considerably smaller than the pores of the material. It has been shown by repeated experiments that none of the many forms yet tried of biscuit earthenware, having practically the same appearance and analogous composition, possess the same efficiency as the Pasteur-Chamberland material; but no adequate reason has been discovered for the circumstance. A diagnostic test for the bacterial soundness of the Pasteur-Chamberland tubes is to compress air within them at a pressure of one-half to one atmosphere when the tube has been steeped in water, or is freshly taken from service. If held beneath a body of water, no air will escape from a sound tube; but a stream of bubbles will issue from any spot capable of passing bacteria. This test apparently does not apply to other forms of earthen filters, and for this reason they should not be used for the filtration of serum, or during an epidemic, unless a portion of the filtrate is cultivated, and the bulk retained until it has been proved sterile. This applies particularly to filters made in the Pasteur-Chamberland form, in which a softer material, such as infusorial earth, is used, and a fresh filtering surface is accordingly exposed after each cleaning. Thus, the Berkefeld filter in infusorial earth, of which the tubes may initially be capable of preventing the direct passage of organisms, has a small portion of its outer surface removed each time it is cleaned. The consequence is that, sooner or later, a faulty surface is exposed, and the tube is liable to pass organisms even before the time when it is worn away sufficiently to break. The Pasteur-Chamberland tubes remain unaffected by cleaning or sterilisation for an indefinite period. They may be sterilised by boiling water, or by saturated steam

under pressure; or, alternatively, a suitable liquid disinfectant may be passed through them, with the advantage of dissolving at the same time the whole of the colloid substances deposited in its pores. When used for the filtration of blood serum, it is found convenient to allow the mass of blood to drip in ice for forty-eight hours, so as to obtain a serum as free as possible from specks of fibrin; and the serum given will be readily sterilised by filters at a temperature up to 40° to 50° C.

The Bacteriological Examination of Water-filters.—The large majority of water-filters at present in use are incapable of preventing organisms from being washed through into the filtrate. In order to ascertain whether this is the case with any particular filter, it should be sterilised in the steam-steriliser, and water containing known organisms should be passed through it for twenty-four hours. This water and the filter should, during the time of the examination, be maintained at a temperature below 5° C. This will almost invariably prevent any growth or multiplication of the organisms. Samples should be taken immediately after the filtration has begun, and at intervals during the day, and again at the end of twenty-four hours. If they are all sterile, the filter is capable of preventing organisms from being directly washed through. In the case of filters of very great density or depth of filtering medium, it may be necessary to prolong the period of examination beyond the first day; but most ordinary filters which permit organisms to be washed through do so within the first few hours. It must be remembered that it is no advantage for a filter capable of permitting this passage of organisms to postpone it for a day or more, as the organisms will ultimately find their way into the filtrate, and in the meantime are likely in practical use to have increased in numbers.

In the case of water-filters which resist this examination,

and may be taken, therefore, to prevent organisms from being directly washed through, the further examination is a matter of some difficulty, and at the present time can only be conducted inferentially, or by comparison with a standard. The object of such examination is to discover whether pathogenic organisms in water can grow through the walls of the filter; and the difficulty in making the examination is that our information as to the circumstances which favour the multiplication of organisms in water, and which determine the maximum extent to which such multiplication may proceed in natural conditions, is quite incomplete. It is impossible to state of any given water whether it offers the maximum assistance to the growth of organisms that may be found in natural water, or to say whether a specimen under examination is capable of multiplying to the same extent as other specimens of the same organism might multiply in a natural water. In many researches, indeed, in which filters appeared to resist penetration of organisms by growth, it was not even certain whether the organisms under examination could grow in the water at all. The method which must, therefore, be employed is to take water containing known non-pathogenic organisms known to multiply in it at suitable temperatures with sufficient freedom to ultimately penetrate the Pasteur-Chamberland tube, and to examine specimens of the filter of which the efficiency is to be determined simultaneously and with the same water-supply as specimens of the Pasteur tubes themselves. The water must be kept at the optimum temperature, and the filtrates examined periodically. If the filter under examination retains the organisms for as long a time as the Pasteur, it must be considered as possessing the same efficiency. If, on the other hand, it passes the test-organisms before the Pasteur tube will do so, it is less efficient, and must for the present be

considered insufficient for the prevention of infectious disease. There is an extremely large body of evidence to justify the conclusion that the resistance offered by the Pasteur-Chamberland tube is sufficient to prevent the passage of disease organisms from natural water. This evidence has been collected mainly in all parts of the French possessions, and published by the French Government; and since the filters have been introduced into this country and India, similar evidence has arisen. There is, however, no evidence to show that the resistance which it offers exceeds that which is necessary for affording trustworthy protection against water-borne disease. It is, therefore, not possible to accept any filter of less efficiency as affording a trustworthy guarantee against infection. In experiments of this kind, care should be taken to procure several specimens of the filter under examination, and to ascertain that they fairly represent those intended for ordinary use. It is also desirable, when special test-organisms are artificially introduced, to avoid the simultaneous introduction of small quantities of culture material.

It has been found that water and other fluids sterilised by heat may retain a toxic capacity, setting up, for instance, suppuration on inoculation into suitable animals; while the same liquid sterilised by filtration through a Pasteur-Chamberland tube produced no effect. At the present time these phenomena and the conditions which determine them are not sufficiently worked out to make it possible for filters to be adequately examined as to their capacity to produce similar results.

A very full and interesting report by Drs. Woodhead and Cartwright Wood upon the efficiency of the various types of filters in use will be found in the *British Medical Journal*, vol. ii., 1894, pp. 1053, 1118, 1182, 1375, 1486.

THE EXAMINATION OF MILK.

From the fact that milk forms such an excellent nutrient material for the growth of nearly all bacteria, it follows that this article of food is almost invariably contaminated with bacteria from various sources. The milk in the udder of a cow in perfect health is absolutely free from micro-organisms, but when the cows are suffering from disease, the milk as it leaves the udder may contain the tubercle or other pathogenic organisms which may be, and generally are, the specific cause of the particular diseased condition. It is unquestioned that many diseases, such as scarlet fever, typhoid, tuberculosis, diphtheria, etc., are in very many cases conveyed by milk. Sources of infection are to be found in the many insanitary conditions which surround the milk-supplies in many parts of the country. The cow-sheds in which the cows are milked are usually saturated with excremental filth, the animals themselves are kept in a very dirty condition, their hind-quarters and udders are frequently soiled with dejecta, as is also the straw on which the animals stand, which in itself forms an admirable forcing-ground for micro-organisms. Other sources of contamination are want of personal cleanliness on the part of the milkers, and dirty dairy utensils, which are possibly 'cleaned' out with water from a surface-well which is probably polluted with farmyard drainage. Again, other risks of bacterial contamination are introduced by want of proper care and sanitary precautions when consigning the milk to the consumers. The milk is cooled in open 'coolers'; it is sent long railway journeys in loosely-covered churns, and, lastly, is exposed for a considerable period of time on counters in open vessels exposed to all kinds of street dirt and dust.

From the above, which is by no means an overdrawn picture, it is easy to see that many millions of bacteria find their way into the milk-churn, and it is remarkable that, with so many sources of pollution, more epidemics are not traced to the milk-supplies, considering the fact that milk forms the staple article of food of young children. Milk forms such an excellent medium for the growth and multiplication of bacteria that they increase in this medium with excessive rapidity. Dr. Freudenreich examined a sample of milk purchased in Berne, and determined the rate of the multiplication of the microbal contents on keeping the sample at 15·5° C. The sample at starting contained 27,000 organisms per c.c.; these after four hours increased to 34,000 per c.c.; after nine hours the increase was to over 100,000, which became over 4,000,000 after twenty-four hours. S. Rowland, after examining a number of milks purchased in various shops in London, found that they contained on the average 500,000 organisms per c.c. Drs. Stewart and Buchanan Young have recently examined the milk-supply of Edinburgh. Since November, 1894, they have examined three hundred samples of milk from fifty dairies scattered throughout the city. It was found that three hours after milking there were in the winter, on an average, 24,000 bacteria per c.c.; in spring and early summer 44,000; in late summer and autumn 173,000. It was found that in dairies supplied with milk from the country the average number of micro-organisms contained therein five hours after milking was 41,000 per c.c., while in dairies supplied from town cowkeepers the average was 352,000 per c.c. Numerous other investigators have published similar results, which show how universally milk-supplies are bacterially contaminated as the result of the primitive and insanitary methods employed in their collection and storage.

