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APPLIED BACTERIOLOGY

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APPLIED BACTERIOLOGY

IN PREPARATION, BY THE SAME AUTHORS.

THE CHEMICAL AND BIOLOGICAL EXAMINATION OF WATER.

SEWAGE: ITS TREATMENT AND ANALYSIS.

APPLIED BACTERIOLOGY.

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

BY

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PREFACE TO THE FIRST EDITION.

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whole mass of conflicting observations which have been published, of which, indeed, a large number must ultimately be superseded.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.

T. H. P.
C. G. M.

July 10th, 1896.

PREFACE TO THE SECOND EDITION.

The preparation of this edition has furnished us with the opportunity of enlarging and improving the arrangement of several of the articles, and of submitting the whole to thorough revision. In the first edition we avoided inserting references to foreign publications, but, as in many cases readers may desire to refer to such, we have now frequently given them.

In view of the growing importance of bacterial methods of sewage treatment, we have included a short account of the bacteriology of sewage.

We have great pleasure in acknowledging our indebtedness to Dr. Patrick Manson for kindly revising the article on Malaria, also to Dr. Ladds, Mr. F. W. Stoddart, and others, for kind assistance.

T. H. P.

C. G. M.

May 1*st*, 1898.

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APPLIED BACTERIOLOGY.

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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. The continued life of the vegetable and the animal kingdoms is due to their activity, and is modified by it. The science of bacteriology investigates their life-history and its results.

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.

The history of the science of bacteriology may be said to commence with the observations of Antony 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 Müller named and described some three hundred organisms occurring in the waters about Copenhagen. Müller attempted to classify these small organisms, and first used the terms monas, proteus, bacillus, vibrio and spirillum. 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 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.' These conclusions, although not accepted at the time, have subsequently proved to be correct.

Henle in 1840 came to the conclusion that the cause of all contagious diseases must be of a living nature, and furthermore he pointed out the necessary steps in a demonstration of the casual connection between organisms and disease.

The theory which was so long sustained by the authority of the great chemist Liebig, that all fermentative and putrefactive changes were due to purely chemical changes, and that all albuminoid bodies, if left to themselves, would sooner or later decompose spontaneously, owing to their unstable chemical equilibrium, was responsible for the slow progress of bacteriology during the next few years.

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. This apparently small discovery did much for the forwarding of bacteriological research. It remained for Pasteur to make the greatest advance in the study of micro-organisms 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 this. Those on one hand 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, Klebs and Löffler the bacillus of diphtheria, and Koch the bacillus of tubercle. Koch, again, in 1884 published his discovery of the spirillum of Asiatic cholera (Koch's comma bacillus). The specific organism of influenza was discovered simultaneously by Pfeiffer, Kitasato and Canon in 1892, while, perhaps, the latest discoveries of importance are those of Bubonic plague, by Kitasato and Yersin, during the epidemic at Hong Kong in 1894, and of yellow-fever by Sanarelli in 1897.

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, and from the knowledge thus gained plan new and efficient methods for combating and preventing disease. This new field of study has not yet been by any means perfectly explored. In so young a science, it is inevitable that much must be accepted provisionally, and with the reservation that it is more likely to be corrected by later knowledge than are the facts of the older sciences. In many cases it is difficult to avoid being drawn into generalizing the

results of individual experiments into a statement of general law. This error is as dangerous as it is hard to avoid; and the effort to avoid it is the first and not the least important duty of a student of bacteriology. 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 short rodlets are often known as bacteria—if fairly long, as bacilla; 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 million 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 somewhere, 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 $\frac{1}{40}$ of a hollow cube, with edges $\frac{1}{25}$ of an inch long, but at the end of the following day they 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 realized 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 through 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 somewhat 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) *Tetracoccus, or Merismopedia.*—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.

Cocci may also occur singly, in groups, and in chains, surrounded by a gelatinous envelope, which often cause the organisms to agglutinate together in a mass. This form is known as a Zooglæa.

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) *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.

Certain bacilli, as in the case of cocci, also occur singly and in chains which are surrounded by a gelatinous envelope, which often causes the organisms to become agglutinated into masses. This form is known as a Zooglæa.

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.

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 'thermophilic' 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. It may be said in general terms that at the present time knowledge of the life-history of facultative parasitic bacteria in saprophytic conditions is relatively less than knowledge of their parasitic existence. The importance of further investigation in this direction is nevertheless very great, and its progress may lead to conclusions on many important questions—such, for instance, as the persistence of infection outside the human body, in regard to which existing data are inconclusive.

Some of the more important saprophytes play 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 matters 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 or excreted 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 intensely poisonous bodies, which are the cause

of the cases of which we frequently hear of individuals, 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.

Some 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. 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.

Variation of Bacteria. — The capacity of bacteria to produce the manifestations described above is dependent on the presence of suitable conditions, which in some cases can be defined, and in others have yet to be worked out. Thus it may happen that different races of the same

organisms may have different capacities for producing pigment or toxine and different resistance to external influences. Even different specimens of the same culture may vary if they are subcultured in different conditions or even at different ages of the original culture. The failure to recognise this fact has led to much confusion and error, and it must be constantly borne in mind, as among the most important and fundamental data of bacteriology.

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, however, 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 analine 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 sterilize 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 many pathogenic bacteria are quickly killed by a very short exposure to 100° C. (=212° F.). There are others, such as those of tuberculosis, infantile diarrhœa (Flügge), puerperal fever, and symptomatic anthrax, which, after being dried, may resist boiling for many hours. Spores of certain non-pathogenic species may resist the boiling-water temperature (100° C. = 212° F.) for as much as sixteen hours. In view of the fact that the existing organisms of some of the most dangerous infectious diseases, such as small-pox and scarlet fever, are as yet undiscovered, no distinction can be drawn for practical purposes between the resistance of pathogenic and that of non-pathogenic organisms, and measures of disinfection must, so far as possible, be chosen so as to be capable of destroying the most resistant organisms.

Steam for disinfecting purposes must always be saturated, and not superheated; that is to say, it must not be raised above the temperature at which under the existing pressure

it can condense. '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. Saturated steam under pressure, either confined or as a current, is now recognised as the only trustworthy form of steam for the purposes of general disinfection.

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 110° to 115° C. (=230° to 239° F.) for at least fifteen minutes.

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 1887, 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.

CHAPTER II.

BACTERIOLOGICAL APPARATUS—PREPARATION OF NUTRIENT MEDIA, ETC.

The apparatus used in bacteriological research—The microscope—Hot-air and steam sterilisers—High pressure steam sterilisers—Intermittent sterilisation—Sterilisation by means of chemical agents and filtration—Freezing and other microtomes—Incubators, warm and cool — Centrifugal machines — Other bacteriological apparatus — 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

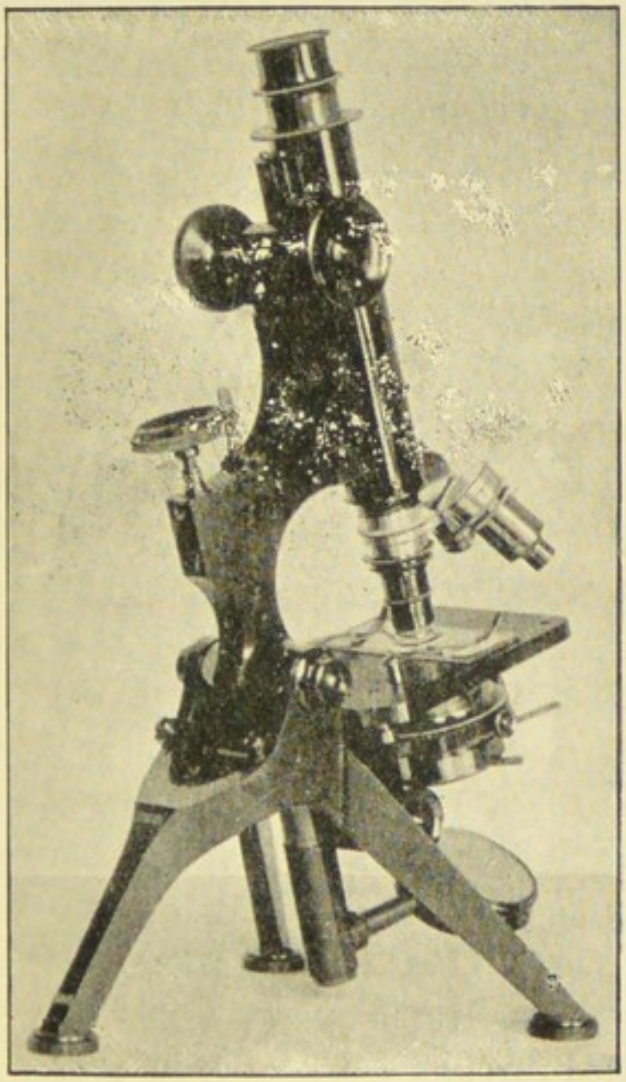


FIG. 1.—THE MICROSCOPE.

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

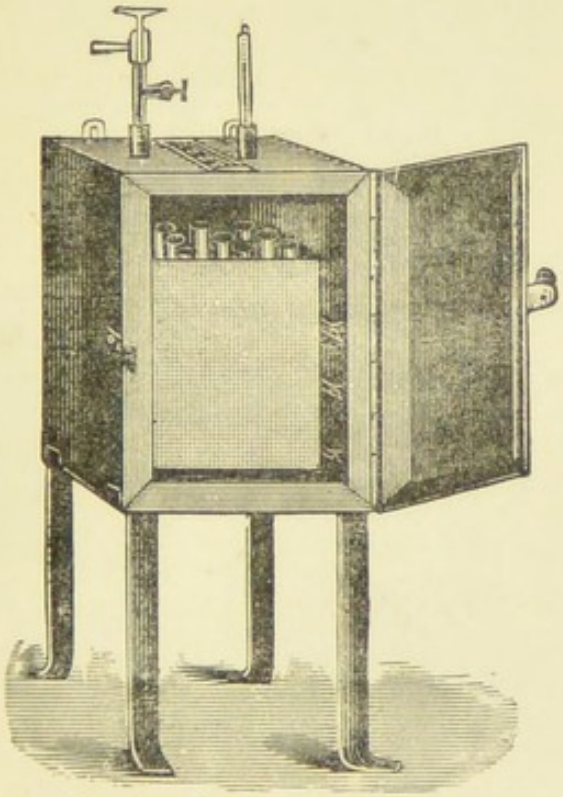


FIG. 2.—HOT-AIR STERILISER.

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

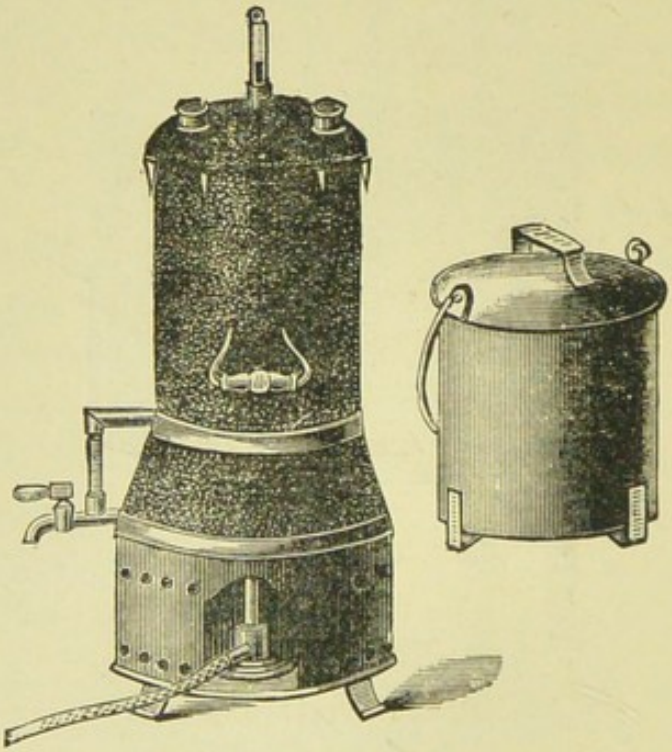


Fig. 3.—Steam Steriliser.

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.

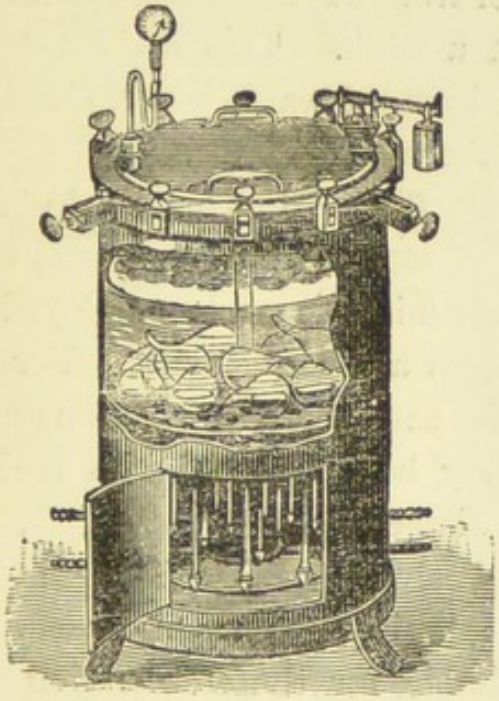


FIG. 4.—PRESSURE STERILISER.

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.

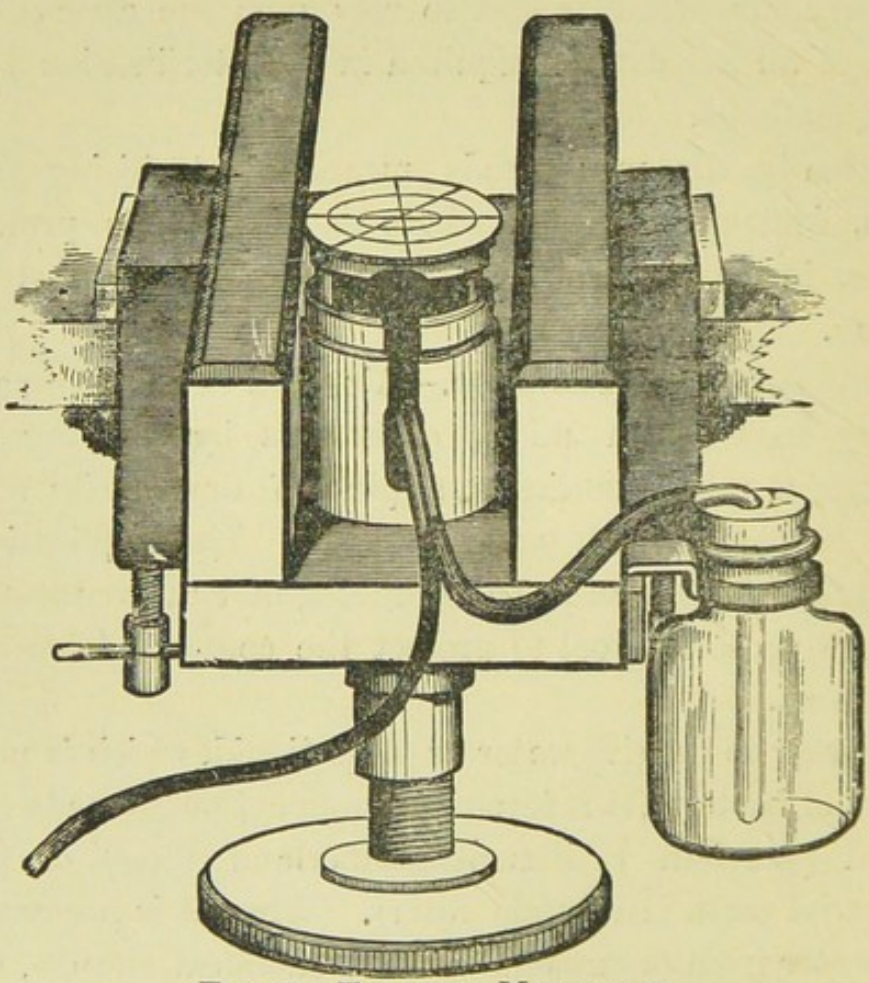


Fig. 5.—Freezing Microtome.

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

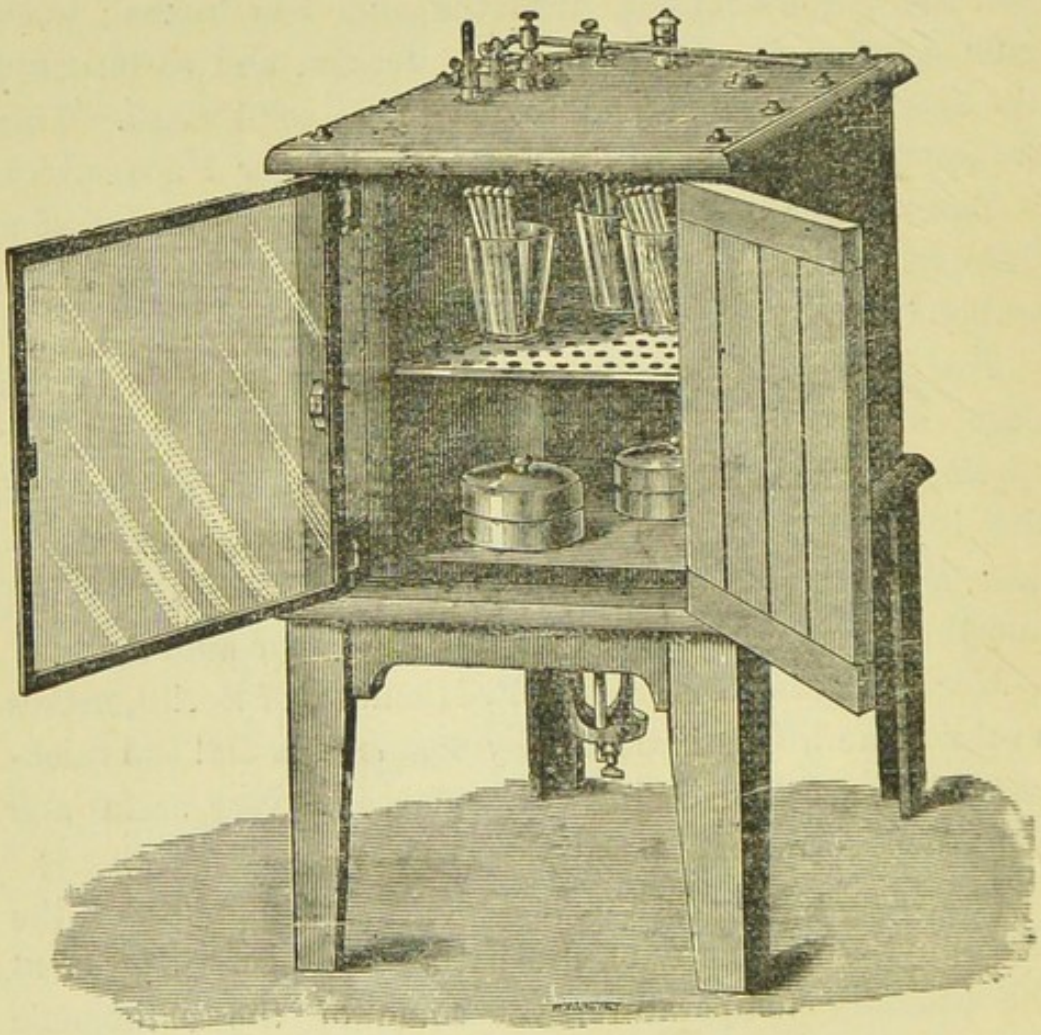


Fig. 6.—Incubator.

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

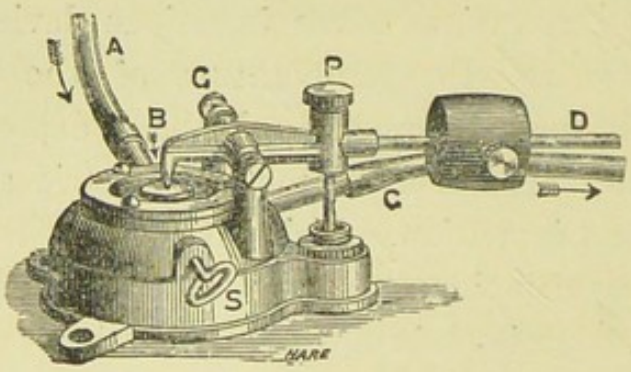


FIG. 7.—EXCELSIOR GAS-VALVE.

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.

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 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

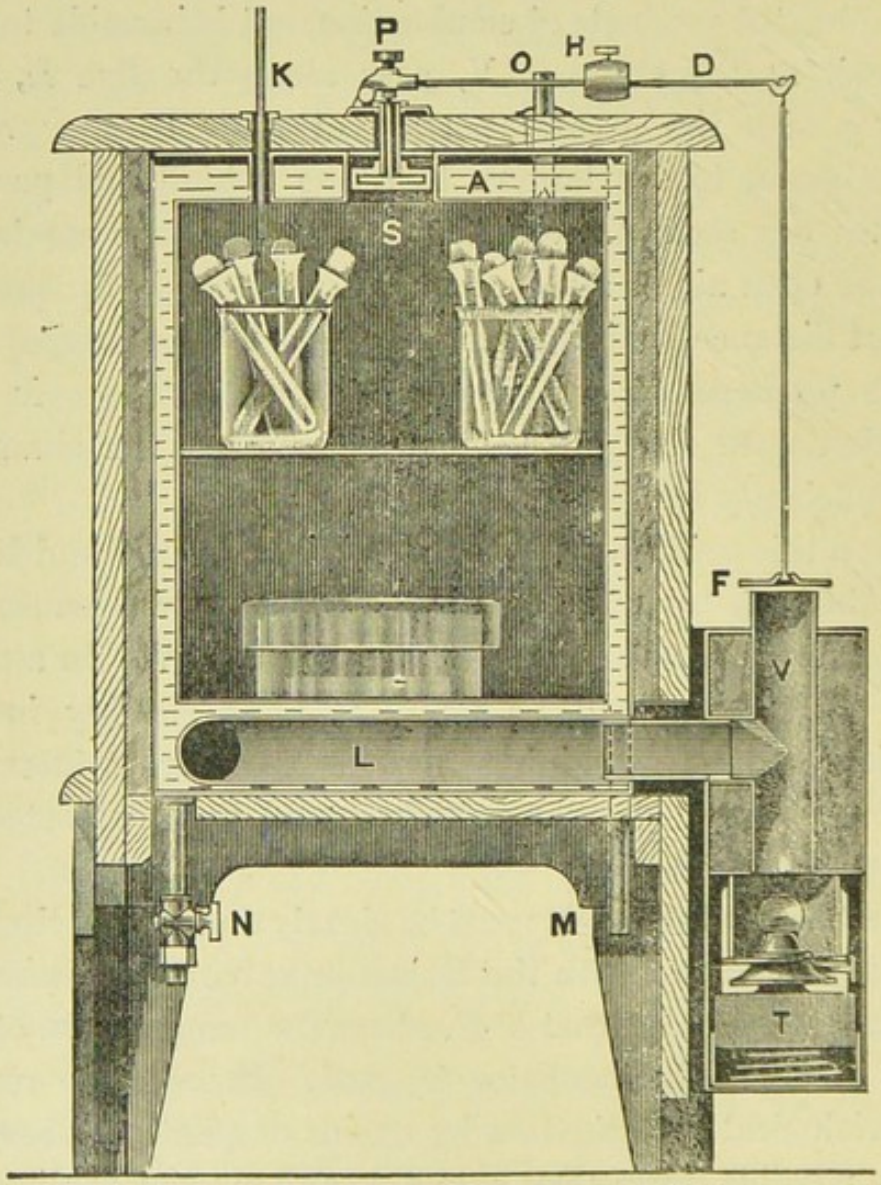


FIG. 8.—INCUBATOR HEATED BY A LAMP.

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

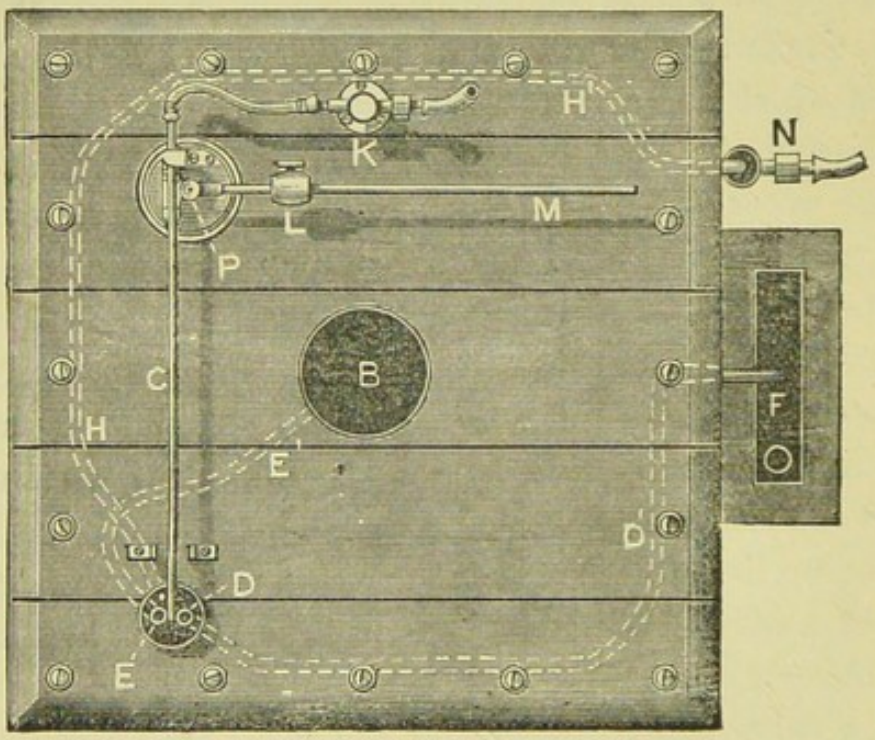


Fig. 9.—Plan of Cool Incubator.

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 Dr. Gerber for the

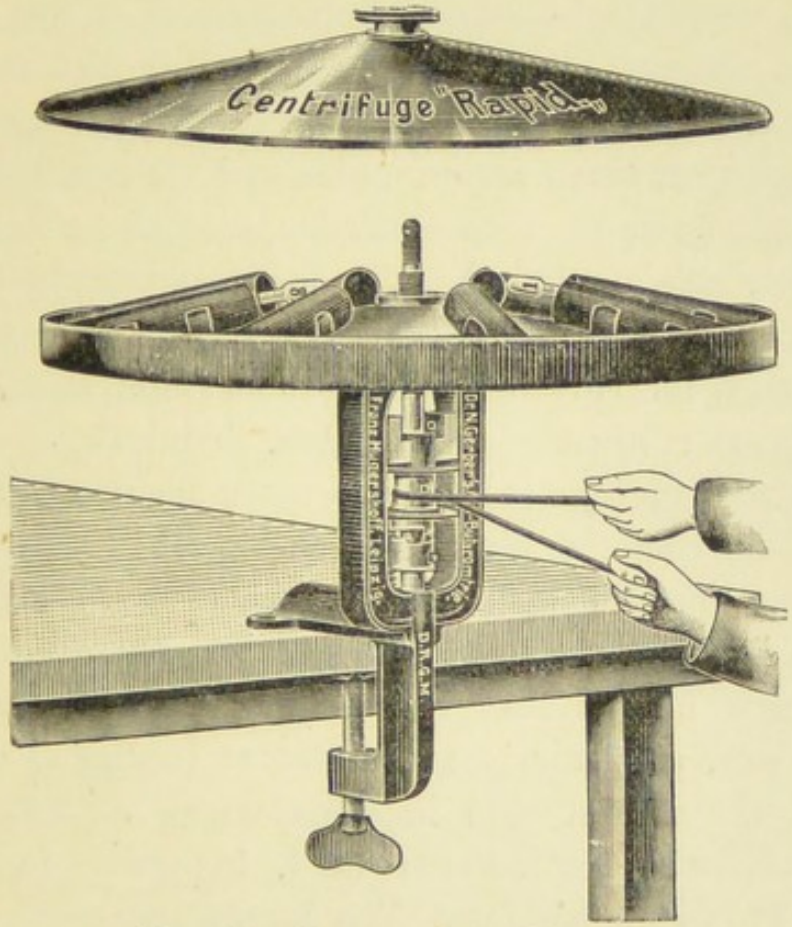


FIG. 10.—CENTRIFUGAL MACHINE.

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 embedding, 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 employed in general use 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.

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.

(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.

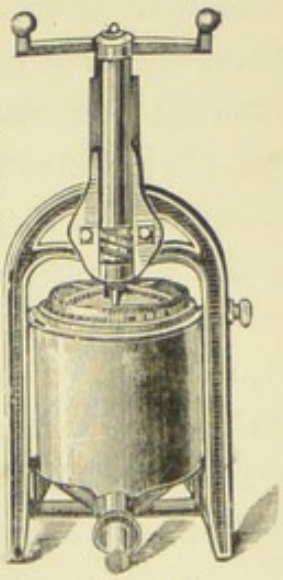


FIG. 11.
MEAT PRESS.

The mixture is now boiled for five minutes, and then very carefully neutralised with a solution of sodium carbonate, making the solution very faintly alkaline to litmus-paper. The alkaline solution is added carefully, drop by drop, shaking the flask well between each addition. 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.

When neutralising nutrient media under certain circumstances, depending upon the relative proportions of the different phosphates of potassium and sodium present, what is known as the amphoteric reaction is obtained—*i.e.*, red litmus is turned blue, and blue litmus red by the same solution. Owing to this difficulty, and that due to the varying sensitiveness of different samples of litmus-paper, making the true neutralisation point somewhat indefinite, we prefer the use of phenol-phthalein as indicator. The procedure is as follows :

An aliquot portion of the nutrient medium is taken, say 20 c.c. ; this is diluted with warm distilled water, a few drops of a solution of phenol-phthalein added, and *normal* solution of sodium hydrate added drop by drop from a burette until a pink coloration is obtained. The correct volume in c.c. of *normal* soda solution to be added to the

* 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 sterilized as above directed.

bulk is calculated and added; the reaction of the medium will then be exactly neutral. The medium is then rendered faintly alkaline by the addition of 1 c.c. of *normal sodium carbonate* solution to each 100 c.c. of nutrient medium. This degree of alkalinity, we find, gives the best results in practice.

(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 sterilised 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 sterilised 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 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,

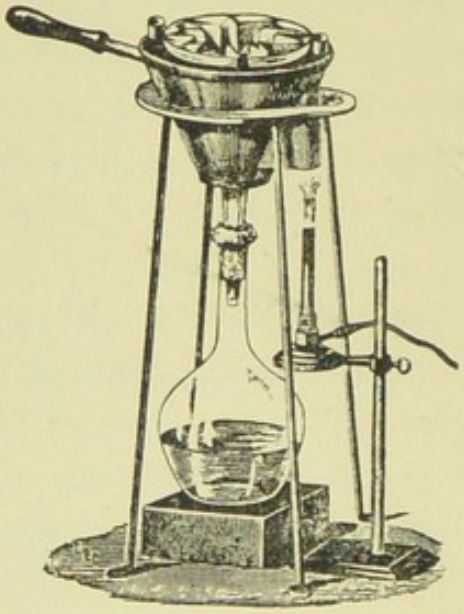


Fig. 12.—Apparatus for Filtering Media.

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 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 sterilization.

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 sterilised 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 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

* 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.

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 coli.

(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) **Potato-Water Gelatine.**—This medium is used for the differential separation of the typhoid and coli bacilli. It is prepared as follows: 500 grammes of potatoes are thoroughly washed, peeled, finely grated, and squeezed through a linen cloth by means of a small laboratory press. The opaque juice is then heated (with or without treatment with a little pure animal charcoal to decolorise) in the steam chamber for one hour. It is then filtered, and 10 per cent. of 'gold-leaf' gelatine added to the clear fluid; this is again heated in the steam chamber, filtered if necessary, poured into test-tubes, and sterilised on three successive days.

(16) **Potato-Water Gelatine (Elsner's modification).**—This is the same as above, with 1 per cent. of potassium iodide added. The best way of preparation is to add a sterilised solution of potassium iodide in requisite amount to the potato-gelatine which has just been made ready for use.

(17) **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 slaughtermen. 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.

(18) **Löffler's Medium.**—This medium, which is used for 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.

(19) **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.

(20) **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.

(21) **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.

(22) **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.

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

(24) **Uschinsky's Solution.** — Uschinsky's solution, as simplified by Voges and Fränkel, has the following composition:

Sodium chloride - - -	5 grammes.
Neutral sodium phosphate - -	2 ,,
Ammonium lactate - - -	6 ,,
Asparagin - - - - -	4 ,,
Distilled water - - - -	1000 ,,

Many bacteria will grow very well in this simple non-albuminous fluid. This and similar fluids have been recently used in the study of the metabolic products of several of the pathogenic organisms, notably diphtheria.

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: 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 suphuretted hydrogen.

CHAPTER III.

METHODS OF BACTERIOLOGICAL STUDY— STAINING, ETC.

The study of micro-organisms by means of pure cultures—Gelatine plate cultures—Esmarch roll cultures—Streak, stab and shake cultures—Culture of anaerobic bacteria—Hanging-drop cultures—Permanent cultures — Indol reaction — 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, so that the work has to be done entirely afresh.

In this connection it must also be remarked that bacteria may display an extreme sensitiveness to differences in the composition of nutrient media so minute that they have not in all cases been identified chemically. Too much care cannot be given to preparing such media with the utmost accuracy, and, in particular, all precautions must be taken to assure the identity of composition of media to be used in experiments of which the results may subsequently have to be compared with each other. The variation between different races of an organism, or even between different specimens of the same organism, and the possibility of an effect of symbiosis or antagonism, has an important practical bearing on experimental work. In comparative experiments it is desirable to work with bacteria of the same race which has been grown and subcultured, so far as possible, under similar conditions, and to repeat the experiments with specimens of another race. In plate cultures of possibly mixed bacteria, the inoculated material should be diluted, so as to avoid crowding of colonies.

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 into 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 into 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 below is used. This consists of a glass plate supported by a tripod.

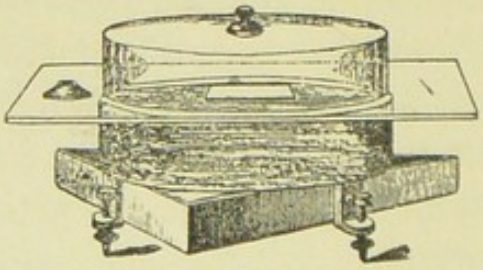


Fig. 13.—Plate Culture Apparatus.

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, 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. 14.—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 that they are quickly made, they do not occupy much room, and are well protected against atmospheric germs.

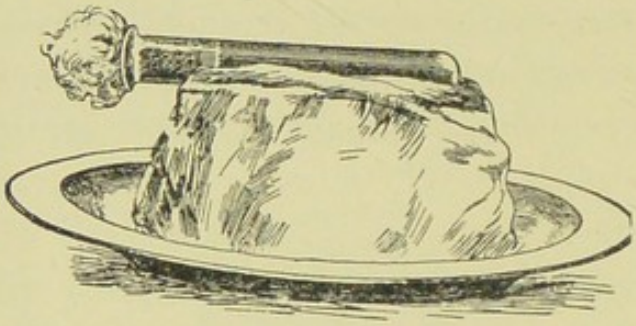


Fig. 15.—Method of making Esmarch's Roll Culture.

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.

Character of Bacterial Colonies.—The gelatine plate cultures are kept at 22° C., 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 procedure 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

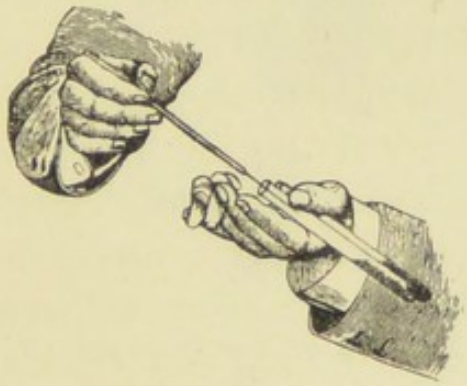


FIG. 16.—METHOD OF MAKING A 'STREAK' CULTURE.

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.