Milk may be a very prolific source of infection in many different ways. For instance, in the case of tuberculosis, the milk may become infected from outside sources, such as dust containing dried-up phthisical sputum. It may and is more frequently directly infected from the animal yielding it suffering from tubercular disease of the milk-glands. Cows with apparently sound udders, but affected with tuberculosis of the lungs, have been known to yield milk containing tubercle bacilli. In Copenhagen and Berlin, where all animals before going to the slaughter-house are examined by experts, the percentage of the oxen and cows affected with tubercular disease, from 1890 to 1893 inclusive, was found to be 17·7 and 15·1 per cent. respectively of the total number examined.* This is in accordance with Hirchberger's observations, who found that 10 per cent. of the cows living in the neighbourhood of towns suffer from tuberculosis, and 50 per cent. of these yield milk containing tubercle bacilli.

Drs. Woodhead and Macfadyen found the tubercle bacillus in six samples of milk out of six hundred samples examined.

The question of the use of tuberculous milk has received much more attention on the Continent than it has in this country. In Denmark a most thorough and complete system of inspection has been instituted with excellent results; cattle found to be tuberculous are at once isolated, and, if necessary, slaughtered and the body destroyed.

The great mortality amongst young children, due to tubercular intestinal affections, is undoubtedly due to the use of milk containing the tubercle bacillus. Delicate children are the most susceptible, as, owing to imperfect nutrition and other causes, the system is unable to resist the attack of the organisms. Brouardel cites a case where five out of fourteen young girls living together in

* Royal Commission on Tuberculosis (1895).

a boarding-school became consumptive subsequent to the daily use of milk from a tuberculous cow.

That the tubercle bacilli occurring in milk are virulent has been proved by subjecting animals to subcutaneous injection, and by feeding them with the infected milk.

Dr. Martin writes*: 'The milk of cows with tuberculosis of the udders possesses a virulence which can only be described as extraordinary. All animals inoculated showed tuberculosis in its most rapid form.' Dr. Woodhead, after investigating the effects of unboiled tubercular milk, speaks in similar terms of this virulence of milk derived from tuberculous udders and inoculated into test animals. These two observers had occasion to use milk from a cow that had tuberculous disease in one quarter only of the udder; and they found the milk from the other three-quarters to be perfectly harmless on inoculation; but the mixed milk from the four teats was to all appearance just as virulent as the milk from the diseased quarter. Butter, skimmed milk, butter-milk, obtained from the milk of a cow having tuberculous udders, all contained tubercle bacilli.

To some extent the chances of infection are reduced in actual practice, as the milk as usually supplied to the consumers is the mixed milk of a herd of cows, whereby a tuberculous milk suffers considerable dilution with the milk from healthy cows; but this dilution, as shown by recent experiments, only reduces the risk of infection, but does not entirely do away with it.

Freudenreich examined twenty-eight samples of mixed milk, and found out of this number four that proved to be virulent when inoculated into guinea-pigs. Two of these samples came from dairies where from twenty to thirty cows were kept, and where in each case only one cow was suspected to be affected with tuberculosis. The

* Royal Commission on Tuberculosis (1895).

other two samples, which were more virulent, came from dairies where there was more than one suspected cow, and where the udders of some of the animals were visibly tuberculous.

Affected milk may not, under ordinary circumstances, induce tuberculosis, owing to its not containing a sufficient number of organisms to constitute a 'toxic dose,' but in the case of persons rendered susceptible owing to disease or weak health, or who have a constitutional predisposition to consumption, the use of tuberculous milk constitutes a very grave danger to health.

Many other diseases are conveyed by the agency of milk. According to Dr. Ernest Hart, fourteen epidemics of scarlet fever and seven of diphtheria have been traced in this country to the use of infected milk, as have also a number of epidemics of typhoid fever and cholera. As already shown (see p. 149) the typhoid bacillus and the cholera spirillum are capable of rapid multiplication in milk, without perceptibly changing it, but they are both destroyed when the lactic acid fermentation sets up. In addition to the contamination with various pathogenic organisms which may give rise to disease in man, milk is liable to attacks of certain non-pathogenic bacteria which are the cause of certain milk-diseases known as 'blue milk,' 'red milk,' 'yellow milk,' 'bitter milk,' 'stringy milk,' 'slimy milk,' 'soapy milk,' and a number of others.

Blue Milk.—This is a common disease of milk, and consists of the formation of blue patches on the surface of the milk, which condition may be produced in from twenty-four to seventy hours, according to the temperature. Steinhoff, as early as 1838, showed this disease of milk to be infectious, and Fuchs, in 1841, stated that the disease was caused by a microbe, which, however, he was unable to cultivate owing to the imperfect bacteriological methods

employed at the time. The bacillus of 'blue milk' is known as the *Bacillus cyanogenus*.

B. cyanogenus.—The bacillus of blue milk consists of small motile rods, which are provided with abundant flagella. The organism is pale blue in colour, does not liquefy gelatine, which, however, is stained bluish-green, finally becoming of a dirty grayish tinge. On potatoes the growth occurs as a thick, dirty-yellow layer, which afterwards becomes blue; the medium is discoloured.

Red Milk.—Several organisms may give rise to this disease in milk, the chief of which is the red milk bacillus of Hueppe (*B. lactis erythrogenes*).

B. lactis erythrogenes.—This organism was isolated by Hueppe and Grotenfeldt from red milk. It occurs as short rods, the growth of which liquefies gelatine. The colonies are of a yellow colour when first seen on the plate, but after liquefaction they become rose-red. A yellowish deposit occurs on agar, which soon changes to yellowish-red. The cultures give rise to an unpleasant, sweet smell. Other organisms which give rise to red milk are the following: *B. prodigiosus*, *Sarcina rosea*, *Saccharomyces ruber* (pink torula). A red colour in milk may be due to the presence of blood, as a result of disease of the udders.

Yellow Milk.—According to Freudenreich many organisms, especially those of putrefaction, can produce a yellow colour in milk, but this is rare in practice, as the milk is very seldom kept long enough for this change to take place. The best-known organism which gives rise to a yellow colour in milk is the *Bacillus synxanthus*. This organism was first found in a sample of boiled milk which had assumed a yellow colour. It is a motile rod, which curdles milk by means of a rennet-like ferment, which afterwards re-dissolves the curd and produces a yellow pigment.

Bitter Milk.—This fault may be produced in milk by the

cows eating certain plants, but there are a number of bacteria which give rise to bitterness in milk; the chief of these are the butyric acid ferments. Other organisms giving rise to bitterness in milk have been described by Weigmann, Conn, Duclaux and Freudenreich.

Stringy Milk.—Owing to the action of micro-organisms, milk frequently becomes filamentous or stringy in character. This milk disease is much deprecated in Switzerland, where milk so diseased cannot be employed in the manufacture of certain cheeses. The milk, after twelve or fourteen hours, assumes a sticky character, which sometimes is so marked that the liquid can be pulled out into strings if the finger be dipped into it. The Norwegian national drink, known as *tættemælk*, is a preparation produced with the aid of the 'stringy' milk bacillus. Amongst the many organisms producing stringiness in milk, the following are perhaps the most important:

Bacillus lactis pituitosi.—This organism was isolated by Löffler, who describes it as a stout, slightly curved rodlet, which does not liquefy gelatine.