'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 best to always hold the tube during the inoculation in an

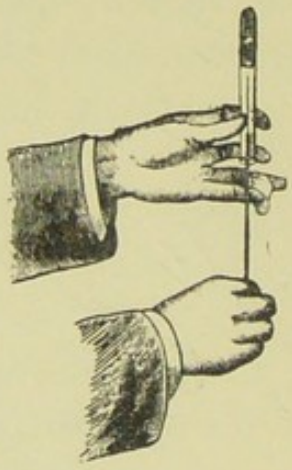


FIG. 17.—METHOD OF MAKING A 'STAB' CULTURE.

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.

All the ordinary culture media contain traces of free oxygen, and will absorb more on standing. For the growth of anaerobes, this oxygen may be got rid of by one of the following methods: (*a*) By growth in a vacuum; (*b*) by growth in an atmosphere of an inert gas, such as hydrogen or carbon dioxide; (*c*) by additions of a reducing substance to the media which does not interfere with the bacterial growth. Such additions take up any free oxygen there may be in the medium, and prevent further absorption. 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, formate of soda, and sulphindigotate of sodium have also been employed as additions to culture media for the purpose of extracting oxygen.

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

Grüber's method of effecting the removal of oxygen is as follows: 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 indiarubber 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.

The most generally used and satisfactory method for the cultivation of the anaerobic bacteria is the following modification of the Esmarch roll-tube:

A large test-tube has as much glucose-gelatine medium put into it as would be required in the ordinary roll culture. It is then corked with an indiarubber stopper, through which pass two angle tubes, one of which passes to the bottom of the tube, both of the external limbs being plugged with cotton-wool. Three or more such tubes are prepared and sterilised in the ordinary way in the steam steriliser.

The liquid or other substance to be examined for anaerobic bacteria is attenuated by dilution with ordinary melted gelatine medium, as in an ordinary plate culture. The prepared sterilised tubes are inoculated from these attenuations. The tubes are placed in water at 30° C., to keep the media in a molten condition. Hydrogen gas is now passed through the long tube which dips into the media for about 15 to 30 minutes. The gas supply tubes are then sealed off by means of a blowpipe flame, and the media in the tube is rolled and cooled as in the ordinary Esmarch tube. The gelatine is thus enclosed in an atmosphere of hydrogen, in which the colonies are free to develop. When the colonies have grown sufficiently, they can be subcultured into 'deep' tubes of glucose-gelatine. The needle may be prevented from carrying air into the stab by first melting the upper half-inch of the medium. From such 'stab' or 'deep' cultures anaerobic growths may be maintained almost indefinitely by successive subcultures in similar tubes.

The supply of hydrogen gas for the above purpose is best generated from a large Kipp's apparatus, using pure zinc and sulphuric acid, of course, with the usual precautions to prevent admixture with air. The gas, before passing to the culture-tube, should be washed by passing through a wash-bottle containing water.

Perhaps the simplest method for the cultivation of the

anaerobic bacteria is that devised by Buchner, who abstracts the oxygen from the air by means of pyrogallic acid and caustic potash. All that is necessary is to enclose the inoculated culture-tubes in a larger tube, provided with a closely-fitting and well-greased rubber stopper. Into the bottom of the larger tube from a quarter to half an ounce of pyrogallic acid is first introduced; then, immediately before closing it, a few c.c. of 10 per cent. caustic potash solution is poured in by means of a funnel. In a short time an atmosphere practically free from oxygen is produced in the apparatus, and one in which strict anaerobes will give evidence of good surface growths. Greater certainty may be attained by the addition of 0·5 per cent. of sodium formate to the culture medium. We have had no difficulty in obtaining even good plate cultures of anaerobic bacteria by this means.

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.*

Stoddart (*The Analyst*, May, 1897) applies the test as follows: A warm solution of nitrous acid prepared by adding one part of pure sulphuric acid to ten parts of 0·02 per cent. solution of potassium nitrite, is poured over an agar culture. If the organism gives the reaction, a pink coloration appears in the superficial layers.

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

The following are the most commonly used basic aniline stains, dyes used for staining bacteria:

Gentian violet (syn. benzyl violet, pyoktanin).
Methyl violet (syn. Hoffmann's violet, dahlia).
Methylene blue (syn. phenylene blue).
Thionin blue.
Fuchsine (basic fuchsin, basic rubin, magenta).
Bismarck brown (syn. vesuvin, phenylene brown).

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 6. 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. Stains should always be filtered before use, otherwise granules of colouring matter will be deposited upon the preparation which it is impossible to wash off.

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 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.

* 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.

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. Some workers prefer to use the actual glass slip instead of cover-glasses for the staining process.

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 a 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 standing, 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 recommends 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 pubblished* 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 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.

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

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 re-

maining 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 dis-

solve 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 sixty minutes. Superfluous 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 of 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 next 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 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

* 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.

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—IMMUNITY—BACTERIAL TOXINS—SERO-THERAPY.

Methods of spread of disease—Epidemics—Methods of bacterial action—The antagonism of micro-organisms—Immunity—Hypotheses of immunity—The exhaustion or pabulum, antidote or retention, and acquired tolerance hypotheses—Methods of producing artificial immunity—Metabolic products of the growth of pathogenic bacteria—Ptomaines—Isolation of ptomaines—Toxalbumoses, bacterial proteins—Sero-therapy—Researches of Behring, Kitasato, Tizzoni and others on antitoxins—Defensive proteids or alexins—Preparation of diphtheria antitoxin—Preparation of the toxins—Immunisation of the animals—Standardisation and preparation of the serum—Preparation of antitetanic, antistreptococcic, antivenomous and other sera.

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.

The more important diseases may be roughly classed under the above four headings as follows:

1.	2.	3.	4.
Actinomycosis.	Anthrax.	Actinomycosis.	Leprosy.
Anthrax.	Cholera.	Anthrax.	Malaria.
Diphtheria.	Leprosy.	Diphtheria.	Measles.
Influenza.	Malaria.	Erysipelas.	Pneumonia.
Pneumonia.	Scarlet fever.	Glanders.	Relapsing fever.
Tuberculosis.	Typhoid.	Gonorrhœa.	Scarlet fever.
	Tuberculosis.	Hydrophobia.	Small-pox.
		Syphilis.	Syphilis.
		Tetanus.	Whooping-cough.
		Tuberculosis.	Yellow fever.

Epidemics.—Many of the contagious diseases attach themselves more or less permanently to certain districts, and are termed endemic. Thus leprosy is endemic in the Sandwich Islands, the Cape, etc.; cholera is endemic in the delta of the Ganges; small-pox in the Soudan, and to a lesser extent such diseases as diphtheria, typhoid and scarlet fever, are endemic in some parts of England. From time to time some of the specific diseases, particularly cholera and plague, become widely spread over certain areas; they are then said to be epidemic. When a disease spreads more or less over the globe, as in the case of influenza, it is said to be a pandemic. The causes contributing to epidemics are still but little known. They have been attributed to meteorological and climatic conditions, imperfect and filthy sanitary conditions, particularly in the case of cholera, to facilities for convection, accumulation of susceptible individuals, etc. There are at least three causes contributing to the rise of an epidemic, of which only two are at present known. The first factor, which we may call x, is the specific micro-organism of the disease in question; the second factor, z, is individual idiosyncrasy, climatic and other predisposing causes; but the third factor, y, the cause of epidemics, still remains to be determined. This is the x, y, z theory of Dr. Von Pettenkofer, and it is

believed that when the real nature of *y* is discovered it will be in our power to prevent to a great extent epidemics of all kinds.

Methods of Bacterial Action.—When the pathogenic bacteria invade the animal body, they may exert their pernicious power in either or both of two ways. They may by their excessive multiplication cause stopping up of the minute capillaries, or they may produce substances called toxins, which exert poisonous effects upon susceptible animals. These toxins are the result of the metabolic processes of the organisms ; they are in some cases secreted in the intercellular tissue of the organism, and in others excreted by them.

The result of the infection of the body by the pathogenic organisms varies very much in different cases, and is manifested by the various symptoms which characterise disease, such as disturbances of the nervous system, fever, tissue changes, etc. With the lower animals various forms of septicæmia may be produced, as in the case of rabbits infected by the pneumococcus. In man septicæmia is not usually produced, nor is there the same multiplication of the organisms in the blood, although in some cases, notably in that of the organism of relapsing fever, the multiplication of the organisms is liable to be exceedingly rapid. Generally in the human subject the organisms remain strictly local, as in the case of diphtheria and tetanus, or proceed by the blood or lymphatics to various organs, where they produce characteristic lesions, as in tuberculosis.

The Antagonism of Micro-organisms (Symbiosis).—The mutual antagonism or influence of growth of one species upon the growth of another has been specially studied by Freudenreich and Sirotinin. When several organisms are associated in a liquid culture one species may take pre-

cedence, 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 metabolic products of some organisms, *e.g.*, *Bacillus typhosus*, are capable of restraining the growth of the organism itself. Correlative phenomena are the beneficial alterations which are produced in the behaviour of an organism by the presence in the culture medium of other organisms. Thus the presence of streptococci appears to enhance the virulence of the diphtheria bacillus; or attenuated cultures of *Bacillus anthracis* may reacquire virulence, if injected simultaneously with a culture of *Bacillus prodigiosus*. And in some cases bacteria thrive better in the presence of certain others than without them.

Certain chemical actions cannot be effected by some bacteria alone, while they can be done by two forms in combination: for instance, the decomposition of nitrates into gaseous nitrogen.

It has also been observed that among certain soil bacteria the single varieties are harmless, but in combination they are found to be pathogenic to animals. This fact should merit special attention when investigating new or obscure diseases. Some writers have assumed cholera to be produced by the combination of two organisms (diblastic

theory). Again, some feeble pathogenic organisms, as in the case of attenuated tetanus bacilli, become greatly exalted in virulence when cultivated with the *Proteus vulgaris*.

Immunity.—Immunity means the protection which is afforded to the animal body against pathogenic organisms under certain conditions, which immunity may be either natural or artificially acquired. When an animal is non-susceptible to a given disease, the immunity is said to be *natural*, but when the protection is afforded by a previous attack of the disease, it is said to be *acquired* immunity.

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 may, 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. Cases, however, occur in which such lesions are followed, sometimes after considerable intervals, by generalised or local tuberculosis.

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.

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 in 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.*

Artificial Immunity.—We will now proceed to briefly consider the various means by which artificial immunity may be induced in the animal body. As has already been stated immunity may be 'active' and 'passive.'

Active Immunity.—This may be produced by an injection or a series of injections of the organisms in a *virulent* or *attenuated* condition, or by sub-lethal doses of their metabolic products.

Thus active immunity may be produced by one of the following methods:

1. By the injection of the virulent organisms in non-lethal doses.

2. By injection of the dead organisms.

3. By the injection of the toxic products prepared from filtered broth cultures of the organism.

4. By the injection of the living, but attenuated, organism prepared by one of the undermentioned methods:

(*a*) By passing through one animal, whereby it becomes attenuated for another animal.

(*b*) By growth at normal temperatures.

(*c*) By growing in the presence of oxygen or air.

(*d*) By frequent and prolonged sub-culturing.

(*e*) By growth in the presence of very weak antiseptics, or by the injection of an antiseptic with the organisms.

Passive Immunity.—This condition may be attained when the serum of an animal which has been immunised by one of the previous methods is injected into another animal. This is due to the fact that the serum of a protected animal has a very powerful neutralising or antagonistic effect upon the virulent bacilli if injected at the same time or shortly afterwards. With some infections high resistance can be obtained in certain animals by repeated and increasing doses of the toxines obtained from broth cultures of the organism; the serum of animals so treated will protect other animals against lethal doses of the toxines, or of the virulent organisms themselves. This serum also exercises a protective action against a future infection, although the immunity thus conferred lasts but a short time. Thus these sera become very valuable curative agents, and are the basis of the modern system of serum-therapeutics. The serum of an animal highly immunised against a particular toxine is properly known as 'antitoxic serum'; that of an animal highly protected against a particular organism in a virulent condition is known as 'antimicrobic serum.' This method of inducing immunity was first worked out in the case of diphtheria and tetanus, and has since been applied with varying success to the treatment of several diseases.

A combination of the above methods has been successfully employed to immunise animals—for instance, by repeated injections of cultures, first attenuated and afterwards of very high virulence, a high degree of immunity

is acquired. The well-known anticholeraic treatment of Haffkine belongs to this class.

Bacterial Toxins.—As has already been stated, micro-organisms produce, as the result of their vital processes, a number of complicated bodies, which are variously known as ptomaines, cadaveric alkaloids, 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 or metabolism.

Many of these bodies, when introduced into the animal body, give rise to similar symptoms to those caused by the organisms which elaborated them, so that we may say that such bacteria affect the body chiefly through certain toxic principles which they elaborate.

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.

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*, are bodies of an albumin or proteid-like nature, and, like the ptomaines, are products of the vital activity of bacteria. When separated from the bacteria, by which they have been produced, and introduced into the animal body, they give rise to symptoms similar to those produced by the bacteria themselves.

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.

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 synthetically.

In their physical and chemical characters the ptomaines present a very close resemblance to the vegetable alkaloids. The liquid ptomaines have a penetrating and persisting nauseous or cadaveric odour. This characteristic corpse-like or musky odour is frequently to be met with in bone caverns of the Stone Age, in guano beds and in other seats of ancient putrefactions. The solid ptomaines are generally crystallisable, colourless, soluble in water, but insoluble in alcohol, chloroform and benzene. All the liquid ptomaines are soluble in ether-alcohol, amylic alcohol, and in some cases chloroform.

Most of the ptomaines are powerful nitrogenous bases having an alkaline reaction, and combining with acids to form salts, which are soluble in water but less so in alcohol. In many cases they yield striking reactions with the usual alkaloidal reagents, but these are rarely characteristic.

The chemical constitution of the ptomaines, like the plant alkaloids, is very complex. They can be mainly classed as (*a*) Amines; (*b*) Ammonium bases; (*c*) Pyridine derivatives; (*d*) Imido-bases; (*e*) Amido-acids, in addition to a large number of bodies, the constitution of which is at present unknown.

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, $C_5H_{14}N_2$—*Pentamethylene-diamine.*—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.

Putrescin, $C_4H_{12}N_2$ (*Dimethyl-ethylene-diamine*).—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.

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 saprin and cadaverin, and is said to be 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. It is described as crystallising in needles, which gradually decompose on exposure to moist air. It has a 'dry' taste and smells of stale cheese. Tyrotoxin is not precipitated by the majority of the usual alkaloidal reagents. In constitution Vaughan believes this compound to be diazobenzene—potassoxide, $C_6H_5.N_2.OK$. 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.

Vaughan found that three months are required for the formation of tyrotoxicon in milk kept in tightly-stoppered bottles, but that its formation was hastened by the presence of the *B. butyricus*, the poison then being produced in about ten days. This has been confirmed by Frith, who found that if a piece of rancid butter were suspended in the liquid, the poison can be detected in as little as five days.

For the detection of tyrotoxicon in suspected milk, cheese or ice-cream, Vaughan recommends the following process: The curdled filtrate from the milk or ice-cream, or the filtered cold water extract of the cheese, is neutralised with sodium carbonate, transferred to a separator, and shaken with its own volume of pure ether. The mixture is allowed to stand for twenty-four hours, or until the ether has separated, when it is allowed to evaporate spontaneously in an open dish. The residue is dissolved in a little water, and the liquid again shaken with ether, the ethereal liquid again separated and allowed to evaporate spontaneously.

The residue is then taken up in a few drops of distilled water. The liquid which contains the tyrotoxicon, if present, is tested as follows: A little of the liquid is poured upon a porcelain tile with a few drops of a freshly-prepared mixture of equal parts of phenol and pure sulphuric acid, free from nitrous compounds. In the presence of tyrotoxicon a coloration varying from yellow to orange-red, and ultimately violet, will be obtained. The remainder of the liquid should be tested by placing upon the tongue of a suitable animal, and the physiological effects observed.

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. 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 Weyl. This base was also obtained by Brieger from an amputated arm of a patient with tetanus. It is a strongly-alkaline deep-yellow liquid base, permanent in alkaline solution, but quickly decomposes in presence of acids. 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.—Brieger isolated two toxins from cultures of this organism. One induces cramps and muscular tremors in all small animals, the other diarrhœa and symptoms of collapse.

Separation of the Ptomaines. — The chemical methods employed by many of the workers upon these bodies in the past are open to severe criticism. The processes used were very complicated, involving treatment of the raw material with acids under heat, boilings at the ordinary temperature, etc., this alone being sufficient to cause alteration in albuminous bodies present. Owing to the unstable nature of the ptomaines, and their susceptibility to oxidation, all evaporation of solutions containing them should be carried on under reduced pressure, and at a temperature not exceeding 45° C.

Several of the ptomaines, like the vegetable alkaloids, can be obtained from solutions containing them by extraction with ether, after setting them free with alkali. The solution should, of course, be previously freed from other bodies soluble in ether by extraction with this solvent in acid solution.

Brieger's Method.—The liquid culture of the organism, or the filtered decoction of the ptomaine-containing body, is rendered faintly acid with hydrochloric acid, and gently warmed. 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 under reduced pressure. A solution of mercuric bichloride is then 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 production of black sulphide of mercury; this is then filtered off. The filtrate is then carefully concentrated by very careful evaporation, under reduced pressure, until

crystallisation begins to take place. All the inorganic salts crystallise out first; these are removed, and (*a*) in case of the solid ptomaines 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. (*b*) In the case of the liquid ptomaines the mother-liquid is rendered faintly alkaline, the ptomaine extracted with a suitable solvent and separated. This is then evaporated in vacuo, when the base is obtained in a free condition.

The Stas-Otto process,* such as is used for the extraction of strychnine, may also be used for the extraction of ptomaines from animal matters.

Great care should be taken to use perfectly pure reagents in any work connected with the extraction of ptomaines. Ether and amylic alcohol are very liable to contain traces of pyridine and other bases, which are liable to be mistaken for ptomaines.

Toxalbumoses, Bacterial Proteins, 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.

* For a description of this somewhat lengthy process, and also a further account of the chemistry of the ptomaines, see Allen's 'Commercial Organic Analysis,' vol. iii., parts ii. and iii.

The introduction of the use of porcelain filters, whereby liquids could be rendered bacteria free, encouraged the further inquiry into the nature of their toxins.

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.

The virulence of some of these toxins is very considerable. A tetanus toxin has been prepared, of which 0·00005 milligramme killed a mouse weighing 15 grammes; a man weighing 70 kilogrammes, with the same susceptibility, would be killed by 0·23 milligrammes. This would make the poison about 300 times more potent than strychnine.

Much work has yet to be done on these bacterial toxins

* *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.

to determine their true character and significance. A very important research has recently been carried on by Brieger and Boer (*Zeitschrift für Hygiene*, xxi., 268), who, working on filtered cultures of diphtheria and tetanus, have isolated characteristic toxic bodies by a process of precipitation by certain metallic salts. These bodies do not give the reactions of either peptone or albumose, and their composition is at present quite unknown. They may be entirely separate bodies, or maybe the toxicity of the toxalbumoses of Fränkel, Roux, Martin, and others, may be due to these bodies which have been carried down with the albumoses during their precipitation, which, from the chemical point of view, is quite possible. In support of this theory are the facts that the organisms of diphtheria, tetanus, tubercle, and cholera produce toxines in non-albuminous culture liquids, and therefore the toxines may be formed within the body of the organism, and not as a result of the breakdown of the proteids of the culture medium.

Intracellular Poisons.—Klein,* Buchner, and others have 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 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

* Local Government Board Report, 1893-94, p. 469.

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, 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 is also concerned with two kinds of poisons. But neither tetanus nor diphtheria is due to the action of 'intracellular' poisons; in these diseases the specific organisms remain 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.

Sero-Therapy.—The results of the outcome of bacteriological research in the past have been, generally speaking, towards the promotion of the principles of preventive medicine, but the recent discovery of, and the application of, antitoxins in combating disease must be ranked as the greatest advance of the century in the realm of curative medicine.

It was pointed out by Fedor and Nuttall that anthrax bacilli were killed by admixture with fresh-drawn blood. Bouchard found that, although normal blood could be used to cultivate the *Bacillus pyocyaneus*, the serum of a rabbit which had been rendered immune to this organism will attenuate or positively destroy the pathogenic characters of the bacillus. Jasuhara and Ogata obtained similar results with anthrax bacilli. Similar researches were made by Emmerich and Mastbaum with the bacillus of swine fever, and they found that the blood serum of an immune

rabbit could be used to prevent the progress of this disease in animals already suffering from it.

It was found by Ehrlich in the case of the vegetable albuminoid poisons, ricin (from *Ricinus communis*) and abrin (from *Abrus precatoris*), and in the case of serpent venom by Fraser and Calmette, that the serum of animals immunised against these substances conferred a protective effect when injected with these toxins into other animals. Ehrlich found that the serum of a mouse which had been immunised by feeding with very minute but gradually increasing doses of ricin had the power of protecting another mouse against as much as forty times the lethal dose of that poison.

The greatest advance in this direction was made by Behring, Kitasato, Tizzoni, and others, whose experimental researches showed that by repeated injections of animals with small and at first weakened but gradually increasing and non-virulent doses of either the living culture or the specific toxins a state of gradually increasing resistance is acquired by these animals against these diseases, and that this resistance is proportionate to the amount of antecedent injections; further, that as the resistance increases the blood attains an immunising power in increasing proportion, not only as regards the subject from which the blood is derived, but also as regards a new subject; that is to say that the formation and presence of these anti-bodies become not only increasingly marked in the animal which is being immunised, but if injected into a fresh animal they are capable of furnishing this latter with a proportionate degree of resistance against subsequent infection. They have further shown that the immunising power of such blood serum comes into action even after infection has already taken place, that is to say, the blood serum has a curative therapeutic action.

The great amount of work that has already been done in connection with the antitoxin treatment of diphtheria, tetanus, erysipelas, typhoid, pneumonia, and plague, has been the outcome of this line of research.

The view that the condition of acquired immunity is due to specific substances in the blood is not new; but what is new, however, is the experimental proof that such 'anti-bodies' do exist in the blood of an animal that has been artificially immunised, as also in the blood of human beings that have acquired immunity by having passed previously through an attack of the disease.

Many of the antitoxic sera possess a most marked 'specificity.' That is to say, although a given serum has a most marked protective or curative action on cases infected with the corresponding microbe, yet its immunising action is not entirely confined to the one affection. Diphtheria antitoxin has some protective action not only against the diphtheria bacillus, but also against the bacillus of typhoid and against streptococcic infection; not only this, but that a given animal, once immunised against one organism, resists another species of organism far more readily than a normal control animal. Starting with this knowledge, several workers have for some time past been engaged in increasing the natural resistance of a single animal against more than a single infective process, and strong hopes are expressed of eventually obtaining by this means a serum of exalted immunising powers against each of the original microbes.

But little is at present known with respect to the nature of the 'anti-bodies' present in the blood serum of immune animals, or of the means whereby they exercise their property of neutralising toxins or bactericidal powers.

The chemical nature of these anti or protective bodies is

probably that of a proteid, possibly a globulin. Hankin isolated a proteid body from the blood serum of a naturally immune rat, which possessed the property of destroying the pathogenic power of anthrax bacilli. Furthermore, the injection of this proteid, together with anthrax spores, into mice prevented the development of the disease. Hankin has suggested the term *defensive proteids*, and Buchner the term *alexins* (from ἀλεξω, I defend), to denote these antibodies. They are destroyed by a temperature of 60° C., and are precipitated by alcohol and ammonium sulphate, and correspond generally in character with the enzymes or unorganised ferments.

Preparation of Diphtheric Antitoxin.—The steps in the preparation are as follows: First, the preparation of a powerful toxin; second, the estimation of the power of the toxin; third, the gradual immunisation of a suitable animal by the inoculation of gradual and increasing doses of toxin; fourth, the testing and standardisation of the resulting blood serum.

Preparation of the Toxin.—The diphtheria toxin is prepared by growing a pure virulent culture of the Klebs-Löffler bacillus in slightly alkaline beef broth at 37° C., in flat-bottomed flasks, supplied with a regulated current of sterile air. Some observers state that the current of air is quite unnecessary. Liquid blood serum may be used instead of the broth for the preparation of the toxin. 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. The growth gradually slackens, and the operation is usually complete at the end of three weeks. The culture is then filtered through a Pasteur-Chamberland filter, to separate the organisms from their toxic products. The toxic strength of the filtrate is next ascertained experimentally by finding

what volume is required to produce the death of a guinea-pig weighing about 500 grammes in forty-eight hours. This should be effected by $\frac{1}{10}$ c.c., and if the toxin is at or near this standard, it is ready to begin the process of immunising an animal.

Immunisation of the Animal.—Horses are the best and most convenient animals for this purpose, since they are able to yield large quantities of serum without injury to their health. It is a matter of the greatest importance that the animals should be in a condition of perfect health. They should be particularly examined to ascertain their freedom from glanders and tuberculosis. The horses should not 'react' when tested by injections of malleïn and tuberculin.

Small but gradually increasing doses of the diphtheria toxin are now injected from time to time into the animal, the place chosen for the inoculation being the apex of the shoulder. A slight swelling makes its appearance, and after a few days subsides, when the operation is repeated, using a larger quantity of toxin. A swelling again appears; when this has in turn subsided, further injections are again made, till it is possible to inject so large a quantity as 500 c.c. of the toxin without injury to the animal's health. Such a degree of resistance as this is developed generally after about five or six months' treatment. As pointed out by Behring, immunisation takes place most rapidly when each injection of toxin produces a reaction in the form of localised inflammatory swellings; that is to say, the dose should be as large as possible, so long as general injurious effects are not produced. When the animal has reached a sufficiently high state of immunisation, it is ready to furnish supplies of antitoxic serum. For this purpose the blood is drawn off from the animal under due aseptic precautions. This is done by placing a sterile cannula in the jugular

vein, and drawing off the required quantity of blood into sterile glass bottles. The bottles containing the blood are placed in an ice-chamber to allow the clot to separate, or the clot may be more expeditiously removed by submitting the blood to centrifugal treatment in a suitable machine. The clear serum is then separated and preserved for use by putting up in small sterile, stoppered bottles. Some makers add a small quantity of an antiseptic, such as phenol, camphor, etc., but this should be unnecessary with carefully prepared serum. One of the firms supplying this article evaporate the serum to dryness in vacuo, and send it out in the form of golden-yellow scales, which dissolve in three or four parts of water.

Louis Cobbett (*Journal of Pathology and Bacteriology*, January, 1896) has arrived at the conclusion that the blood serum of normal horses may possess a certain amount of 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 by injecting the horse with a culture of living bacilli.

The preparation of a horse to give serum of very high antitoxic value by the above method involves long treatment, extending to six months or more. Dr. Cartwright Wood has, however, recently devised a method for rapidly producing diphtheria antitoxin, by which he claims that an animal can be rendered immune towards large quantities of diphtheria poison, and also that such animals can be made to produce powerful diphtheria antitoxin. The distinctive feature of the method consists in the use of the products produced by the growth of the diphtheria bacillus in

albuminous fluids made by the addition of serum to ordinary peptone broth. The fluid is allowed to grow for three or four weeks at a temperature of 37° C., and, after filtration, heated for an hour at 65° C. It is claimed for this method that powerful antitoxic serum can be easily produced in a shorter space of time than has hitherto been possible, and that as a consequence the amount of serum necessary to be injected is greatly reduced. Its greater strength will permit of the patient receiving at the beginning of treatment a sufficient quantity of the serum at one injection, by which experience has shown that curative action is exerted in the most marked manner.

During the process of immunisation of an animal by the above methods, a small quantity of blood is withdrawn from time to time, and its antitoxic power tested by the undermentioned method.

Standardising the Serum.—The process of standardising or estimating the antitoxic power of the serum consists of testing the serum against a certain amount of the toxin, conveniently ten times the lethal dose—*e.g.*, 1 c.c. of toxin, of which 0·1 c.c. is the lethal dose. The two chief methods employed for this purpose are those of Behring and Ehrlich and that devised by Roux.

The method usually employed is that of Behring and Ehrlich. The principle of this method consists in estimating precisely the amount of serum required to completely neutralise the effects of ten lethal doses of toxin, when these have been mixed together previously to subcutaneous injection into a guinea-pig.

A serum that contains *one normal antitoxin unit per c.c.* is one of such a strength that $\frac{1}{10}$ of a c.c. completely neutralises the action of ten lethal doses of toxin. Taking this as a basis of calculation, it is easy to determine the number of antitoxin units contained per c.c. in any sample

recorded in the tables by simply dividing the fraction of a c.c. that protects by 10—*e.g.*, when $\frac{1}{1000}$th of a c.c. of a serum protects, the serum contains 100 normal units per c.c.; when $\frac{1}{600}$th c.c. protects, the serum contains 60 normal units per c.c., and so on.

The lethal dose of toxin is determined by estimating the amount of toxin required to kill per 1,000 grammes weight of guinea-pig. Of this toxin, ten times the amount required to kill a guinea-pig of from 200 to 300 grammes weight is injected, together with the antitoxin to be tested, and the effect observed. By noting the absence or presence of local reaction and the increase or loss of weight, it is stated that an opinion may often be formed after twenty-four hours, but that after the lapse of forty-eight hours a decisive conclusion can always be arrived at. When the toxin is completely neutralised, as it should be, the animal should not only live, but there should be no trace of local reaction (œdematous swelling). This swelling may not be apparent in the first twenty-four hours, but a rapid fall in weight will at that time frequently indicate its probable occurrence within the next twenty-four hours. In this connection it should be borne in mind that guinea-pigs, taken from stock and put into small cages, usually rise in weight when not injured by the action of the toxin.

Roux's method of standardising anti-diphtheric serum consists in defining the proportion of serum in relation to the weight of the animal which would protect a guinea-pig against a lethal dose of culture or toxin of the diphtheria bacillus. This method is still made use of at the Pasteur Institute and by other makers, the strength of serum which they consider suitable for use in the treatment of diphtheria cases being one which contains sufficient antitoxin to protect an animal of from 100,000 to 200,000 times the weight of the

serum. Such a serum is stated to contain from 20 to 40 normal units of Behring per c.c.

Antitetanic Serum.—The antitoxic treatment of tetanus has received a great deal of attention, and much experimental work has been done upon the question by Behring and Kitasato in Germany, and by Tizzoni and Cattani in Italy. The former investigators found that immunity could be conferred upon an animal by the injection of small but increasing doses of the toxins, but the degree of immunity so conferred was not very great. More successful results were obtained by injections of toxin mixed with small amounts of iodine terchloride.

Tizzoni and Cattani conferred immunity by injections of broth cultures of the living organisms, which had been attenuated by heat and other methods. Both these methods were very successful in immunising susceptible animals not only against very large amounts of tetanus toxin, but also against injections of the virulent bacilli. The immunity so conferred lasts for several months at least, and the serum of these protected animals also confers immunity on other animals.

Roux and Vaillard immunise horses as follows, the treatment lasting about three months: The virulent tetanus bacilli are cultivated in a broth culture, in an atmosphere of hydrogen, in specially constructed flasks. After about fourteen days' growth, the culture is filtered through a porcelain filter to free it from bacilli. Injections are then made with this toxin daily, subcutaneously or intravenously, starting with 1 c.c. of iodised toxin, gradually increasing the dose until the pure toxin can be injected without danger. The serum is standardised from time to time, and the blood is drawn off and the serum prepared by the methods already described in the case of antidiphtheric serum. It must be admitted that the clinical results

attending the use of antitetanic serum in man have been somewhat disappointing. The reason is not far to seek: the number of immunisation units has probably not been sufficiently high, and the treatment has not been put in operation at a sufficiently early stage in the disease. It is clearly useless in acute cases with a short incubation period, unless employed almost immediately after infection.

The importance of dose and of early employment of the treatment will be seen from the following: Behring advocates the employment of a serum of which 1 gram will protect 1,000,000 grams of body weight. A guinea-pig weighing 200 grams would therefore require 0·0002 grams of serum to protect it against the minimum lethal dose of the toxin, when injected simultaneously. But if infection had already commenced in the animal, 1,000 times this dose would be necessary; a few hours later 10,000 times this dose would be the minimum amount of serum to protect the animal. The antitetanic serum at present sent out by the Pasteur Institute in Paris has a strength of 1 : 1,000,000,000. It is suggested to inject 50 to 100 c.c. in one or two doses.

Antistreptococcic Serum.—The use of antimicrobic serum in the case of streptococcic infection has recently come into extensive use. This serum was first prepared by Marmorek as follows: He found that the virulence of a culture of *Streptococcus pyogenes* could be enormously 'exalted' by passing through a number of guinea-pigs, by intraperitoneal injection. By this means the virulence became so greatly increased that if only one or more individual organisms were introduced into a rabbit, by subcutaneous or intravenous injection, a fatal septicæmia was rapidly produced. Small but gradually increasing doses of these highly virulent streptococci were then injected into a horse, and the injections continued over some consider-

able period of time. The blood serum is standardised against the 'exalted' organisms from time to time, until a sufficiently high antimicrobic power is developed.

Bokenham has also prepared a similar serum by cultivating the streptococci in the first instance in a mixture of broth and human blood serum. Horses and asses were inoculated, and a considerable degree of immunity was established.

This antistreptococcic serum has been used with a very considerable degree of success in erysipelas, puerperal fever, and other cases of streptococcic infection in the human subject.

This serum is a true example of an antimicrobic serum, in contradistinction to an antitoxic serum. It possesses but little or no power of protection against the toxins of *B. streptococcus*.

Anti-typhoid, anti-cholera, anti-plague, anti-pneumococcic sera have been prepared in an analogous manner to the above; an account of these will be found under these respective diseases.

Antivenomous Serum.—Although snake venom has no relation to bacterial toxins, it is important that a short account of antivenomous serum should be given here, more particularly as this important discovery is the direct outcome of the application of bacterial methods. The importance of this discovery, due to the researches of Drs. Calmette and Fraser upon snake venom, may be estimated when it is stated that in British India alone nearly 23,000 individuals are killed yearly as the result of cobra bites.

The difficulty in the past has been to obtain sufficient quantity of the venom for scientific investigation, owing to the fact that the venom glands of the full-grown cobra only contain about 1 c.c. of the liquid venom. But while in charge of the Bacteriological Institute at Saigon, Cochin

China, Dr. Calmette, during the rainy season of the autumn of 1891, was able to obtain a large number of the reptiles known as the cobra di capello, which provided a sufficient amount of venom for the subsequent investigations.

It was found that the venom exercised remarkably rapid toxic effects upon all classes of animals, the serpents themselves only proving refractory; but even these succumbed to very large doses of the venom. Unlike the toxins of bacterial origin, serpent venom can stand a considerable amount of heating without injury to its virulence. The toxic character of cobra venom is in no way diminished by half an hour's heating at 97° C., but its toxic character is destroyed if the temperature is raised to 98° C. The venom of some species of reptile will endure even a greater amount of heat than this without injury, but others lose their virulence at a somewhat lower temperature. The method employed by Dr. Calmette for the preparation of antivenomous serum, as described by him in his paper before the British Medical Association Meeting of 1896, is as follows: The method of procedure for the purpose of vaccinating large animals, such as horses, consists of injecting them with gradually increasing doses of the venom of the cobra, mixed with diminishing quantities of a 1 in 60 solution of hypochlorite of lime. The condition and weight of the animals are watched, so that the injections may be lessened if the animals do not thrive well. Injections of stronger and stronger venom are made, first considerably diluted, and then more concentrated. In order that the horses may yield a thoroughly efficient serum, when they have acquired a sufficiently perfect immunity, it is necessary to inject the venom of as many species of reptile as possible. The process of immunisation lasts at least fifteen months before the animals yield a sufficiently active serum for practical purposes.

The serum now supplied by Dr. Calmette from the Pasteur Institute of Lille is active to the extent of 1 to 200,000, that is to say, it is sufficient to inject as a prophylactic dose a quantity of serum into a rabbit equal to $\frac{1}{200000}$ of its body weight. 0·5 c.c. of this serum is sufficiently active to protect a rabbit against a dose of venom, which otherwise would be lethal in three or four hours, if it is not injected later than half an hour after the bite. The usual dose of the antivenomous serum for a human being is about 10 c.c., and in order to insure success it must be injected as soon after the bite as is practicable.

A large number of cases have been reported from India and other places of the successful treatment of human beings suffering from snake bites by means of this antivenomous serum.