Bacillus lactis viscosus.—This organism, which renders milk very stringy and is known as the viscid-milk bacillus, was first isolated by Adametz. It is a very short rodlet, aerobic, and does not liquefy gelatine. At the ordinary room temperature the milk does not become markedly stringy for some time.

Streptococcus Hollandicus.—This organism of stringy milk is used in Holland in the manufacture of Edam cheese. The organism is a coccus which occurs in the form of chains. It does not liquefy gelatine; it renders milk stringy within twelve to fifteen hours at a temperature of 77° C., the milk becoming sour at the same time.

Soapy Milk.—Milk which first appears to be normal often acquires a disagreeable soapy taste in from twelve to twenty-

four hours. Weigmann has discovered the cause of this soapy taste to be due to an organism, which he also found in the straw used as litter, from which it appears that the milk becomes infected when the litter is changed at milking-time.

Milk is also liable to a number of other obscure changes and diseases, the cause of which may be traced to bacterial agency. Micro-organisms also play a useful part in connection with dairy products, various organisms being employed to bring about required changes in the milk. Green mould (*Penicillium glaucum*) is the chief agent employed in the ripening of Roquefort and Gorgonzola cheese. Moulds also play an important part in the production of Brie and other soft cheeses; the growth of certain organisms is encouraged upon the surface of these cheeses, so that the special ferment which they produce can penetrate the body of the cheese and bring about certain characteristic changes. A certain yeast is used in the production of *kephir* (the national drink of the Caucasus), which is made by the fermentation of cows' milk, similar to koumiss, which is produced by the fermentation of mare's milk. White mould (*Oidium lactis*) is very frequently met with in milk and other dairy products. When the milk becomes sour, a white thick skin is found on the surface, which is wholly formed of the mycelia and hyphæ of this mould.

In the report of a special analytical and biological commission on milk-supply, held under the auspices of the *British Medical Journal* (1895), the following reforms were suggested for the better management of dairies, to secure a pure milk-supply:

1. That all milking be carried on in the open air, the animals and operators standing on a material which is capable of being thoroughly washed, such as a floor of

concrete or cement. Such a floor could be easily laid down in any convenient place which can be found. The site chosen should be removed from inhabited parts as far as possible, and should be provided with a plentiful water-supply. Only in this way does it seem possible to avoid the initial contamination with the colon bacillus.

2. That greater care be expended on the personal cleanliness of the cows. The only too familiar picture of the animal's hind-quarters, flanks, and side being thickly plastered with mud and fæces is one that should be common no longer. It would not be difficult to carry out this change; indeed, in the better-managed of our large dairy companies' farms such a condition no longer prevails, but in the smaller farms it is but too frequently met with.

3. That the hands of the milker be thoroughly washed before the operation of milking is commenced, and that after once being washed they be not again employed in handling the cow otherwise than in the necessary operation of milking. Any such handling should be succeeded by another washing in fresh water before again commencing to milk.

4. That all milk-vendors' shops should be kept far cleaner than is often the case at present. That all milk-retailing shops should be compelled to provide proper storage accommodation, and that the counters, etc., should be tiled.

To these valuable suggestions we would point out the great necessity which exists for proper and thorough systematic veterinary and bacteriological inspection, whereby any animal suffering from any tubercular or other disease could be at once isolated and, if necessary, destroyed.

Examination of Milk for the Tubercle Bacillus.—This can be best done by Van Ketel's method, as follows: To 50 c.c. of the suspected milk add 10 c.c. of liquefied colourless

carbolic acid. The mixture is well shaken for a few minutes, and poured into a conical test-glass to settle for twenty-four hours. A little of the deepest layer of the sediment is then removed with a fine pipette, from which cover-glass preparations are prepared as usual, by rubbing a droplet between two perfectly clean cover-glasses. The films are then air-dried and 'fixed' by passing through the flame three times. The cover-glasses are then passed through a mixture of equal parts of alcohol and ether. The cover-glasses are now dried and stained by the Ziehl-Neelsen method, as described under 'The Staining of the Bacilli in Sputum' (p. 124).

When a sample is required to be examined at once, the milk can be 'whirled' in a centrifugal machine, and the sediment taken for examination as above. This method could probably be improved by the addition of alcohol to the milk to reduce its viscosity, thereby facilitating the separation of the organisms.

Examination for the Typhoid Bacillus.—The milk can be examined by one of the methods described under 'The Examination of Water.'

Determination of the Number of Organisms.—The numerical determination of the bacteria present in milk can be determined by the method already described under 'The Examination of Water,' except that the dilution, owing to the much larger number of organisms present, requires to be carried to a much greater extent. If the samples cannot be plate-cultured at once, they should be allowed to remain in an ice-safe, otherwise the results, owing to the rapid multiplication of the bacteria, will have but little practical value.

THE BACTERIOLOGICAL EXAMINATION OF AIR.

The air does not normally contain any characteristic bacterial flora, as the organic matter required for their growth is not found in the air to any considerable extent. Their presence is due to the fact that they are blown about with the dust by air currents and winds from surfaces where they exist in a dried-up condition. Bacteria do not themselves unaided rise into the air; when air-currents are absent they always sink under the influence of gravity to the ground, where they always find better conditions for their growth and development. Wherever the greatest quantity of dust exists will be found the greatest number of bacteria; therefore the air in the summer always contains a larger number of bacteria than it does in the winter. A larger number of bacteria is always found in the air of towns than in the country. At high elevations, at the tops of hills or mountains, the air is almost free from micro-organisms; whereas on plains and low-lying places bacteria are almost always found in greater or lesser numbers. Again, the atmosphere of the open sea far out from land is almost free from bacteria. By far the greatest number of micro-organisms are found in the air of rooms and crowded public places, when they are whirled up from the ground with the dust.

The micro-organisms generally found in the air are the spores of moulds, yeasts, and bacteria, particularly the spores. Pathogenic organisms are sometimes found, particularly where a number of patients are collected together for treatment. The tubercle bacillus has frequently been found in the air of hospital wards containing phthisical patients, whose sputa have been allowed to dry.

A great number of researches have been made by various investigators as to the number of organisms found in the air in various parts of the world, but the results, although

of interest, are of little practical importance. The number of organisms present in the air is largely determined by the amount of moisture present, there being a much larger number of bacteria in dry than in moist air. The air of sewers has been shown to be remarkably free from micro-organisms by Carnelley and Petri, and more latterly by Laws and Arthur. All these observers obtained, roughly speaking, half the number of organisms from the sewer-air that they found in the external air. From this fact it can be argued that these organisms were derived from the outside air, the damp walls of the sewer acting like a Hesse's tube, thus accounting for the diminished number of micro-organisms present. This theory was proved to be correct by Laws, who examined the number and species of bacteria found in London sewer-air; these he found to be the same as those in the external air, while those organisms which were normal to sewage were found to be comparatively rare.

Filtration of Air.—It has been found that cotton-wool arrests in a trustworthy manner all organisms conveyed in air, which passes through it so long as the wool is moderately dry. Hansen has also found that the Pasteur-Chamberland tube, which when wet will not permit the passage of air, allows it to pass, and frees it from all organisms, when dry. A sufficient number of bends of narrow tube, whether wet or dry, is found also to reliably sterilise air which does not pass through them at too great a velocity. For bacteriological purposes cotton-wool is ordinarily employed for filtration of air. Glass-wool, powdered glass, sand, asbestos, sugar, and a number of other substances, have been employed from time to time to render air free from micro-organisms.