It has been asserted that small doses of cobra venom would immunise animals against all other snake poisons of whatever sort. Dr. Kanthack has recently investigated this matter and reports that there is some reason to believe that cobra antivenomous serum may be capable of neutralising the poison of many kinds of venomous snakes, for the reason that the physiological action of the poisons of these other snakes is the same as, or not greatly different from, that of cobra poison. On the other hand, there are certain venomous snakes whose poison has a different physiological action to that of the cobra, and recorded experiments do not support the view that cobra antivenomous serum will neutralise the poison from these varieties. Dr. Kanthack also records experiments which tend to disprove the statement that chlorinated lime solution prevents the absorption of the snake poison within the animal body.

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—Infection by air, dust, meat, milk, etc.—Evidence given before Royal Commission on Tuberculosis—Resistance of the bacilli to desiccation—Pathogenesis—Special regulations in force in New York and Germany—Presence of tubercle bacilli in air of hospital wards, etc.—Identity of human, avian and bovine tubercle bacilli—Bang's method for eliminating tuberculosis from cattle—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 ; 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 or two distinct methods : (1) *Direct staining ;* (2) *demonstration of the presence of the tubercle bacillus 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. *Counterstain 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 counterstaining 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.

Until ten or a dozen slides have been made and carefully examined with negative results, we cannot safely assert that the bacillus is absent. In doubtful cases it will be safest to inoculate a guinea-pig with sputum, when positive results will be obtained.

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.

It has been shown 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.

Milk is without doubt a very prolific source of infection, as the following facts will show: The milk may become infected from outside sources, such as dust containing dried-up phthisical sputum, but the milk 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 M'Fadyean 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

* Royal Commission on Tuberculosis (1895).

five out of fourteen young girls living together in 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

* Royal Commission on Tuberculosis (1895).

suspected to be affected with tuberculosis. The 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.

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 Kossel, Brouardel, and Picini, the disease was found latent, post-mortem, in 40 to 60 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. 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 leprœ*, 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. Theodore Williams 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 recog-

nised, 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.

In any experiments made to test the resistance of the bacillus of tubercle to high temperatures or to the action of disinfectants, the organism may be so attenuated that, if inoculated on to media, it may not be possible to demonstrate its vitality by culture within a reasonable time, and in such cases it is much preferable to inoculate the material to be tested for the tubercle bacilli into an animal such as a guinea-pig or a rabbit—the former for preference.

Nocard considers that the bacillus tuberculosis is the same organism in man, cattle and birds, though modified by its surroundings. To support this view, he states that tuberculin can be prepared from all three varieties of the bacillus which has the same properties and activity. The following are the characteristic differences observable in cultures: The bacilli derived from birds seem to be a little longer than those met with in tuberculous mammalia, they are stained by the same reagents and flourish on the same media. The bacillus of birds seems to be more vigorous in growth, and it grows at 43° C., at which temperature the bacillus of human tuberculosis is arrested. Its cultures on solid media are softer and more greasy, and less tightly packed than those of human tuberculosis. Tuberculosis avium is, according to Nocard, difficult to inoculate into the guinea-pig, the dog, the sheep, and the horse, but the rabbit is readily infected.

Professor Bang's method for eliminating tuberculosis from a herd of cattle depends on the fact that calves are

rarely tuberculous, and become so by being infected through being kept with diseased cows. He therefore tests the whole herd by means of tuberculin, and isolates those that react as infected or suspicious. The herd is divided into two sections, which are separated from one another and have separate attendants and separate buildings; they are, however, allowed to mix when out in the fields. Every six months the healthy side were tested with tuberculin, and any that were found to react were placed on the infected side, while all calves were placed on the healthy side. The animals in the tuberculous side which were obviously tuberculous were got rid of, but those that were apparently healthy were used for breeding. After this system had been carried on for three years, the number of the tuberculous animals in the herd had been reduced to a very considerable extent, and the results seem to show the disease may be to a large extent eliminated by the simple plan of separating the animals that react to tuberculin from those that do not, and removing all calves, as soon as they are born, from their infected surroundings.

Koch's Tuberculin Treatment.—Koch found that if a local tubercular infection was set up in a guinea-pig by subcutaneous injection, and later a second subcutaneous injection of the virulent or dead bacilli was made in another part of the body, ulceration set up in the primary tubercular nodule, the wound healing, and the animal did not die of tuberculosis. From the result of prolonged investigation Koch found that there appeared to exist in tubercle bacilli an albumose-like body which exerted a healing action in tuberculosis. He prepared a glycerine extract of this body and called it 'tuberculin.' It was prepared as follows: A peptone broth containing 5 per cent. of glycerine was inoculated with virulent bacilli, and incubated at blood-heat for about eight weeks. The culture

was then evaporated down to one-tenth of its bulk on a water-bath. The result was tuberculin, and would contain the bacterial products such as would not be destroyed at 100° C.

The injection of 0·25 c.c. of tuberculin into a healthy individual induces in three or four hours laboured breathing, malaise, and moderate pyrexia, all of which effects pass off in twenty-four hours. The injection, however, of as little as 0·01 c.c. into a tubercular person gives rise to similar symptoms in a very much more aggravated form, great inflammatory reaction and necrosis occurring round the tubercular focus, resulting in a casting off of the tubercular mass. This is well seen in the case of lupus.

The publication of Koch's results in 1890 raised great hopes that a means of combating tuberculosis had been discovered, and in the case of lupus the treatment certainly appeared to be attended with some success. This remedy, although 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 antitoxin treatments in diphtheria, cholera, etc.

It has also been found of use in the diagnosis of tubercle. 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 failures being only about 2 per cent. The injection of 0·05 c.c. causes a rise of temperature of 2° to 3° F. above the normal in from eight to twelve hours.

Koch has recently (April, 1897) published the results of his later researches on tuberculosis. He found that tubercle bacilli contain two fatty acids of different solubility in

alcohol, and he found that it was apparently due to these substances which rendered the reabsorption of the bacilli so difficult in the animal body. As a result of his investigations, Koch announced that he has prepared three new modifications of the original tuberculin. These he names respectively Tuberculin A, O, and R, and for their preparation it is necessary to employ virulent cultures. Tuberculin A is prepared by extracting the bacilli with decinormal soda solution, neutralising, and filtering. This acts very much like ordinary tuberculin, but the reaction is much more severe. Tuberculin O is prepared by drying and pounding tubercle bacilli, and extracting with distilled water; the emulsion is then centrifugalised. The residue left in the centrifuge from the last operation is dried, pounded in an agate mortar, and extracted with water as before, these processes being repeated until hardly any solid residue is left. The whitish opalescent liquids resulting from these operations are mixed, and the result is Tuberculin R.

Tested on animals, the Tuberculin O produced almost identical effects with the alkaline extract (Tuberculin A), except that no abscesses are produced at the seat of inoculation. It had, however, but little immunising effect. The Tuberculin R, on the contrary, possesses very decided immunisation effects, with but little reaction. This comparative absence of reaction is a great improvement upon the original tuberculin. The preparation by hand of the last preparation is not unattended by risk, but it can be perfectly well made by machinery, and is manufactured under Dr. Koch's direction by the firm of Meister Lucius and Brüning, of Höchst-on-the-Main. The use and doseage of the preparation (Tuberculin R) is simple. The fluid is made to contain 10 milligrammes of solid matter in the cubic centimetre. This is diluted with sterile salt solution

to bring it to the required strength. The dose commences with $\frac{1}{500}$ milligramme. If reaction occurs, this must be further diminished. Injections are made every other day with slightly increasing doses, so that there is never a rise of temperature of over 1° F. Koch has by this treatment found great improvement to take place in cases of lupus, and satisfactory results have also followed the employment of the remedy in tuberculosis of the lungs. But we must beware of expecting, at present, an absolute cure for consumption. In early cases of phthisis the treatment promises to be useful and is worthy of careful trial, but, of course, much further work must be done before we can judge of the measure of success of this new treatment.

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 leprœ* was first described by Hansen in 1871. 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 aqueous 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 sub-cultured 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 appears to indicate that leprosy is exclusively a human disease. It is unknown how the bacillus is conveyed: and the *causa*

* Leprosy Commission Report, p. 425.

vera of leprosy still remains unsettled. There seems to be some evidence to prove that it is not spread by contagion or heredity, though there are examples which appear to favour both. As recently as 1897, Ehlers of Copenhagen has re-affirmed his belief in the contagiousness of leprosy, whilst Virchow has declared that it is not highly contagious. 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.

(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 in 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.'

At the great Leprosy Congress held in Berlin, October,

1897, Hansen again defended segregation, which he maintained had largely caused the decrease of leprosy in Norway. At the same time he advocated more humane isolation after the proposals of our own Leprosy Commission in India. At this same Congress, Besnier suggested that the nasal secretion played an important rôle in spreading the disease.

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 (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-

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

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.

Sticker (*Münch. med. Woch.*, 1897, Nos. 39 and 40) states that (1) lepers eliminate with the nasal secretion the leprous bacillus often in extraordinary numbers, and during the greatest period of the disease, and (2) the front part of the nasal mucous membrane, and mostly that covering the nasal septum, is the place which leprosy first, and perhaps always, attacks. The author has examined 143 lepers from this point of view. In 55 out of 57 cases of tubercular leprosy the leprous bacillus was found in the nasal secretion, and yet in only 2 cases were there any leprous nodules in the nose. In 45 out of 68 cases of anæsthetic leprosy, and in 27 out of 28 of the mixed form, the bacillus was also found.

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.

The French Academy of Medicine have recently been trying the new serum-therapy in leprosy, at the St. Louis

Hospital, Paris. The preliminary report of this committee which was presented on September 28, 1897, is on the whole unfavourable.

ANTHRAX.

Discovery and morphology of the organism—Growth on media—Staining of the bacilli—Resistance of the bacilli and spores to external influences—Pathogenesis—Report of the Anthrax Committee—Infection from imported hides — Protective vaccination for cattle — '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 in 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 favour-

able 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

* Unless the frog is heated to 37° C.

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 pleurisy.

In November, 1896, the Home Office appointed a committee upon anthrax to make inquiry into, and report on, the conditions of work as they affect the health of the operatives in the industries in which anthrax is alleged to occur, and to report what, if any, special rules should be made or special requirements enforced under the Factories and Workshops Act, 1895, for the protection of persons employed in those industries. The committee having taken the evidence of a large number of persons, including official and other experts, as well as employers and employed, and having visited numerous works, have now presented their report. They say that two principal forms of the disease have to be recognised—external anthrax or malignant pustule, and internal anthrax. The period of incubation is short, and does not usually exceed three days. Among wool-sorters both varieties are met with, but in the sorting of dry hides and skins—another dusty process—only the external variety is heard of. Amongst rag-sorters there is no special incidence of anthrax, although in

Hungary it is met with so often that the disease has become known as "rag disease." A résumé is given in the report of the numerous outbreaks which have occurred in this country, and the opinion is expressed that many non-fatal cases have not been reported. The evidence produced shows that the principal classes of material by means of which anthrax has been generally conveyed to human beings in British factories and workshops are (1) wool (including camel-hair and goat-hair); (2) hides and skins; (3) horse-hair and bristles. The various trades in which these articles are manipulated are considered in detail, and in summarising their conclusions the committee say that there are three principal directions from which the question of prevention may be approached—the exclusion of infected material from use, disinfection, and the employment of insusceptible persons. As in many countries the disease is endemic, we cannot count upon due care either in preventing the spread of the malady or even in excluding infected materials from those forwarded for sale and export. The suggestion of disinfection, promising as it may appear at first sight, does not bear examination. Not single bales (known to be dangerous) are in question, but thousands of bales, any one of which may be dangerous. Hence, it is evident that upon such an enormous scale anything worth calling disinfection is hopeless.

A number of cases of anthrax, resulting in a number of deaths, have recently been reported in various parts of the United States, from tanneries dealing with hides imported from China. Also a number of cattle have been infected as the result of drinking water from rivers and creeks receiving the waste liquors from these works.

The virulence of *Bacillus anthracis* 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.

Up to the end of 1890 over three million head of cattle have been inoculated with attenuated anthrax virus prepared at the Pasteur Institute, Paris.

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.' Lately a process of destroying carcases has been introduced in which the entire animal is dissolved in sulphuric acid.

CHAPTER VI.

TYPHOID.

Discovery and morphology of the organism—Method of staining—Growth on media—Pseudo-typhoid organisms—Occurrence and distribution of enteric fever—Conveyance of typhoid by water, milk, dust, shell-fish, vegetables, etc.—Pathogenesis—The bacteriological diagnosis of enteric fever—Widal's serum reaction—Elsner's method of diagnosis—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. 89, *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 often has a bluish iridescence.	Dirty yellowish-white expansion, which does not spread 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.	Gives no indol reaction.	Gives a well-marked indol reaction after from twenty-four to forty-eight hours.
Widal's typhoid serum reaction.	Agglutination of bacilli.	No change.

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 in 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 has been steadily 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

* 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. See also reprinted report.

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 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 sought to be 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. For the methods employed to isolate the typhoid bacillus, see under The Examination of Water.

According to some 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 apparent fact that water contaminated with fæcal matter may be instrumental in causing typhoid fever without the recognition 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. Germano and Maurea,† after a very prolonged investigation, have isolated no less than thirty varieties of typhoid-like bacilli. 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

* *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.

as to their vitality, and as to the retention of their differential 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 con-

siderable 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.

Forty-eight epidemics of typhoid fever have been recorded since 1882 as directly due to contaminated milk supplies by Dr. E. Hart, in the pages of *The British Medical Journal.* See the reprinted 'Report on the Influence of Milk in spreading Zymotic Diseases' for the detailed reports of the outbreaks.

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.

Dr. E. Cautley (Local Government Board Report, 1896-7) has recently investigated the behaviour of the typhoid bacillus in milk. The following are his conclusions:

The typhoid bacillus will live in milk under the conditions that ordinarily prevail in a household. When this bacillus has been artificially added in large amount to milk in the condition in which it commonly reaches the consumer, the presence of the microbe *in the living state* may be demonstrated after the milk thus treated has been kept several days.

There is no indication from the above investigations that this microbe is capable of multiplication under the conditions in question. Judging from the results obtained, it is

very probable that the number present rapidly diminishes in milk which is kept.

It has been proved that the typhoid bacillus will live in sterile milk which is curdled by the addition of bacillus lactis.

It will also live in milk which has turned sour at the temperature of the room in which it is kept.

These latter results indicate that it is quite possible for the typhoid bacillus to exist in curd cheeses.

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 observations 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 in 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.

The Serum Diagnosis of Typhoid.—This new addition to our diagnostic resources depends on the fact that the serum of an individual immunised against the typhoid bacillus exerts a clumping or agglomerating action on actively motile typhoid bacilli, when suspended in a suitable medium. The reaction was discovered independently by Widal and Grünbaum, and the idea of applying the reaction for the

diagnosis of typhoid fever was at once taken up by several investigators.

To carry out the test two things are required—the blood serum to be tested in a diluted condition, and a broth culture of typhoid bacilli, not more than twenty-four hours old, containing the organisms in an actively motile condition. In place of the last a little of the growth on an agar culture of the same age may be taken, and rubbed up with a little broth in a watch-glass so as to produce a uniform emulsion. Several observers have published some modification of the test, but the following method as described by Grünbaum* is probably the best: The lobe of one of the patient's ears is washed with ether or some other suitable antiseptic. The ear is preferable to the finger because it is more easily cleaned and the epidermis is thinner (especially among the working classes); it is also more vascular, yet less sensitive. With a straight spear-pointed surgical needle, or with one half of a fine steel nib, a prick is made into the lobe and the blood collected in a capillary tube. By suitable pressure and a little patience an almost indefinite quantity can be collected from this source. The capillary tube should not be too narrow; an ordinary vaccine tube is a little too wide, but can be used without much disadvantage if no other is available. On the whole, capillary tubes bent into V-shape are the most convenient. Care should be taken that the blood forms a continuous column in the tube; if it has flowed a little way down the tube in the interval required to press out a fresh drop of blood from the ear, it should be made to flow back to the end before collecting again, so that there may be no air space in the tube. The blood having been collected, the ends of the tube should be cautiously sealed in the flame, care being taken that the blood is so far away from

* 'Treatment,' vol. i., p. 73.

the ends as not to become heated. It is preferable for the capillary tubes to be sterile, in case of its being impossible to examine the blood soon after collection; but it is not essential if the interval does not exceed a few hours. It is best if the tube can be centrifugalised so as to obtain a clear serum; if this cannot be done, it will be found that, after a few hours, on breaking off the ends of the tube (and in the case of a V-tube, in the middle also), a string of clot and some serum can be blown out on to a glass slide, and the serum with some corpuscles collected in another capillary tube, or in the diluting pipette to be described immediately. If the tube has been centrifugalised it is broken off at the junction of serum and corpuscles, and the clear serum blown out.

A diluting pipette is easily made from a small capillary tube with a small bulb, with the aid of the graduated pipettes of a hæmocytometer. These are graduated for 5 c.mm. and 20 c.mm.; a mark is made on the diluting pipette at 5 c.mm. and 80 c.mm., so that we can dilute the serum in the proportion of 1 to 16. This is done by sucking the serum from the glass slide up to the first mark, and then bouillon or ·6 per cent. NaCl solution after it up to the second mark, blowing the whole out again on to the glass slide and thoroughly mixing. It is well to suck up and blow out two or three times to ensure thorough mixing.

If a diluting pipette is not available, the following method as recommended by Delépine may be adopted. Fifteen drops of broth or salt solution are successively taken up in a loop of platinum wire, and placed on a glass slide; close to them is placed one drop of the serum, and the sixteen drops are then thoroughly mixed.

A small drop of this diluted serum has then to be mixed on a cover-glass with an equal-sized drop of a broth culture

of the typhoid bacillus. The culture should preferably be from a specimen of low virulence, but it is not an essential point. It is also better (but again not essential, if free from clumps) for the culture not to be over twenty-four hours old. If no broth culture is available, an agar culture rubbed up in broth or salt solution will do as well—and for comparative experiments, even better. A control cover-glass preparation of the culture should always be made and examined.