Examination of Air.—A large number of methods have been described from time to time for the bacteriological

examination of air. Some observers simply expose plates covered with nutrient medium to the air for a given time, and then count and examine the various organisms as soon as they have grown sufficiently well. Other investigators have used various filtering materials, sugar, sand, etc. A certain volume of the air to be examined is aspirated through a tube containing one of these materials, which

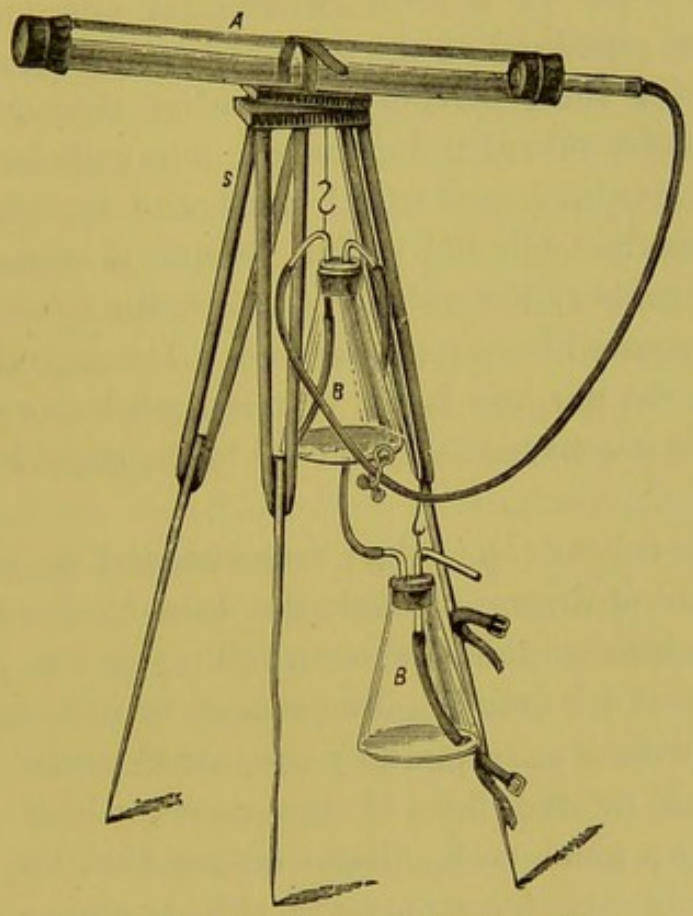


FIG. 31.—HESSE'S APPARATUS.

is afterwards treated with sterile water; this is then examined by the methods as given under 'The Examination of Water.' The most satisfactory method is a modification of the Esmarch roll culture, devised by Hesse.

Hesse's Method.—The apparatus consists essentially of a glass cylinder about 70 centimetres long and 3·5 centimetres in diameter; this tube is covered at one end by two rubber

caps, the inner one having a hole in its centre 10 mm. in diameter, and at the other end a rubber cork fits in the cylinder; through this cork a glass tube 100 mm. in diameter passes, which is plugged with cotton-wool. The cylinder is sterilised by placing for one hour in the steam-steriliser. The apparatus is then prepared as follows: The rubber stopper is carefully removed, and 50 c.c. of sterile nutrient gelatine in a fluid condition is introduced into the tube and rolled out on the sides as in the preparation of an Esmarch's tube, leaving a somewhat thicker coating along the under side of the cylinder. The cylinder and its fittings are mounted on a tripod stand, and the glass tube which passes through the rubber stopper is connected by means of a rubber tube with an aspirator, the cotton having first been removed from its outer end. The aspirator most suitable for the purpose is the double wash-bottle arrangement, which is conveniently attached to the stand by means of hooks.

The outer rubber cap is then removed, and the aspirator started. Air is drawn through the tube by suction, the micro-organisms contained therein falling on the gelatine. The amount of air entering is estimated by the capacity of the flasks forming the aspirator; the rate at which it enters is controlled by the flow of the water, which can be regulated by a pinch-cock. Hesse advises that the amount of the rate of flow for rooms and closed spaces should be about 1 to 5 litres, passed at the rate of half a litre a minute; for open spaces 10 to 20 litres is passed at about four minutes per litre. The tube is then capped and the colonies allowed to develop, after which they can be further examined by subcultures.

BACTERIOLOGICAL EXAMINATION OF SOIL.

Surface-soil, particularly that which is rich in organic matter, is very rich in micro-organisms. That it is only the surface-soil that contains any quantity of micro-organisms is shown by the fact that at as short a distance as about two metres in depth the soil contains but few organisms. This was shown by Koch in 1881, who showed that in soil which had not been disturbed, even at a depth of only one metre, but few bacteria are to be found. This fact has since been confirmed by the extended researches of Fränkel and others. The number of bacteria in undisturbed surfaces has been estimated by many observers; the results vary greatly, as would be expected, in different places, the number generally running to several thousand per gramme of earth.

In an investigation by Fränkel of the soil of a fruit orchard, he found the superficial layers contained from 50,000 to 350,000 organisms per gramme of soil. The greatest number was not immediately upon the surface, but at one-quarter to one-half a metre below the surface. At a depth of from three-quarters to one and a half metres there was a very abrupt diminution in the number of bacteria.

From 200,000 organisms at a depth of half a metre, the number fell to 2,000 at a depth of one metre, from 250,000 at three-quarters of a metre to 200 at one metre. At a depth below one and a half metres, generally speaking no more bacteria were found. The most important fact established by these researches is that in virgin soil there is a dividing-line at a depth of from three-quarters to one and a half metres, below which very few bacteria are found, thus showing the ground-water region is quite free, or nearly free, from micro-organisms, notwithstanding the vast number upon the surface of the soil. Buchanan Young has

investigated the nature of the soil in graveyards, and he finds, on the whole, that the bodies do not greatly influence the number of micro-organisms found. According to Kirchner, the freedom of the ground water from bacteria is due to the great porosity of the soil, which acts as a very efficient filtering medium.

Examination of Soil.—When the deeper layers are to be examined, care must be taken to prevent contamination with the other portions, particularly the upper layers. Fränkel has devised an ingenious instrument for taking samples of earth from various depths. This takes the form of a borer, which contains at its lower end a small cavity, which can be closed up by turning a handle, or opened by turning in the opposite direction. The borer is pushed down to the necessary depth; the handle is then turned, with the result that the earth enters the cavity; the handle is again turned, enclosing the sample of earth completely; the borer is then withdrawn. The soil can now be examined by thoroughly mixing a very small quantity of the earth with melted nutrient gelatine, which can then be poured on a plate, or better, made into a roll culture by Esmarch's method. Another method is to wash the soil with sterile water, which is examined as usual by the plate method.

CHAPTER XIV.

THE CHARACTERS OF SOME COMMONLY OCCURRING ORGANISMS NOT DESCRIBED IN THE PREVIOUS PAGES.

Micrococcus aerogenes—*M. agilis*—*Bacillus aquatilis*—*B. arborescens*—Black torula—*B. erythrosporus*—*Spirillum Finkler-Prior*—*B. fluorescens liquefaciens*—*B. fluorescens non-liquefaciens*—*B. gasoformans*—*B. jacinthus*—Magenta bacillus—*B. megatherium*—*Spirillum Metschnikovi*—*B. mesentericus fuscus*—*B. mesentericus vulgatus*—Bacillus of mouse septicæmia—Peat bacteria—Phosphorescent bacteria—Pink torula—*B. prodigiosus*—*Proteus vulgaris*—*Proteus mirabilis*—*B. ramosus*—*Spirillum rubrum*—*Sarcina alba*—*Sarcina lutea*—*B. subtilis*—*M. tetragenus*—*B. tholoeideum*—*Spirillum tyrogenum*—*B. violaceus*—*M. violaceus.*

Micrococcus Aerogenes.—Forms large oval non-motile cocci. It is very resistant to the action of acids, and in nutrient media containing sugar it produces a large amount of gas. This organism occurs in the intestine and in polluted water. Cultural characters:

Gelatine Plates.—Forms circular gray-white colonies.

Gelatine Tubes.—Forms a flat, gray-white, button-like growth on the surface; in the depth a brownish-yellow growth appears. After some time slight liquefaction of the gelatine takes place.

Agar-Agar.—A yellowish-white expansion forms.