The mixed drop of diluted serum and of culture should have very little depth, as the 'clumps' have a tendency to sink and get beyond the focal distance of the lens. The cover-glass is now placed on a glass slide having a central hollow, which is surrounded by vaseline to prevent evaporation, or on an ordinary glass slide covered with a piece of moistened blotting-paper with the central portion cut out, and examined with an immersion lens. At first the bacilli may be moving actively about, but if the case is one of enteric fever they soon slow down or stop, then gradually groups of two or three form; these groups soon hang on to each other until nearly all are collected into various crowds or 'clumps' with very few isolated bacilli left. If the reaction is complete within thirty minutes the case is certainly one of enteric fever. Generally it is not complete, and there may be groups of only ten or twenty, but the occurrence of grouping and loss of movement are in themselves almost as decisive.

The dilution of the serum is necessary because the serum of normal individuals will often, undiluted, evince an agglutinative power, but not, so far as my experience goes, in a total dilution of less than one to sixteen. Therefore neglect of sufficient dilution may lead, and has already led, into error.

The time limit is necessary for a similar reason. Very

many normal sera will act, even when diluted, if left an indefinite time.

Elsner's Method of Diagnosis.—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.

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, 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. 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, 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

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

one for the other. By this method the author examined thirty different colon and typhoid cultures, and in each case obtained the same result. 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. 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, 1896, 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 protective 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.

Widal and Sanarelli have also succeeded in immunising guinea-pigs against the subsequent intraperitoneal injection of virulent typhoid bacilli by repeated and increasing

subcutaneous doses of typhoid cultures, in which the living bacilli had been killed by the action of heat or of antiseptics.

Widal, Grünbaum, Pfeiffer, and others, have noticed that the serum of individuals convalescent from enteric fever has an inimical effect upon the typhoid bacilli, it apparently containing a body which exercises a devitalising action on the bacilli. Upon these results several anti-typhoid serums have been recently introduced by various workers. Drs. Wright and Semple of the Army Medical School, Netley, have recently introduced a protective vaccine treatment against typhoid, very much on the same lines as the anti-choleraic treatment, for which they claim a great measure of success.

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 im-

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

munity. (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 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—Varieties of organisms—Pseudo-diphtheria bacilli—Other organisms accompanying diphtheria—Distribution and occurrence—Transmission by means of milk, dust, water, etc.—Pathogenesis—Antitoxin treatment—Method of injection of antitoxin serum—Results and advantages of the treatment—Need for proper dosage and standardisation of serum—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 will retain its virulence for months under certain conditions. 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*. 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 considerable periods of time, even amounting to seven and eight weeks after complete apparent recovery from 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.

According to Dr. Cartwright Wood, an immediate diagnosis can be made by direct staining in 60 per cent. of cases of diphtheria.

Pseudo-Diphtheria Organisms.—Peters (*British Medical Journal*, 1895, vol. ii., p. 1557, Path. Soc.) has described the following varieties of the diphtheria bacillus: (1) Long Klebs-Löffler bacillus; (2) short diphtheria bacillus; (3) short pseudo-diphtheria bacillus. The latter is widespread and non-virulent, and he regards it as possibly being unconnected with the diphtheria bacillus, though closely resembling it.

Hoffman (*Wiener med. Wochenschr.*, 1888, Nos. 3 and 4), Roux, and Yersin (*Ann. de l'Institut Pasteur*, iv., 1890), and others, have also described pseudo-diphtheria bacilli, which are non-virulent in character, and are shorter and plumper than the normal Klebs-Löffler organism, but are otherwise similar in cultural characters.

Klein (*Local Government Board Report*, 1889) has described a pseudo-diphtheria bacillus which might be confused with the Klebs-Löffler bacillus, 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. He states, however, that its occurrence is so rare that it is usually neglected.

Roux and Yersin have obtained attenuated varieties of the diphtheria bacillus by cultivating it at a temperature slightly above blood-heat and freely supplied with air, and they have suggested that a similar attenuation of

pathogenic power may occur in the fauces of convalescence from the disease, and that possibly the bacilli of low pathogenic power which have been described by various observers may have in this way been produced from the true diphtheria bacillus. They also found that cultures of diphtheria obtained from false membrane which had been kept by them in a dry condition for some months grew readily, but were destitute of virulence.

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. Viru-

lent Klebs-Löffler bacilli have been occasionally met with by several investigators in the normal throats of apparently perfectly healthy individuals.

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.

Professor Smith, in a recent report for the School Board of London, states that in his opinion elementary schools do not play so great a part in the dissemination of diphtheria as is usually supposed, and he proves this point by the collection of a mass of statistics dealing with cases which have come under his notice as Medical Officer of the School Board. In no case, he says, has it been necessary to order the closure of the school on account of the outbreak of diphtheria. Accompanying his report are a series of coloured maps, illustrating the prevalence of diphtheria in each county during the past six years, which go to show that diphtheria has, for some reason which we do not at present understand, become concentrated in the neighbourhood of Middlesex.

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.

Hewlett (*Trans. Path. Soc.*, Lond., 1896, p. 360) describes a case in which diphtheria bacilli were present in the throat for about twenty-two weeks after the commencement of convalescence from an attack of diphtheria. The bacilli were obtained repeatedly in cultivations from the throat during the whole of this period, and up to the last were markedly virulent as tested by inoculation experiments. The case was that of a schoolboy, who remained in good health all the time, and but for the bacteriological examination would have returned to boarding-school, where he would undoubtedly have proved a possible, if not a probable, source of infection to his schoolfellows.

As infection may be transmitted in this way, strict isolation of convalescents should, where possible, be insisted on, until the bacillus has disappeared, particularly in the case of children attending school. 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.

The spread of the disease by the agency of milk is a matter deserving special attention, particularly as the establishment of creameries in so many parts of the country may prove to be factors of great importance in the dissemination of the disease. At these creameries the milk of a very large number of cows is received twice a day, and mixed in a large tank before passing through the separator. Thus, if the milk from one cow or one dairy contains the bacillus of diphtheria, it would be impossible to avoid the infection of the whole. The skim milk so obtained is at some factories condensed in tins, when the bacillus would probably be killed; but at others the milk is sold for human consumption, and as the organism multiplies in milk at an enormous rate, it is easy to understand how epidemics of great magnitude might be caused. The only remedy for this would be to insist on the sterilization of all milk leaving the dairy factory, or the constant supervision and inspection of all cows and employés.

An epidemic of diphtheria due to the milk-supply will exhibit features similar to those of a typhoid epidemic produced in the same way. (1) The outbreak is sudden, and many attacks occur together. (2) The greater proportion of households attacked will have a common milk-supply. (3) The incidence of the disease will fall chiefly on the principal consumers.

Fifteen epidemics of diphtheria have been recorded as directly due to contaminated milk-supplies by Dr. E. Hart in the pages of the *British Medical Journal* since 1881. See reprinted 'Report on the Influence of Milk in spreading Zymotic Diseases' for the detailed reports of the outbreaks.

Dust is undoubtedly a very important source of the conveyance of the disease, although it has been stated by

Flügge (*Zeitschr. f. Hyg.*, 1894) that the diphtheria bacillus perishes at that degree of dryness which is necessary to permit the formation of dust. The accuracy of this statement is a matter of considerable importance, as it practically determines the extent to which the air can be regarded as a means of distributing the infection. The experiments of Reyes (*Annali d'Igiene Sperimentale*, 1895, v., 501), however, contradict this statement. Working with virulent agar cultures, he suspended small portions in sterilised distilled water, and infected with the emulsion, cloth, silk, blotting-paper, earth and sand. He exposed these in various experiments, both in sunlight and in the dark, and both dry and wet. In some experiments the drying was conducted with sulphuric acid, and in others mere evaporation, as would occur in practice, was employed. In one case, with the object of directly examining Flügge's conclusion, a quantity of infected powdered mud was placed in a sterile drying-stove, through which a current of air passed continually, watch-glasses containing sterilised water being placed at various heights in the stove. All these glasses proved to be infected with the organism, which was of full virulence. The net outcome of this research is to establish that the presence of moisture favours the growth of the organism, although it does not immediately perish on desiccation. Thus, when desiccated in the presence of sulphuric acid, it may survive as long as forty-eight hours. When, however, it is desiccated in the ordinary way, it will remain alive in cloth, silk or paper, for several days; in sand for over two weeks; and in powdered mud up to a hundred days. The survival is still longer when these media are moist, and extended in the case of sand to more than 120 days. Exposure to diffused sunlight only reduces the life of the organism by a few days, and temperature within ordinary atmospheric limits exercises no appreciable

difference. Without assuming that these results would be quantitatively true of all specimens of the diphtheria organism—an assumption which is carefully to be avoided in all inferences from bacteriological experiment—it is clear from these results that even after an amount of desiccation permitting the organisms to be freely carried by the atmosphere, dust may contain living and virulent diphtheria bacilli, and the air must be regarded as a powerful means of spreading the disease.

Several researches have demonstrated the fact that the diphtheria organism may be capable of growing in water. The experiments of Démétriadès (*Archives de Médecine Expérimentale,* 1895) are the best evidence of this capacity in the diphtheria bacillus. He inoculated it in sterilised distilled water, and found that it began to diminish only on the seventh day, and did not disappear until the twenty-eighth day. In such results the question always rises whether the water was not rendered more favourable to the growth of the organism by the accidental introduction or inoculation of minute quantities of the nutrient matter on which the organisms had been cultivated.

With sterilised spring water the diminution of the bacillus did not occur until the nineteenth day, and it survived till the thirty-first. With various unsterilised spring waters the organism survived and multiplied still longer for times and to extents which appeared to vary with the impurity of the water, and in particular with the amount of dissolved organic matters which it contained. It is thus evident that the diphtheria bacillus has a capacity of growth in distilled water, and that in unsterilised natural waters its vitality in the presence of other micro-organisms is entirely exceptional in a non-aquatic microbe. Taken as a whole, the experiments show that the bacillus may survive for some weeks in spring waters of little organic impurity,

although during this time the virulence is gradually attenuated. If, however, at any time previous to its ultimate disappearance the organism be transplanted into a suitable culture medium, it can re-acquire its full initial virulence. Such a medium may be a human throat in a suitable condition, and the research therefore establishes the fact that drinking water must be added to the list of the means capable of spreading diphtheria. Its importance in this respect is the greater in the light of the entirely independent results of Reyes, showing the great capacity of the organism to survive in damp earth, which, when once infected, may for very long periods continue to infect any water flowing through it.

Faulty sanitary conditions may also 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.

Kanthack and Stephens ('Transactions Pathological Society,' London, 1896, p. 361), contrary to the generally stated view, find that in cases of fatal diphtheria, almost without exception, diphtheria bacilli are to be found in the lungs, and generally in the cervical and bronchial glands and spleen, and in two out of three cases examined in the kidney. They think these observations are of importance since they prove the necessity of using the antitoxin energetically in all serious cases of diphtheria, the amount of toxin to be counteracted being always enormous when the bacilli have gained access to the lungs or other organs. The existence or suspicion of broncho-pneumonia should always excite us to action, and the antitoxin should not be spared when this complication arises. In laryngeal cases prompt and copious injections should be administered to circumvent the dangers of a diphtheritic broncho-pneumonia.

There is a considerable body of evidence to show that the intensity of the infection may be aggravated by the presence of other organisms, as the streptococcus, less often the pneumococcus, and still more rarely the staphylococcus. De Blasi and Russo-Travalli have found considerable increase

in virulence in a few cases where the *B. coli* was present. Investigating this subject experimentally on guinea-pigs, they obtained the same result, which is important, owing to the ubiquity of the organism, and probably lends force to the direction of using antiseptics locally as much as possible.

The Antitoxin Treatment of Diphtheria.—The discovery by Behring and Kitasato, in 1890, of the existence of the antitoxic properties of the serum of the blood of animals rendered immune to diphtheria, and their application of this antitoxin to the cure of the disease, must be regarded as one of the most important advances of the century in the medical treatment of infective disease. The advantages of the treatment, particularly on the Continent, have been fully recognised by medical practitioners, and the results that have been obtained have approximated in some cases to the prophecy of the discoverers, that the mortality from the disease would be eventually reduced to five per cent.

The preparation and standardisation of diphtheric antitoxin has already been fully described under the section on sero-therapy. See p. 128, *et seq.*

The most suitable place for injection of antitoxic serum 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.

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 im-

mediately 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.

As far as the strength of antitoxin is concerned, we are met with the difficulty that a uniform standard of strength 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 standard is adopted, it will be impossible for clinical observers to agree upon the proper dose to be employed in any individual case.

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. There is a good deal of evidence in favour of the idea that local disturbances and erythematous rashes, etc., when they occur, may be in great measure due to the albuminous constituents of a serum; they may appear even after injection of normal horse serum. 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 the serum in an extended number of cases.

Unquestionably the most important factor in the antitoxin treatment is the dose, as will be seen from the following observations by Wernicke and Behring, who have shown that it is necessary to inject ten times as much antitoxin into a guinea-pig that has received subcutaneously a lethal dose of diphtheria toxin eight hours previously, as when it is treated immediately after the injection of the toxin, whilst after twenty-four hours fifty times the initial quantity is necessary to effect a cure. That the importance of this fact is fully recognised is indicated quite clearly by Behring in the directions sent out with the serum prepared under his supervision. Thus he recommends as sufficient a dose of 600 'normal antitoxin units' 'in cases where the serum treatment is commenced on the appearance of the first symptoms of the disease,' whilst at a more advanced state of the disease doses of 1,000 or 1,500 'normal units' are required. Dr. Sidney Martin is of opinion that in the case of patients suffering from a severe attack of diphtheria, it is necessary to give as much as 4,000 'normal immunisation units' as a dose.

In the issue of the *Lancet* of July 18, 1896, is the report of the *Lancet* special commission on the relative strengths of diphtheria antitoxins. The report is most valuable, and should be carefully studied by everyone who intends to employ antitoxin.

In this report attention is drawn to the fact that the employment of antitoxin in this country has not been attended with anything like the success that has attended it abroad, and that this is, at least in part, due to the quality of the serum manufactured and distributed in this country. It is impossible to use sufficient volume of a serum of low immunising value to do any good, and hence

those serums are to be preferred in which the requisite number of 'units' are contained in a small volume, say 10 c.c. or less.

The conclusions arrived at are as follows:

1. 'That a common standard of estimating the strength of antitoxic serum should be agreed upon by the English manufacturers.

2. 'That no serum should ever be sent out containing less than 60 normal antitoxic units per c.c.

3. 'That antitoxic serum of higher strengths must also be provided to meet the requirements of treatment in more severe cases of diphtheria.

4. 'That every sample of antitoxic serum sold should be plainly marked with the antitoxic strength of the serum (number of normal antitoxic units per c.c.), the quantity of serum present in the bottle, and the date of issue.'

In many cases the injection of the serum 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.

In taking into consideration the great reduction in the mortality from diphtheria, it is necessary to take into calculation the age of the patient, this being a very important factor. Again, 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.

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. No doubt 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 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.

The following are the general conclusions drawn from the statistical and clinical observations, as drawn up in the reports by the Medical Superintendents of the Metropolitan Asylums Board, upon the diphtheria antitoxin treatment.

(1) A great reduction in the mortality of cases brought under treatment on the first and second day of illness.

(2) The lowering of the combined general mortality to a point below any former year.

(3) The remarkable reduction in the mortality of the laryngeal cases.

(4) The uniform improvement in the results of tracheotomy.

(5) Diminution of the faucial swelling, and the subsequent distress.

(6) Lessening or entire cessation of the irritating and offensive discharge from the nose.

(7) Uniform improvement produced upon the clinical and general condition of the patients.

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.

All clothes, sheets, blankets, etc., should of course be thoroughly disinfected in a steam disinfector.

CHOLERA.

Discovery and morphology of the organism—Growth on media—Distinction from the Finkler-Prior bacillus—Bacteriological diagnosis of cholera—Indol reaction—Pathogenic effects on animals—Variations in organisms—Occurrence and distribution—Transmission of the disease—Methods of conveyance—Conditions of growth—Pathogenesis—Specificity of organism—Haffkine's vaccine 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.

Cultural Characters.—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 one of the organisms which is likely to be confounded with Koch's 'comma.' It is occasionally found in the stools in English cholera (cholera nostras), cholera infantum, etc.

The general behaviour of the two organisms on different media is distinctive, as is seen in the following table:

Cultural Characters.	Koch's 'Comma' Bacillus.	Vibrio proteus ('Finkler-Prior' Spirillum).
Gelatine Stab Culture	Slow liquefaction	Rapid liquefaction.
Agar Streak Culture	Greyish-white growth	Dirty-yellowish growth.
Potatoes*	Slow light greyish-brown growth at room temperature	Rapid slimy yellow growth at room temperature.
Milk	Slowly coagulated	No change.
Egg Albumen (Plover's)	Whitish growth	Bright-yellow growth.
Broth*	Indol reaction in twelve hours	Indol reaction only after three days.

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

* Care must be taken that the potatoes and broth are faintly alkaline.

the microscopic appearance is doubtful or negative. In true cholera the ileum presents a characteristic appearance, the mucous and serous coats being congested.

Within recent years spirilla have been cultivated from cases of true cholera from different localities, and although they very closely resembled Koch's comma organisms, yet they differed slightly in cultural characters. Therefore 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.

Bacteriological Diagnosis.—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; (4) in its pathogenic effects on animals.

'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, but after subculture they are usually found to possess only one terminal flagella. 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. 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 lie with their long axis in the same direction, and present what is known as the 'fish-in-stream' appearance. Klein has, however, found the same appearance to be given by the *B. coli communis* and the *Proteus vulgaris* in cases of so-called English cholera.

Such 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.

Many other organisms besides Koch's 'comma' also produce indol and nitrites 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.

In the diagnosis of cholera the most valuable information is afforded by the intraperitoneal injection of guinea-pigs with pure cultures. It must be noted that the lower animals do not suffer from the disease under natural conditions, but symptoms simulating human cholera infection have been produced in animals as the result of the interference of the stomachic and intestinal processes by the administration of alkalies, opium, etc. The most prominent symptoms in the intraperitoneal injection into guinea-pigs are subnormal temperature, distension of the stomach, and ultimately profound collapse. The peritoneal effusion may be clear, or may be somewhat turbid, due to its containing flakes of lymph. The organisms are practically confined to

the peritoneum, although if the dose is a large one they may be found in the blood and small intestine.

The positive pathogenic effects of the cholera spirillum on guinea-pigs are best demonstrated, according to Pfeiffer, by taking a full needle-loop of the surface growth of an agar culture, distributing this in 1 c.c. of sterile broth or salt solution, and then injecting into the peritoneal cavity of a guinea-pig. This should be a lethal dose for an animal of average size—about 300 grammes—but for an animal larger than this a somewhat larger dose should be employed.

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 subculture, 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 alka-

line, 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.

Sanarelli (*Zeitsch. f. Hygiene*, vol. xi.) has recently isolated no less than thirty-two vibrios from water, morphologically distinct from each other, all of which gave a distinct indol reaction. Four of these organisms he found to be extremely pathogenic to animals, producing symptoms in guinea-pigs undistinguishable from those given by the true cholera spirillum. Sanarelli is of opinion that many varieties of spirilla may exist in water capable of producing a disease in man and animals practically identical with true cholera, and that the assumption hitherto held that this disease is produced by only one particular variety of vibrio must be abandoned.

Occurrence and Distribution.—The disease is endemic in many parts of India, particularly the Delta of the Ganges; 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 Pettenkofer, Emmerich, Hasterlik, and others, who have 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.

As already stated, 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 Vaccine Treatment.—This treatment has been tried on several thousands of 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.

Haffkine administers two injections—the first of attenuated 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—Pathogenesis—*Streptococcus pyogenes*—*Staphylococcus pyogenes albus*—*Staphylococcus epidermidis albus*—*Staphylococcus pyogenes citreus*—*Staphylococcus cereus aureus*—*Staphylococcus cereus albus*—*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 *Staphylococcus 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.

Ulcerative Endocarditis, Acute Suppurative Periostitis, and Osteomyelitis.—These and other suppurative conditions are caused by the pyogenic cocci and streptococci.

ERYSIPELAS.

Fehleisen's streptococcus—Growth on media—Method of staining—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*—Serum treatment of streptococcic infection—Treatment of malignant growths by means of metabolic products of growth of *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 most 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, and also by Gram's process.

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 Cobbett 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.

The probable identity of the *Streptococcus erysipelatis* and the *Streptococcus pyogenes* is supported by Jordan, Frankel, and Von Eiselberg (Schenk), Würtz, Bokenham, Bulloch and others.

Serum Treatment.—Antistreptococcic serum was first prepared by Marmormek. This has been used with great success in puerperal fever and other disease conditions due to streptococcic infection.

The method of preparation has already been dealt with (see p. 134).

It has been a not uncommon observation by many that an attack of erysipelas supervening upon a malignant growth often led to the disappearance of the latter. This fact led to the use of sterilised cultures of the *Streptococcus pyogenes* in the treatment of inoperable tumours. This treatment has been used with varying success. Coley, of New York, claims to have met with the best success with this remedy, and to have obtained even better results from the use of a filtered culture, containing the mixed metabolic products of the growth of the *Streptococcus pyogenes* and the *Bacillus prodigiosus*.

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 frequently washed with an antiseptic, as in the case of scarlet-fever.

GONORRHŒA.

Specific organism first discovered by Neisser—Morphology—Cultivated by Bumm—Method of staining—Special media necessary for culture—Occurrence and pathogenesis—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-fuchsine 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 the toxins 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.'

Malleïn is prepared as follows: The organism is grown in broth for about six weeks; the cultivation is then filtered through porous porcelain, and the bacilli-free filtrate put by in tubes and bottles, and then carefully sterilised. If about 1 c.c. of malleïn be injected into a healthy animal, nothing, or only a slight febrile reaction occurs, in the horse not exceeding about 102°, the normal being about 100°; but if glandered ever so little the temperature runs up to 105° or even 106°. At the seat of inoculation a large swelling appears, and any local lesions, if present, become much enlarged. This swelling is of more importance diagnostically than the rise of temperature.

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.

Both in England and on the Continent the results obtained with malleïn have been most successful; as a diagnostic agent it is practically infallible; it seems, how-

ever, to act but feebly as a curative agent, although a few cases of apparent cure after its use have been reported.

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, or boiled under a pressure of 2 or 3 atmospheres, 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—Stassano's researches—The Contagious Diseases Act—Necessity for again putting the Act into force.

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

* *Lancet*, January 4, 1896.

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.

Stassano (*France Méd.*, August 6 and 13, 1897) draws attention to the gradual tendency of the parts affected by syphilis to become immune to further attacks, chiefly as regards the skin. The early secondary lesions invade the skin symmetrically; later eruptions are more and more limited as parts of the skin previously affected become immunised. The primary chancre he regards not as a local result of the syphilitic virus, but as a secondary lesion caused in the following way. The swelling of the inguinal glands forms the first line of defence against the syphilitic virus, and owing to the barrier caused by them, the virus is diverted into the blood-vessels of the same region instead of getting into the general lymphatic circulation. The virus takes action in the superficial capillaries of the skin and mucous membrane, resulting in the formation of the chancre.

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 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.

More than half of the English army in India are at present, it appears, regularly incapacitated by venereal disease. The actual numbers are 522 men per thousand, as compared with an average of only 30 in the armies of the great Continental Powers, who have the wisdom to enforce strict sanitary regulations. These figures indicate a very serious state of things in India, and, in a lesser degree, in this country as well, partly through India, but also from similar causes operating in our garrison towns and great centres of population. Hundreds of men are being turned loose from every troop-ship that returns from India unfit for military duty or any other efficient work, but not unfit to disseminate the disease they themselves have contracted. In the interests of the community generally this state of affairs calls for the immediate attention of the Legislature.

CHAPTER VIII.

INFLUENZA.

The specific organism was discovered in 1892—Morphological characters—Method of staining—Growth on media—Occurrence—Distribution—Pathogenesis—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 not exceeding 1·5 μ in length and 0·3 μ in thickness. It has rounded ends, and is generally found in pairs, but on cultivation grows out into strings in a similar way to anthrax. The limits of growth are from 25° to 42° C., the optimum temperature being that of the body. It is aerobic and its powers of resistance to outside influences are of a very low order.

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 per-

ceptible with difficulty. Growth on ordinary agar media is slight and uncertain, but it grows better in broth containing grape-sugar and glycerine. The organism must be subcultured every eight days at least, on blood agar, 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, characterised 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 frequently during the eighteenth and early part of the nineteenth century. From 1847 till 1888 England was free from its ravages. In May, 1889, 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 slight epidemics have occurred 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—Serum treatment.

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. It possesses a considerable number of flagella, either terminal or all round.

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. 87 *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 pro-

duced 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.'

Serum Treatment.—The serum treatment and the preparation of antitetanic serum have already been noticed (see p. 133).

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 μ 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 Chamberlan, be induced by the injection of filtered cultures, or of serum from animals that have died of the disease.

BUBONIC PLAGUE.

Discovery and morphology of organism—Method of staining—Growth on media—Distribution and occurrence of disease—Filth disease—Pathogenesis—Characters and types of disease—Methods of conveyance—Antitoxin treatment—Serum treatment of Yersin—Protective treatment of Haffkine—Preventive measures—Conditions favouring occurrence of disease—Steps to be taken to prevent and retard progress of disease.

The bacillus of Bubonic or Oriental Plague was discovered independently by Yersin and Kitasato, when investigating the character of the disease during the epidemic at Hong Kong in 1894. The plague bacillus presents very few special biological features; it might be best described as a cocco-bacillus. In the animal body it occurs as a short, almost ovoid rod, generally linked in pairs, measuring on the average about 2·3 μ by 1·7 μ; but longer forms are to be seen, measuring as much as 5 μ. In cultivation the young bacilli are so short as to be almost coccoid or slightly oval, but in older cultures rod, thread, and involution forms occur. In broth-culture the organism forms chains appearing like a streptococcus. The organism

is non-motile, and does not form spores. According to some observers, the organism is encapsulated.

Method of Staining.—The organism is easily stained by the ordinary dyes, but is not stained by Gram's method.

Kolle (*Deut. med. Woch.*, March 4, 1897) says that in cover-glass preparations from the pus of the buboes there are, besides pus cells, detritus, and red blood cells, abundant bacilli with rounded ends. Both ends of this micro-organism stain deeply, whereas the central portion hardly stains at all. This peculiarity of staining is not really seen in any other microbe pathogenic to man.

Growth on Media.—The bacillus grows slowly at a temperature from 18° to 20° C., and very rapidly at 37° C.; in twenty-four hours an abundant growth may be obtained on almost any media. The culture in broth is very characteristic: a flocculent, wavy deposit is formed, which settles to the bottom of the liquid, leaving the upper portion clear.

In old broth cultures there is formed an abundant curd-like deposit, and a thin filmy membrane on the surface. The bacillus grows readily on most of the various media—gelatine, agar, blood-serum, potato, etc. On the surface of gelatine it forms a thick shining white or cream-coloured growth, which is confined to the inoculation streak; the medium is not liquefied.

Cultures appear to quickly lose their virulence, and it has been found that though many of the least virulent cultures grow most rapidly, yet, upon testing them on rabbits and guinea-pigs, they have lost almost all their toxic properties. The culture in broth is alkaline; it does not appear to curdle milk.

Distribution and Occurrence.—The typical bacillus is present in all cases of plague. As in other affections, the streptococcus is found as a secondary infection.

The bacillus is also found in the blood and in the enlarged spleen. Thus plague is a polyadenitis, the glands being the starting-point of a septicæmia. The micro-organism is found almost always in organs where hæmorrhages have taken place.

Until the outbreak occurred in Hong Kong in 1894 but very little was known of this disease, though it seems to have existed in China and India from time immemorial, breaking out periodically in the crowded cities. It appears to be essentially a filth disease, and its existence can therefore hardly be wondered at when one considers the terribly insanitary condition of many Asiatic cities. The general symptoms closely resemble those of the fatal epidemic which visited Old London in the year 1665.

Pathogenesis.—The disease may be regarded as of a specific, acute, and infectious nature, characterised by a severe general febrile condition, accompanied by inflammatory swellings of the internal and external lymphatic glands and of the spleen, by changes in the liver and kidneys, and by inflammatory changes in the cerebral membranes.

Three types of the disease are now recognised—namely, the bubonic, the septicæmic, and the pneumonic.

The period of incubation appears to be usually from three to six days, but, according to Lowson, may extend to nine days. The bacillus probably has access to the body through wounds in the skin, or through the mucous membrane of the alimentary tract. As all the poorer classes of natives wear no footgear, it will be easy to see how the disease might be propagated through scratches or wounds of the foot, and also in other parts of the body in the same manner.

The thermal death-point of the organism appears to be between 58° C. and 60° C. Light and partial desiccation does not have much effect upon cultures, but complete

desiccation kills the bacillus. In sterilised water the bacillus has remained alive for fifteen days at room-temperature.

According to Yersin, flies can serve as propagators of the disease. The injection of liquid cultures into white mice, guinea-pigs, rabbits, and rats, produces plague symptoms, and the animals die in two to five days. An injection of 0·1 of a cubic centimetre of an emulsion of a recent culture of plague will generally kill a white mouse of ordinary size in one to three days. When introduced into the gastric canal, they act much more slowly. A quarter of a centigramme of dried plague toxin may be considered to be a fatal dose to a mouse, half a centigramme is quickly fatal, and one centigramme is rapidly so, generally in about twelve hours.

The bacilli are found in the viscera and lymphatic ganglia of inoculated animals, but are not very numerous in the blood.

Antitoxin Treatment.—Since the time that the bacillus has been isolated attempts have been made to adopt the serum treatment in cases of plague. Yersin has succeeded in immunising horses, and thus obtaining a serum of which one-tenth of a centimetre protects white mice against an otherwise fatal dose of the toxin or of the culture of the bacillus. This serum he has since used on plague patients in China and at Bombay with very good effect; but in order to obtain the best results, it would appear necessary that the disease should be taken in its earliest stages. The serum also possesses great curative properties. Haffkine, too, has been experimenting at Bombay, but, so far as can be gathered, his efforts have been chiefly aimed at protective measures. His method consists in the injection of killed cultures. He states in his report to the Bombay Corporation 'that he inoculated himself in the flank with

10 c.c. of a culture which had been heated for an hour at 70° C. The symptoms produced consisted of pains at the seat of the inoculation, and a rise of temperature. The highest point reached was 102° F., 8·5 hours after the injection, which was accompanied by a slight headache and a feeling of faintness. The temperature was normal twenty-four hours later. The bowels remained normal; the pain at the seat of the inoculation was mostly felt the next morning whilst getting up from bed. A small nodule remained at the seat of the inoculation, but was rapidly absorbed.' The injection of a killed culture—which, to all intents and purposes, is simply a solution of the toxin secreted by the bacillus—could not produce plague, so that from the experiment above mentioned it will be seen that the inoculation would be comparatively harmless; but before this method could be universally adopted, it would be necessary to ascertain the exact dose to produce immunity, how long after injection the highest point of resistance is reached, and, finally, for how long the immunity thus conferred lasts.

Preventive Measures.—These should be such as would be adopted in the event of an outbreak of cholera. Dr. Atkinson, Principal Civil Medical Officer of Hong Kong, in a report to the Government on the plague, dated 1897, states that the general conclusions to be drawn from the experiences of 1894-1896 are as follows:

1. That the occurrence of plague is favoured by:

(*a*) Long prevalence of drought or of abnormally low rainfall.

(*b*) Atmospheric temperature below 82° F.

(*c*) Absence of sunshine.

(*d*) General insanitary conditions, such as obstruction to the free access of light and air to domestic dwellings.

2. That the steps to be taken to retard the progress of the disease are :

(*a*) General cleanliness, and the free admission of light and air to domestic dwellings.

(*b*) The immediate isolation of the sick, and those who have been in close contact with the disease.

(*c*) The careful and systematic disinfection of all premises in which cases occur, and of latrines.

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—Morphology of organisms—Methods of staining—Cultural differences—Method of obtaining pure cultures—The organisms may exist in the healthy throat—Value of bacteriological diagnosis—Pathogenesis—Serum treatment—Dr. Washbourn's researches—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.

Serum Treatment.—Washbourn (*British Medical Journal*, February 27, 1897) was the first to publish an account of the preparation of an antipneumococcic serum on a large scale, and to describe a method by which its strength could be accurately estimated. Pane, also, in a communication made before the Medical and Chirurgical Academy of Naples (March 14, 1897), described an antipneumococcic serum which he had obtained from a cow and a donkey, and with which he had successfully treated a number of cases of pneumonia in the human subject.

In Dr. Washbourn's experiments a pony was the animal selected for the preparation of the serum. After nine months' treatment, first with living and then with dead cultivations, the serum was found to possess marked protective powers.

To accurately standardize the serum he found that it was necessary to determine the minimal fatal dose of the test cultivations, and this entailed the devising of a method for

maintaining the cultivations at a constant virulence. By using a special method of cultivation, it was found possible to preserve the virulence of the pneumococcus at an absolutely constant level for a long period. The medium employed consisted of agar streaked with sterile rabbit's blood. The minimal fatal dose of Dr. Washbourn's pneumococcus for rabbits and mice was 0·000001 loop of the cultivation. (The loop held about 0·5 mg.) Plate cultivations showed that this quantity contained about 200 living cocci. For the purpose of standardization, the serum, in varying quantities, was mixed with a tenfold fatal dose of the cultivation, and the mixture was injected into the peritoneal cavities of rabbits, control experiments with the minimal fatal dose always being made. The smallest quantity of serum which will protect the animals under these conditions is called a unit. The most powerful serum Dr. Washbourn has yet obtained from the pony was of such a strength that 0·03 c.cm. protected against the tenfold fatal dose. A cubic centimetre of this serum, therefore, contained 33 units.

As far as the therapeutic value of the serum is concerned, Dr. Washbourn's experiments show that 2 c.cm. of the serum will protect when injected during the first quarter of the disease—that is to say, within the first six hours in the case of rabbits. The same dose does not protect when injected at a later period. In applying this method of treatment to cases of pneumonia in the human subject, it follows that the earlier the treatment is commenced the greater the chance of success. As far as the dose is concerned, Dr. Washbourn states that at least 600 units should be injected twice a day.

Furthermore, Dr. Washbourn states that from the nature of the serum, no immediate beneficial effect is likely to be observed after injection. It is only after treating a large number of cases that a definite conclusion can be arrived

at. The six cases, some of them exceedingly severe, which have been under my own care, have all recovered; but in the hands of others the treatment has met with a more variable success. Of twenty-three cases treated by Pane with his own serum two died. One of the fatal cases was injected for the first time a few hours before death, and the other case was complicated with interstitial nephritis.

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—Loeventhal's serum diagnosis—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.

Loeventhal, of Moscow (*Deut. med. Woch.*, August 26, 1897), says that the presence of specific bacterial products in the blood during the apyretic stage of relapsing fever, as shown by Gabritschewsky, led this author to make use of a serum test. The technique is easy, but as no cultivation of the spirillum has yet been made, the test can only be applied when there are cases available from which spirilla containing blood can be obtained. The two sorts of blood are intimately mixed together and placed in the warm incubator, a control specimen being always made. The spirilla gradually become motionless and collect together in masses. The reaction is usually complete in half an hour. If none occur within two to two hours and a half relapsing fever as the cause of the preceding febrile attack may be excluded. Gabritschewsky shows that the reaction is a specific one.

Practical Disinfection.—The excreta and secretions should be disinfected in the same manner as recommended in enteric fever.

SCARLET FEVER.

A streptococcus described by Klein is probably the specific cause of scarlet fever—Occurrence and distribution of the disease—Mortality greatest in young children—Conveyance of the disease by milk—Number of epidemics caused by milk infection—Difficulty of exercising sanitary control owing to the nature of the infective material—Pathogenesis—Epidemics—Successful treatment of cases with Marmorek's antistreptococcic serum.

A large number of organisms have been described in connection with scarlet fever by different observers, but the organism discovered by Klein, and called by him the *Streptococcus scarlatinæ*, would appear from the evidence forthcoming to be the specific organism of the 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. The organisms were isolated by smearing sloped gelatine tubes with the infected blood.

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. Milk is a frequent source of the conveyance of the disease. An epidemic 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. Thirty-two epidemics of scarlet fever have been recorded as directly due to contaminated milk-supplies by Dr. E. Hart in the pages of the *British Medical Journal* since 1881. See the reprinted 'Report on the Influence of Milk in spreading Zymotic Diseases' for the detailed reports of the outbreaks. 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 relationship of small-pox to vaccinia—The micro-organisms associated with small-pox and vaccinia—The specific organism not yet definitely discovered—Researches of Klein and Copeman—Klein's bacillus albus variolæ—Probably the specific organism—Copeman's egg culture experiments—Glycerised lymph—Jennerian vaccination—Recommendations of Royal Commission on vaccination—Distribution and pathogenesis of small-pox—Preventive measures.