Potatoes.—A slimy gray-white growth forms.

Micrococcus Agilis.—This organism is a motile coccus, which is found in water. The cocci, which are 1 μ in

diameter, occur as diplococci and in short streptococci. In old cultures they lose their motility, but on inoculating into a saccharine liquid they regain their power of movement. It will grow at 37° C. Cultural characters :

Gelatine Tubes. — A pinkish-red expansion is formed. The gelatine is very slowly liquefied.

Agar-Agar.—A pinkish-red expansion is produced.

Potatoes.—A pinkish-red growth is produced.

Bacillus Aquatilis.—Is found in water. It forms short straight bacilli, three times as long as they are broad, they occurring singly and in chains. This organism is anaerobic, and will not grow above about 24° C. It reduces nitrates to nitrites. Does not stain by Gram's method of staining. Cultural characters :

Gelatine Plates.—Forms mother-of-pearl-like dots, both on the surface and in the depth. By age the colonies become more raised, but do not grow in width. The gelatine is not liquefied.

Agar-Agar.—A moist white expansion is formed, which only grows at room-temperature.

Potatoes.—A gray-white irregular expansion forms, afterwards becoming of a coffee-yellow colour.

Bacillus Arborescens. — This organism was found by Frankland in river-water. It forms slender bacilli about 2·5 μ long by 0·5 μ broad, and occurs in twos and threes, also in long chains. Cultural characters :

Gelatine Plates.—Branching wheatsheaf-like colonies are produced, having a beautiful iridescent appearance. The gelatine is slowly liquefied.

Gelatine Tubes.—The medium is slowly liquefied, producing a yellow deposit.

Agar-Agar.—A dirty orange-coloured growth is slowly produced.

Potatoes.—A deep orange growth is produced.

Black Torula (*Saccharomyces niger*). — This yeast is frequently met with in the air. Cultural characters:

Gelatine Plates and Tubes.—Forms a heaped-up black mass.

Potatoes and Bread.—Grows as a sooty black crust, with a dry furrowed surface.

Bacillus Erythrosporus.—This organism, which occurs in water, etc., is a slender bacillus, which may form chains, and is very motile. From two to eight oval spores appear at the ordinary temperature in each rod, which may extend beyond the walls of the bacillus. They are characterised by their reddish colour; even when the bacillus is stained with methylene-blue, the spores retain their reddish colour. Cultural characters:

Gelatine Plates. — Whitish colonies are formed, which gradually spread over the surface; around them in the gelatine a peculiar fluorescence appears. The centres of the colonies are usually brownish, the outer zones are light yellowish-green. The colonies show radiate markings.

Gelatine Tubes.—Grows abundantly both on the surface and in the depth, and the whole tube assumes a green fluorescent colour. The gelatine is not liquefied.

Potatoes.—Produces a somewhat restricted growth, which is at first reddish, but later becomes nut-brown in colour.

Spirillum Finkler-Prior.—This organism was isolated by Finkler and Prior in 1884, from the stools of persons suffering from cholera nostras. Microscopically, it is very similar to the spirillum of Koch, but is distinguished from it by its ability to grow on potato at room-temperature, while cholera will only grow at blood-heat. It does not produce the indol reaction in so short a time as the cholera spirillum. Cultural characters:

Gelatine Plates. — Grows very rapidly in the form of

small white points, which under a lens appear to have well-defined outlines, and are yellow or yellowish-brown in colour.

Gelatine Tubes. — Liquefaction takes place at an early date, and proceeds rapidly. The liquefaction occurs in the form of a funnel-shaped tube, the liquefied gelatine becoming very turbid.

Agar-Agar.—A yellowish-white film is formed.

Potatoes. — A yellowish white layer is formed at the room-temperature.

Bacillus Fluorescens Liquefaciens.—This organism occurs in water, air, soil, etc., and occurs more frequently than any other form. It forms short bacilli, about 1 to 1·5 μ long by 0·5 μ broad. It occurs chiefly in pairs; is very motile. Cultural characters:

Gelatine Plates.—Forms small white dots; after about forty-eight hours the gelatine becomes liquefied, and a well-defined depression is formed. The whole of the gelatine then very soon assumes a green fluorescence.

Gelatine Tubes.—In the depth is a whitish growth, while at the surface a funnel-shaped depression forms, and finally the whole contents of the tube become liquefied and of a fluorescent green colour, whilst a thick white deposit is formed.

Potatoes.—A brownish expansion is formed.

Bacillus Fluorescens Non-Liquefaciens.—This organism is found in water. It forms short fine bacilli with rounded ends. It is strictly aerobic, and is not motile. Cultural characters:

Gelatine Plates.—The surface-colonies have a fern-like appearance, with a mother-of-pearl-like opalescence. No liquefaction of the gelatine takes place.

Gelatine Tubes.—Produces a fluorescent film.

Agar-Agar.—Forms a greenish expansion.

Potatoes.—A brownish growth is rapidly formed.

Bacillus Gasoformans.—This organism is found in water. It forms small, very motile bacilli. It does not grow at the higher temperatures. Cultural characters :

Gelatine Plates.—Forms very rapidly cup-like liquefied depressions.

Gelatine Tubes.—The gelatine is rapidly liquefied. In shake-cultures much gas is formed.

Potatoes.—Grows rapidly, producing a dark yellow, slimy expansion, which afterwards becomes reddish-brown.

Bacillus Jacinthus.—See under *B. violaceous.*

Magenta Bacillus.—This organism is a very short rod, which gives rise to a characteristic pigment. It is found in water. Cultural characters :

Gelatine Tubes.—Forms a streak of a brilliant carmine or magenta colour. The gelatine after some time is liquefied.

Potatoes. — Similar growth to the above. After some time the culture assumes the peculiar metallic lustre of the aniline dyes.

Bacillus Megatherium.—This organism was first obtained from cooked cabbage leaves. It is also found in water. It forms large bacilli with rounded ends, 2·5 μ broad and 8 to 9 μ long. It is motile, and forms spores, giving rise to clostridium-like forms, and has a great tendency to form involution forms. Cultural characters :

Gelatine Plates.—Forms small liquefying colonies, with small brownish halos.

Gelatine Tubes. — Grows very rapidly, liquefying the medium in a funnel-shaped tube, until the whole of the gelatine is liquefied.

Agar-Agar.—A whitish expansion is formed, the agar becoming dark brown in colour.

Potatoes.—Forms yellowish-white cheese-like expansion, the growth being restricted to the point of inoculation.

Spirillum Metschnikovi.—This organism was first observed by Gamaleia in the intestines of fowls. It is pathogenic in the case of fowls, pigeons, and guinea-pigs, but does not affect mice. It very much resembles Koch's cholera spirillum and the Finkler-Prior bacillus. In liquid medium it gives rise to very long spirals, which are very motile; it gives the indol reaction, as does the cholera spirillum. Cultural characters:

Gelatine Plates.—Small white colonies form, which soon give rise to cup-like depressions.

Gelatine Tubes.—Liquefaction of the gelatine takes place in the form of a funnel-like tube, the whole of the medium eventually becoming liquefied.

Agar-Agar.—A yellowish-white expansion is formed.

Potatoes.—A dirty-white layer is formed.

Bacillus Mesentericus Fuscus.—This organism is found in the air, water, hay, on vegetables, etc. It is a short, motile bacillus, which occurs in twos and fours. It forms small spores. Cultural characters:

Gelatine Plates.—Forms small round white colonies, which show delicate, thread-like projections. The gelatine is liquefied.

Gelatine Tubes.—The medium is quickly liquefied in the form of a funnel-like depression; in the liquefied portion are seen a number of grayish flocculent particles.

Agar-Agar.—Forms a yellowish-brown expansion.

Potatoes.—The surface becomes covered with a smooth yellow expansion, which afterwards becomes wrinkled and brown.

Bacillus Mesentericus Vulgatus (*'Potato Bacillus'*).—This organism is found on potatoes and other vegetables, in water, milk, etc. It occurs in the form of short, thick bacilli, often occurring in threads. It is very motile, and forms large oval resistant spores, which fill the interior of the organism. Cultural characters:

Gelatine Plates.—Small circular yellowish colonies are produced, which rapidly liquefy the gelatine.

Gelatine Tubes.—In the depth the growth rapidly liquefies the gelatine along the inoculation track in the form of a funnel; when the whole contents of the tube have become liquid, a pellicle forms on the surface.

Agar-Agar.—A dirty-white expansion is formed.

Potatoes.—Grows rapidly. At first a moist expansion is formed, which afterwards becomes wrinkled and tough.

Bacillus of Mouse Septicæmia.—This organism was obtained originally in garden earth and from putrefying liquids. The organism is exceedingly small, being only about 1 μ in length and from 0·1 to 0·2 μ in thickness; it is non-motile. Two organisms frequently occur together, and they contain spores. Mice inoculated die in from forty to sixty hours, when the bacilli are found in the blood, particularly in the capillaries of the kidneys and spleen. Probably identical with hog-erysipelas. Cultural characters:

Gelatine Plates.—Grows in the depth as a delicate white cloud.

Gelatine Tubes.—Along the track of the needle in the depth is seen a branching, cloud-like growth, which is more marked in the lower than in the upper layers. No liquefaction of the gelatine takes place.

Agar-Agar.—Pale yellow sharply-defined colonies are formed.

Peat Bacteria.—Two organisms, 'O' and 'Q,' have been

described by Dr. A. C. Houlston (Local Government Board Report, Supplement containing medical officer's report, 1893-4), who obtained them from peat. These two bacteria give rise to acidity when grown in peat-infusion, which has a great solvent action upon lead. It is to these organisms that is attributed the cause of the lead-solvent power in waters from peaty districts.

Photo-Bacillus Balticum — Photo-Bacillus Fischeri — Photo-Bacillus Fluggeri. — These three phosphorescent bacteria give rise to the phosphorescent appearance seen in the sea in various places, on fish, decaying wood, etc. The are short rods, which frequently occur in chains. They first two organisms liquefy gelatine very rapidly, but the last does not. This last organism — the *Photo-bacillus Fluggeri* — has the most marked phosphorescent power. Beyerinck states that these organisms are best grown in fish-broth made with sea-water, to which is added 1 per cent. of glycerine, 0·25 per cent. of asparagin, and 8 per cent. of gelatine. Several other varieties of light-giving bacteria are known. They all, generally speaking, grow best at a low temperature.

Pink Torula (*Saccharomyces rosaceus*).—This organism is very common in air, dust, etc. It is a slightly rounded or oval yeast, the cells ranging from 5 to 8 μ in diameter, which, under the microscope, are seen to contain a delicate yellow pigment, but appear of a pink colour when seen in the mass. Cultural characters:

Gelatine Tubes.—Small white or grayish points are seen along the line of inoculation, which afterwards gives rise to a coral pink mass.

Potatoes and Bread.—A bright coral pink growth forms.

Bacillus Prodigiosus.—This organism is common in the air, dust, etc. It is a very short bacillus, that differs

somewhat in size, the largest organisms being about 1·7 μ long by 1 μ broad. They are frequently seen in pairs, are non-motile. The organism grows well on all the ordinary media. The production of the blood-red colour is governed by the temperature and by the presence of oxygen, as well as by the nature of the nutrient medium. The colour decreases in cultures kept at incubation temperature. By long-continued growth on artificial media, the organism often loses its power of pigment production, which may, however, often be restored by cultivation on potatoes. Cultures give off an odour of trimethylamine. Cultural characters:

Gelatine Plates.—After two days the colonies are visible as circular depressions, each having a red centre. The less developed colonies in the depth are seen to be devoid of colour.

Gelatine Tubes.—The growth is very rapid, the gelatine liquefying in the form of a circular, funnel-like tube, the whole contents of the tube soon becoming liquid. The liquefied gelatine is very turbid, it containing an abundant deposit of a crimson colour.

Agar-Agar.—Grows rapidly, producing a blood-like expansion, the growth being restricted to the surface.

Potatoes. — Luxuriant growth of a beautiful crimson colour, which afterwards develops a metallic lustre.

Proteus Vulgaris.—This organism is found in putrefying animal substances, sewage, water, etc. It forms slightly bent bacilli about 0·6 μ broad and of variable length up to 3·8 μ; also gives rise to snake-like threads, resembling plaits of hair. It has a great tendency to form involution forms. The organism is very motile. Cultural characters:

Gelatine Plates.—The colonies are yellowish-brown in colour, bristling edges, which afterwards throw out irregular

branches. In the depth characteristic zoogloœa forms are met with.

Gelatine Tubes.—The gelatine is rapidly liquefied, when the whole of the contents are liquid; a whitish-gray cloud is visible at the surface, while at the bottom collects an abundant thick crumbly deposit.

Agar-Agar. — A thin-spreading, moist, shining, grayish-white expansion is formed.

Potatoes.—A dirty-white smeary growth is formed.

Proteus Mirabilis. — This organism is found in water, putrefying animal substances, etc. It occurs in rods of different lengths up to 2 or 4 μ long by 0·6 μ broad. It is very motile, and readily gives rise to involution forms. Cultural characters:

Gelatine Plates.—The colonies form circular white expansions, which, under low powers, appear brownish and finely granular. Liquefaction is less rapid than in the case of the *Proteus vulgaris*.

Gelatine Tubes.—Forms a whitish expansion, surrounded by a liquid circular zone, filled with moving bacilli. At the end of forty-eight hours, a moist, thick, shining pellicle is formed. The whole contents of the tube are liquefied in two or three days.

Agar-Agar.—A moist, shining, dirty-white expansion is formed.

Both the above protei are pathogenic to rabbits and guinea-pigs.

Bacillus Ramosus (*Wurzel bacillus*).—This organism is found in the soil and water. Frequently found by Frankland in the water of the Thames and the Lea. It much resembles the *B. subtilis*. It strongly reduces nitrates to nitrites. The bacilli are about 7 μ long and 1·7 μ broad, the ends being rounded. It occurs in long threads and has resistant spores. Cultural characters:

Gelatine Plates.—The colonies are seen as cloudy centres with root-like branches extending in every direction; the gelatine is slowly liquefied.

Gelatine Tubes.—In stab cultures a slight depression is seen after the second day, whilst the needle-path in the depth has a grayish woolly appearance. The whole contents of the tube then becomes liquid, a tough pellicle forming on the surface.

Agar-Agar.—Grows rapidly over the whole surface; in the depth is seen the characteristic woolly appearance.

Potatoes.—A white dry expansion is formed.

Spirillum Rubrum.—This organism is found in water, garden earth, etc. Forms spirilla with two or three twists on solid media, but in broth will give rise to long threads with up to fifty twists. It is about twice as thick as the cholera spirillum. The shorter spirals are very motile. Shining spots are seen in the body of the organism, which are probably spores. Cultural characters:

Gelatine Plates.—The colonies develop very slowly, often requiring eight or ten days to make their appearance. They form gray or pale red centres, with granular contents and a smooth rim. The depth-colonies become wine-red in colour. No liquefaction of the gelatine takes place.

Gelatine Tubes.—Grows in the depth as a wine-red streak, but at the surface, where the air has access, no colouring-matter is formed.

Agar-Agar.—A moist, shining, gray expansion forms, becoming red in the thicker part of the growth.

Potatoes.—Very slowly small red colonies are formed, which do not increase above the size of hemp-seed.

Broth.—The broth becomes turbid, and a red sediment is formed.

Sarcina Alba.—This organism occurs in air, water, etc.,

in the form of small cocci arranged in two, four, and eight elements. Cultural characters:

Gelatine Plates. — Grows slowly, giving rise to small white colonies. The gelatine is slightly liquefied.

Gelatine Tubes.—Produces a white expansion.

Potatoes.—Slow growth, producing a yellowish, white expansion, which is restricted to the line of inoculation.

Sarcina Lutea.—This organism is found in air, water, etc. It forms large cocci, from 1·5 to 2·5 μ in diameter, and arranged in twos, fours, and eights, in the usual packet-like form. The organisms are non-motile, and are very easily stained by the usual aniline dyes. Cultural characters:

Gelatine Plates.—Grows slowly, producing small round yellowish colonies.

Gelatine Tubes. — A slow-growing yellow expansion is produced, made up of a number of raised protuberances. The gelatine is slowly liquefied.

Agar-Agar.—A thick chrome-yellow growth spreads over the surface of the medium.

Potatoes.—Slow growth. The colonies are restricted to the line of inoculation.

Bacillus Subtilis (*Hay bacillus*).— This organism occurs in hay, the air, water, the fæces, etc. It is about 6 μ long by 2 μ broad, about the same length but somewhat narrower than the anthrax bacillus. The organism grows into long threads, and is very motile, having long flagella. Forms ovoid spores about 1·2 μ long by 0·6 μ broad. These spores are very resistant to heat; they will bear exposure to dry heat of 120° C. for one hour. The bacillus is strictly aerobic. Cultural characters:

Gelatine Plates.—The colonies become visible in about two days, as small white dots in the depth, whereas on the surface they show small grayish liquefied circles.

Gelatine Tubes. — Forms a liquefied, funnel-shaped depression, the lower part throwing out lateral feathery extensions. The whole of the gelatine is soon liquefied and a tough pellicle forms on the surface, and a quantity of flocculent matter collects at the bottom of the tube.

Agar-Agar.—A white opaque moist expansion is formed, which afterwards becomes dry and furrowed.

Potatoes.—A moist, cream-like expansion forms over the whole surface.

Micrococcus Tetragenus.—This organism was first obtained by Koch and Gaffky from a cavity in the lung from a case of pulmonary phthisis. It has since been found frequently in normal saliva and in tubercular sputum. It occurs as small micrococci, about 1 μ in diameter, which divide in two directions, forming tetrads, which are enclosed in a transparent, jelly-like envelope, which are especially well-developed in the animal body, but not so well in cultures. It stains quickly with the ordinary stains, when from the animal body the envelope may sometimes be seen feebly stained. It also stains by Gram's method. Subcutaneous inoculation of a culture in minute quantity into mice is fatal in from two to six days. The cocci are then found to be very numerous in the spleen, lungs, liver and kidneys. Cultural characters:

Gelatine Plates.—Small white colonies are developed in from twenty-four to forty-eight hours. When these are examined under a lens they are seen to be finely granular, with a mulberry-like surface.

Gelatine Tubes.—A thick white or yellowish-white expansion is formed. The gelatine is not liquefied.

Agar-Agar.—The growth at first may consist of a series of spherical colonies, which afterwards develops into a spreading expansion.

Potatoes.—A viscous milk-white growth is formed.

Bacillus Tholoeideum.—This organism occurs in the intestinal tract, and is, therefore, invariably found in sewage and polluted water. It forms short rods, with rounded ends. Grows at the ordinary temperatures. It is pathogenic to mice and guinea-pigs, the bacilli being found in the blood and organs. Cultural characters:

Gelatine Plates.—On the surface the colonies form at first nail-like, slimy growths, which are of a dirty-white colour; later they lose this slimy character, and form large circular grayish centres, with concentric rings. The gelatine is not liquefied.

Gelatine Tubes.—Forms a moist, shining, yellowish-brown expansion, which later becomes thick and spreads over the whole surface.

Potatoes.—A yellowish expansion forms, which rapidly spreads over the whole surface.

Spirillum Tyrogenum (*Deneke's Cheese bacillus*).—This organism was found by Deneke in 1885 in old cheese. In microscopical appearance it resembles the cholera spirillum, from which it is distinguished by the absence of indol, when tested by the indol reaction. The organism is a little smaller than the cholera spirillum. It forms long spiral threads, which are exceedingly motile. Cultural characters:

Gelatine Plates.—The colonies are similar to those formed by the cholera spirillum and by the Finkler-Prior bacillus, except that they are brownish in colour.

Gelatine Tubes.—Grows very rapidly, as in the plate-cultivation, giving rise to liquefaction of the gelatine, not so rapidly as the bacillus of Finkler and Prior, but more rapidly than the cholera spirillum.

Agar-Agar.—Forms a dirty yellowish-white expansion.

Potatoes.—A yellow expansion is formed.

Bacillus Violaceus (*B. Jathinus*). — This organism has been found by various investigators in water. It occurs in rods of different lengths, the longer of which may be bent. The rods are from 1·5 to 3·5 μ long by 0·65 μ broad, and are motile. Cultural characters :

Gelatine Plates.—In the depth the colonies are seen as small white dots, but on the surface they form small grayish circular discs. After five days they are seen as shining, drop-like, grayish-yellow expansions. When the colonies are older, delicate concentric rings are visible in and round the colony.

Gelatine Tubes. — Forms a white expansion, which gradually assumes a violet-blue colour. Afterwards the growth sinks, owing to the slow liquefaction of the gelatine. No pigment forms in the depth.

Agar-Agar.—An abundant growth forms, which is yellow or brownish in colour, and after a few days becomes of a deep violet colour.

Potatoes.—Forms a very deep violet expansion.

Micrococcus Violaceus.—This organism occurs in water, and in the air, etc. They occur as small, somewhat ovoid cocci, in the form of streptococci. It is non-motile. Cultural characters :

Gelatine Plates.—Forms slimy, drop-like colonies of a violet colour.

Gelatine Tubes.—Forms a violet expansion. No liquefaction of the gelatine takes place.

Potatoes.—A violet-blue growth is formed, which afterwards becomes darker in tint.

APPENDIX.

THE CONSTRUCTION OF STEAM-DISINFECTORS.

The difference in efficiency between various steam-disinfectors depends not only on the general method of their construction, but also on the proportions and details of their design. In this appendix it is only possible to describe the general construction of those steam-disinfectors which are best known.

Lyon's Disinfector. — This consists of a horizontal chamber, either oval or circular in section, surrounded by a jacket, and closed at either end by a door. Steam is admitted through safety-valves to the jacket and to the central chamber, in which the objects to be disinfected are placed. The pressure usually employed is about 20 lb. in the interior of the cylinder and about 25 lb. in the jacket, the object being to slightly superheat the steam and diminish the extent to which condensation takes place on the objects to be disinfected. The present method of eliminating the air is to apply a vacuum apparatus, whereby the air within the disinfecting chamber is rarefied to 15 to 20 inches of mercury—*i.e.*, one half to two-thirds of the air is extracted before steam is admitted. In some cases a current of warm air is also admitted before disinfection, so as to diminish the extent of condensation. The drying of objects after disinfecting is effected by extracting some part of the vapour by means of the vacuum, and allowing the re-

mainder to evaporate under the influence of the heat from the jacketed walls of the chamber.

The Equifex Disinfector. — The Equifex disinfector for absolute disinfection is cylindrical and has no jacket. Steam is admitted to coils at the bottom, and in some cases also at the top of the disinfecting cylinder at a pressure of about 50 lb., and serves to communicate to the steam so much heat as is lost by radiation through the sides

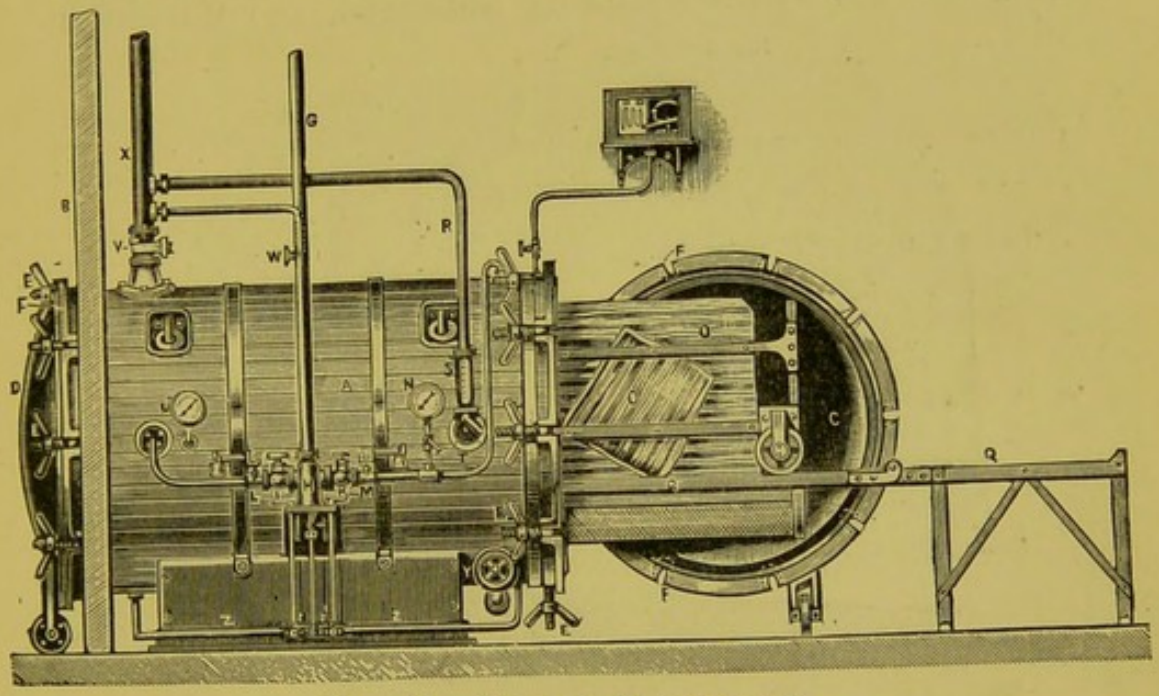


Fig. 32.—Equifex Disinfector.

and doors. Air is eliminated by allowing the steam on first admission to blow off through an outlet-pipe carrying a thermometer, which should register 95° C. before disinfection proper begins. In this way the air from the stove is got rid of; and by intermitting pressure for five minutes the air in the pores of the objects is likewise driven out on the sudden expansion of the volume of vapour condensed in them. The working pressure of steam is 10 lb. per square inch; and, the steam being saturated, its pressure, and therefore its temperature, can accordingly be recorded on an automatic recording-gauge. Objects are dried by

opening a valve at the bottom of the disinfection chamber for the admission of air, and determining a slight aspiration at the upper part of the stove.

The Equifex Low-pressure Disinfector.—This consists of a disinfection cylinder, mounted on either one or two cylinders, which serve as steam-generators. The pressure of steam, which is usually 2 lb. per square inch, is controlled by the use of a water-seal. The air contained in the disinfector is blown out by the steam on admission, and the steam

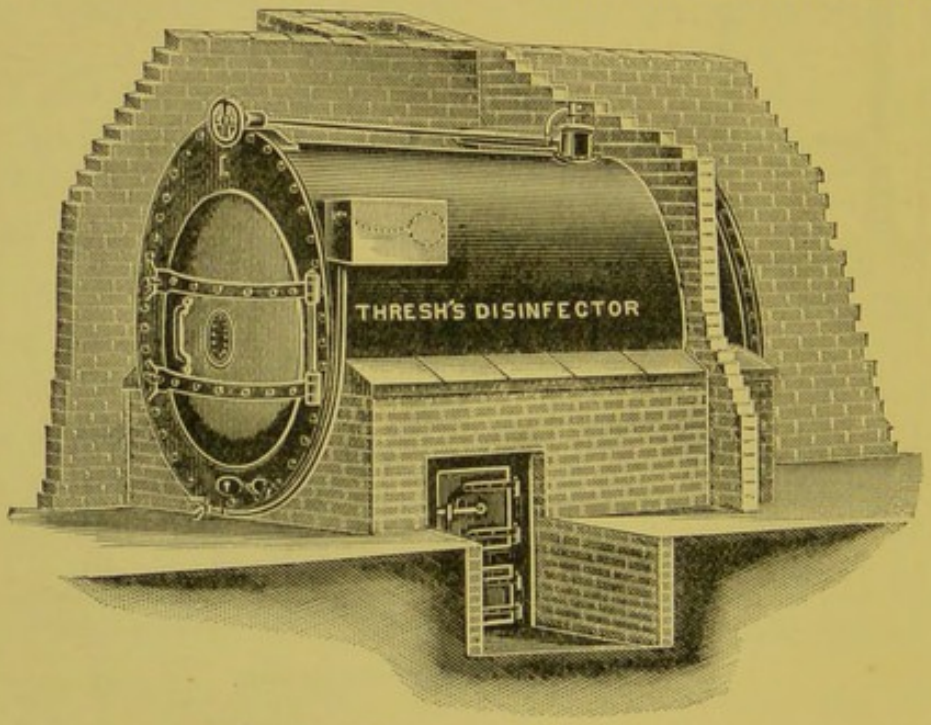


Fig. 33.—Thresh's Disinfector.

during disinfection is allowed continuously to escape. For drying, a current of air heated by passage through pipes fitted in the steam space of the generator is passed over the objects under treatment. A recording-gauge is usually supplied for registering the changes of temperature.

Thresh's Disinfector. — This consists of a disinfecting chamber surrounded by a boiler containing a solution of chloride of calcium, conveyed through a ball-cock from a service-cistern. The steam, which is formed at the top of the

boiler, is conducted down from the cylinder and admitted to the disinfecting chamber at the bottom, being allowed continuously to escape. This stove uses no pressure, and cannot, therefore, be fitted with a recording-gauge. The object of the chloride of calcium is to obtain a higher temperature in the steam than that due to the pressure.

Reck's Disinfector.—This disinfector is made to work with a pressure of one-ninth of an atmosphere, equal to, say, $1\frac{2}{3}$ lb. per square inch. Air is evacuated in a manner similar to that adopted in the Equifex disinfector, but the thermometer recording the temperature at which disinfection is considered to begin is placed at the door of the disinfector instead of in the discharge-pipe. The steam escapes under a safety-valve during the operation, and is more or less condensed at the end by the admission of cold water. Thick objects are removed for drying to a separate closet, which may be heated by the waste steam from the disinfector.

COMPARISON BETWEEN CENTIGRADE AND FAHRENHEIT.

Centigrade.	*Fahrenheit.*	*Centigrade.*	*Fahrenheit.*
60	140·	30	86·
59	138·2	29	84·2
58	136·4	28	82·4
57	134·6	27	80·6
56	132·8	26	78·8
55	131·	25	77·
54	129·2	24	75·2
53	127·4	23	73·4
52	125·6	22	71·6
51	123·8	21	69·8
50	122·	20	68·
49	120·2	19	66·2
48	118·4	18	64·4
47	116·6	17	62·6
46	114·8	16	60·8
45	113·	15	59·
44	111·2	14	57·2
43	109·4	13	55·4
42	107·6	12	53·6
41	105·8	11	51·8
40	104·	10	50·
39	102·2	9	48·2
38	100·4	8	46·4
37	98·6	7	44·6
36	96·8	6	42·8
35	95·	5	41·
34	93·2	4	39·2
33	91·4	3	37·4
32	89·6	2	35·6
31	87·8	1	33·8

The Conversion of Thermometric Scales. — To convert Fahrenheit-heat degrees to Centigrade terms, subtract 32, multiply by 5, and divide by 9.

To convert Centigrade degrees into Fahrenheit, multiply by 9, divide by 5, and add 32.

INDEX

THE END.

Baillière, Tindall and Cox, King William Street, Strand,