Small-pox, or **Variola**, is a specific infectious disease which is caused undoubtedly by a micro-organism, but none of the organisms hitherto found in the pocks have been definitely proved as the cause of the disease.

Vaccinia, or **Cow-pox**, is a disease of cows and calves, and is usually met with in the form of vesicles on the udder. It is believed by most observers that this disease is really small-pox, but so modified in cows as to be a very mild affection. Vaccinia can be inoculated into human beings by means of the lymph taken from the vesicles; the infection so inoculated runs a definite course, and is nearly always a trivial disease.

The question of the relationship of small-pox to vaccinia has been a matter of great dispute and controversy since

the introduction of vaccination. The leading points in connection with the question of the identity of small-pox and cow-pox are the following: There is no doubt that cow-pox can be communicated to human beings, in whom it produces an eruption limited to the inoculation point, and only gives rise to very trifling general symptoms. Against the view that vaccinia is modified small-pox is the fact that in human beings it never gives rise to a general eruption. With regard to the result of inoculating cows with human variola, it has been denied by many authorities, notably Chauveau, that the transference results in cow-pox. Many observers have, however, claimed to have accomplished the transference, including, in this country, Klein, Copeman, and Simpson. The evidence in favour of this is very strong. The general results of the experiments of these workers are as follows: When a series of calves are inoculated with a suspension of variolous crusts in brine, redness and swelling appear at the point of inoculation, but there is not much reaction. If the lymph, however, is squeezed from such reaction as does occur, and is inoculated into other calves, it will be found after a few such passages a local reaction takes place indistinguishable from that caused by true cow-pox lymph. On using this variolated lymph for human vaccination results are obtained identical with those from true vaccine lymph. Thus, it will be seen that the evidence in favour of the identity of variola and vaccinia is very strong. In Germany much of the lymph used for vaccination is that derived from the inoculation of calves with variolous matter in the manner described above.

Organisms Associated with Small-pox and Vaccinia.—Cohn, Weigert, Burdon Sanderson, and others, early pointed out the presence of bacteria in lymph. The most common bacilli found in the crusts and lymph of variola and vaccinia

are the pyogenic organisms and the ordinary skin saprophytes. Klein and Copeman have, however, independently discovered an organism which may prove to have a specific relationship to these diseases.

Two pieces of evidence 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.

It appears that Dr. Copeman has established the pathogenicity of the minute bacilli described by him. He finds that the bacilli in both small-pox and cow-pox are similar morphologically; 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.

Dr. Copeman has succeeded in obtaining growths, 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.

Dr. Klein (*Local Government Board Report*, 1896-1897) has repeated Dr. Copeman's experiments of inoculating hens' eggs with vaccine lymph and variolous matter, but failed to obtain any definite result. He obtained more satisfactory results, however, by the use of solid media. Klein in his experiments made use of glycerine as a means of inhibiting the growth of extraneous organisms present in the variolous crusts. His procedure was as follows: Ordinary nutrient agar and glycerine agar having been melted and poured out into separate sterile Petri dishes and allowed to set, a sample of the emulsion of smashed small-pox crusts was drawn up into a freshly-made glass capillary pipette, and three droplets therefrom were deposited upon the surfaces of each of the media in question. Next, with the flat blade of a sterile platinum spatula (bent

at an obtuse angle against the handle) the three droplets thus deposited were carefully and gently spread over the agar surface, the dishes were duly covered over, and were incubated at 37° C. After two or three or more days, all the colonies able to grow on these media had commonly made their appearance, and could be easily studied. The organisms obtained by Klein from variolous crusts by this method were the following: A number of common saprophytes, organisms of the pyogenic class, a non-pathogenic bacillus known as the *Leptothrix epidermidis*, which organism was first isolated from epidermic scales by Bizzozero, an organism which Klein called the *Bacillus Xerosis variolæ*. This organism closely resembled the so-called pseudo-diphtheria bacilli; but the most important organism isolated was the following:

Bacillus albus variolæ.—This organism gives a pure white growth on agar. The colonies on a plate culture are moist-looking, grow fairly rapidly, and are slightly raised. In stained specimens the organisms are seen as small delicate rods with rounded ends, measuring 0·8-1 μ, the thickness of the rods being about one-third or fourth the length. Subcutaneous injection of agar cultures of this bacillus into guinea-pigs and rabbits produced no result, but cutaneous insertion into calves gave very interesting results, in two or three cases appearances of typical variola being obtained, which in one case at least was obtained after two removes. It is most probable that after further work this will be found to be the true specific organism; but the matter is at present *sub judice*, and the further developments of Dr. Klein's researches will be of the greatest interest.

Jennerian Vaccination.—As is well known, vaccinia can be inoculated into human beings by means of the lymph taken from the vesicles, the operation being known as

vaccination. The disease so inoculated runs a mild course, and it has been conclusively shown that an individual who has had vaccinia is protected against small-pox just as if he had had that disease.

In the East it has been, time out of mind, the practice among the natives to take a flake of skin of a small-pox patient and use it for inoculating others with the disease, as it was well known that small-pox thus produced runs a milder course than if it is acquired by natural means. It was also well known that an attack of small-pox protected against a second infection. This practice was introduced into Europe from the East about the time of the Crusades, and up to the end of the eighteenth century was the only means employed to alleviate the terrible ravages of this disease, which formerly used to ravage all the countries of Europe. The great disadvantage of this method was, however, that the resulting attack of small-pox, though mild, was very infectious.

In 1798 Jenner published his paper entitled, 'An Inquiry into the Causes and Effects of the Variola Vaccinæ,' which contained the results of his remarkable observations and inquiries respecting small-pox and cow-pox. Jenner found that individuals who had contracted cow-pox from affected cows, or a similar disease of horses known as 'grease' or horse-pox, were remarkably insusceptible to small-pox. Jenner produced experimental evidence to show that persons who had suffered from cow-pox did not react to small-pox when inoculated, and further, that persons who had been artificially inoculated with vaccinia were also immune to small-pox.

At first Jenner's views met with the greatest opposition; but when the first prejudice was overcome, the operation soon became very popular.

Although vaccination does not confer absolute immunity,

when a person who has been vaccinated does contract small-pox the symptoms are always modified.

The question of vaccination has recently been investigated in this country by a Royal Commission. The evidence given before this Commission is wholly in favour of vaccination, which very greatly diminishes the liability to attack by small-pox, and when this does happen the disease is always milder and less fatal. The chief point of interest arising out of the inquiry is that vaccination begins to lose its efficacy after the fifteenth year. It was also proved definitely that revaccination restores protection, and the necessity for this is strongly impressed. It is worthy of note that in Germany, where revaccination is the practice, the mortality from small-pox is practically nil.

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 and the Soudan.

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.—As already stated, 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 bedding and clothing in the infected house must be thoroughly disinfected, as far as possible, by a steam-disinfector. The house itself must be well

sprayed with a strong disinfectant solution; the walls are to be subsequently stripped and the ceilings limewashed.

HYDROPHOBIA.

No specific micro-organism yet isolated—Symptoms of rabies in the dog—Raving madness, dumb madness—Postmortem appearances—Incubation period—Pasteur's method of preventive inoculation—Method of preparing the cords—Treatment of patients—Statistics of persons treated—Difficulty of judging how far the results obtained are due to the treatment through want of untreated cases for comparison—Stamping-out system—Returns of the Pasteur Institute—Antirabic serum.

This disease, known as hydrophobia in man, or rabies in animals, has existed since the earliest ages. It appears to originate in carnivorous animals, as the dog, wolf, and jackal, and by them is communicated to man, cattle, horses, sheep, swine, deer, cats, and other animals. The disease is known in almost every part of the world at the present day, and is in all probability due to a specific organism, but no organism has yet been proved to exist, either by staining or by culture.

Rabies in the dog assumes two forms: (1) Raving or furious madness; (2) dumb madness.

Raving Madness.—The earliest symptoms noticed in the dog are a change in the usual habits of the animal. It becomes restless, and may be seen hiding away under chairs and tables, or in quiet corners. The animal's sleep is broken, its appetite is depraved, and it will eat all kinds of refuse, paper, rags, etc. An anxious expression of countenance may be observed, with muscular twitchings of the face, the eyes having a peculiar glassy stare, as if fixed on some distant object, and the animal appears to be generally agitated and disturbed. As the disease progresses

the animal barks and snaps at imaginary objects. The bark of a mad dog is especially characteristic, and once heard, is not easily forgotten. It is a hoarse, low note, not at all like the animal's natural voice, somewhat between a bark and a howl. A mad dog is not afraid of water, as is commonly supposed; on the contrary, it is constantly trying to drink, but is unable to do so owing to the spasm of the muscles of the pharynx. As the disease progresses the symptoms become aggravated; the animal snaps and bites at everything within reach; if chained it will seize and tear at the chain, or if touched with a stick it will bite in the most furious manner. As time goes on and the dog becomes more exhausted by these fits of fury, it falls into a more or less comatose condition, waking up now and then in a fit of passion. This continues until the fourth or fifth day, when, worn out by repeated attacks of fury and want of food, the animal dies from sheer exhaustion and asphyxia. If the dog be at large he may wander a long way from home, biting animals or people indiscriminately, and by-and-by, when tired, his gait will be seen to be unsteady, he totters along as he walks, with his tongue lolling out, his tail pendulous, his coat covered with blood and dirt, and stares about as if partially blind, which in reality he is.

In **Dumb Madness** the animal will be found to have lost its voice, its mouth is constantly open, owing to paralysis of the lower jaw (many people often thinking that the animal has simply broken or dislocated its jaw, never dreaming that it is mad), and its eyes have the same fixed glassy stare as in raving madness. It is very quiet, and evinces a constant desire to sleep. Though apparently much less dangerous than the raving madness, yet the saliva of an animal affected with the dumb form is equally virulent.

Post-mortem Appearances.—The tongue and mucous membrane of the mouth will be found of a dark, blackish colour. The glands are enlarged and congested, the tonsils inflamed, and the vessels of the epiglottis injected. The lungs are congested, while the stomach may contain all kinds of foreign matters, such as rags, straw, pieces of leather, wood, hair, and the like. The brain and spinal cord are congested. To establish the diagnosis beyond doubt it is necessary to inoculate a rabbit under the dura mater in the manner described later in this chapter with a broth emulsion of a small piece of a dog's spinal cord. After positive results have been obtained by this procedure no doubt can be entertained as to the true nature of the disease. The test animal 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.

Incubation Period.—The incubation period in a dog which has been bitten by a mad one will be found to be from about six to eight weeks, though it may extend to six months.

In man, in the majority of cases, the disease usually develops in from eight days to six weeks up to six months, a few cases being quoted where longer periods of incubation have occurred. The incubation period, as applied generally to man and animals, of course depends considerably on the nature of the bites received—the graver the wounds, the shorter the period of incubation.

Treatment.—In man, all that can be done locally is immediate cauterisation, the wound being well opened up and a hot iron or strong nitric acid used freely. The effect

of the treatment is doubtful if applied later than one hour after the bite.

Pasteur's Method of Preventive Inoculation.—Previous to this great discovery and its application to man, in the year 1885, no remedy was known which was of the slightest avail, and the percentage mortality was great; but since this system was adopted in the year 1886, at the Pasteur Institute, the mortality among the patients was ·94 per cent., falling to ·13 per cent. in 1895. From the beginning of 1886 to the end of 1895 over 17,000 persons were inoculated—that is, about 1,700 per year.

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.

Method of Preparing the Spinal Cords and their Application

to Man.—From the brain and medulla oblongata of a mad dog which has been killed or has died of rabies, there are taken, under strict sterile conditions, a small piece of the medulla oblongata from the floor of the fourth ventricle, and also another small piece from the central canal. The fragments removed should be about the size of a pea. These two pieces are put into a sterile test-glass (which has been sterilised for at least twenty minutes at 120° C.), and pounded up with a glass rod, adding about 5 or 6 c.c. of veal broth. It is worked up thoroughly into an emulsion, and then set aside for use. The vessel used for

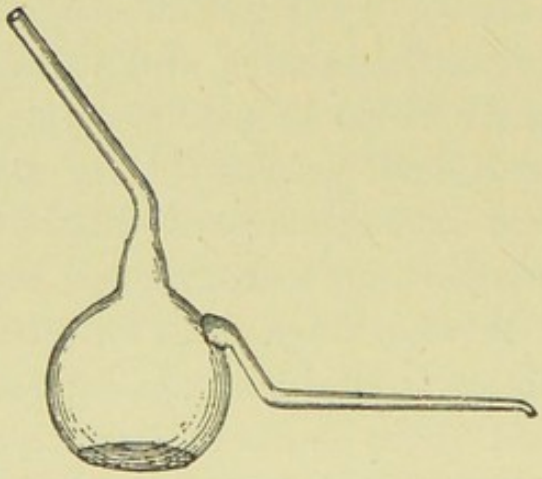


Fig. 18.—Pasteur Flask.

preparing the emulsion in at the Pasteur Institute is an ordinary conical test-glass, holding about half an ounce. Before use these glasses are covered with a piece of filter-paper, which is twisted round the top and sterilised, the paper cover only being removed to admit of the emulsion being made. It is then replaced until the preparation is required for use. The broth is made by using 2 pounds of lean veal to 1 litre of water neutralised with soda or potash if necessary, then filtered and stored in Pasteur flasks. The pipette-end being sealed in the flame, the broth is poured in, and the other opening is closed with a plug of cotton-wool. The flasks are then sterilised at

120° C. for twenty minutes. Having prepared the emulsion of the dog's brain, it is now ready for inoculating into rabbits. A full-grown healthy rabbit is taken and placed upon its stomach on a board which has a hole bored in it at each corner; its legs are stretched out and fastened by cords, or thin strips of leather, to each hole, and thus made fast. An anæsthetic is now administered, either chloroform or ether, and the animal is soon under its influence. The assistant then holds its head steady, whilst

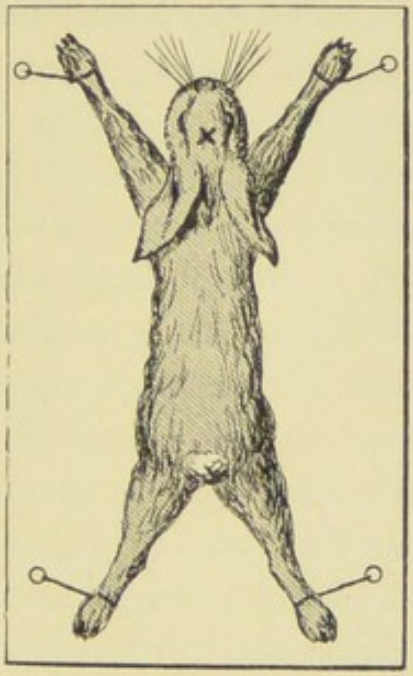


Fig. 19.—Rabbit for Trephining.

the operator, with a sharp pair of scissors, cuts the fur short over the part to be operated upon, and the skin is well washed with a 5 per cent. solution of carbolic acid. Then, with a sharp scalpel he makes an incision of about an inch, extending along the median line at about equal distances between the eyes and ears, cutting down to the bone. A dilator is then inserted to keep the skin apart whilst the operation of trephining is carried out. A small trephine is used of about one-sixth of an inch in diameter. The operator then takes the trephine, and at a point

between half and three-quarters of an inch behind a line drawn between the middle of the two eyes he carefully applies it to the median line and cuts through the bone. The circular piece being lifted up and removed by means of a strong curved needle, the cerebral membranes are now exposed. He then takes a special kind of hypodermic syringe, fills it from the glass which contains the emulsion of the dog's brain, and injects about 1 c.c. under the dura mater. The wound is then freely bathed with the 5 per cent. aqueous solution of carbolic acid, and two or three stitches are put in, to bring the skin together again, the wound is once more dressed with the carbolized solution, and the operation is finished. In skilled hands the process is a very simple one—few fatal results occur, the wound soon heals, and the animal will generally eat directly it recovers from the effect of the anæsthetic. It is usual to inoculate two rabbits at the same time, to avoid accidents, and thus prevent a break in the series of passages from one animal to another. After inoculation the rabbits are placed two together in strong cages, each of which bears a label stating the date, weight of animals, their colour, nature of inoculation, etc.

Rabies in the rabbit assumes the paralytic or dumb form. The hind-legs first become paralyzed, and gradually the paralysis ascends until the whole body is involved; the animal then lies quite helpless, and dies shortly after. Another rabbit is now inoculated from the medulla of the dead one, by the same method as described above, and repeated passages are made from rabbit to rabbit until the incubation period is reduced to seven days. This period will probably become constant after a passage of the virus through about forty or fifty rabbits, though at the first few passages it will be found to be much longer—from fourteen to twenty days in many cases. About 1 cm. of the spinal

cord of the dead rabbit is sufficient for the inoculation of a live one. Having arrived at a time when the incubation period is determined at seven days, and the rabbit dies on or about the twelfth day, the spinal cords may now be used for the inoculation of human beings. The rabbit having died, it is taken whilst quite fresh and laid flat on the abdomen upon a metal tray. The operator then slits up the skin along the dorsal median line, from the head down to the end of the lumbar vertebræ, and dissects it back on either side for about 2 or 3 inches. With a sharp pair of scissors he now detaches the muscles from the skull, the spine, and the ribs, for about 1 inch on either side of the vertebral column. The spinal processes are next cut off, close down, with the scissors. This being done, he now seizes the muzzle of the animal, and holds it firmly with a pair of crab-claw forceps in his left hand, whilst in his right he takes a pair of bone-forceps, and breaks off the skull in pieces, exposing the brain. Then, still holding the muzzle firmly, he proceeds to remove the vertebral laminæ, cutting them right and left alternately, inserting the point of the blade of the bone-forceps into the spinal cavity, taking great care not to injure the cord. The cord is thus laid bare to about the lumbar plexus. The lower end is now seized with the dissecting-forceps and raised from the spinal groove, the spinal nerves which hold it down being cut with the scissors. When a sufficient length of the cord has thus been freed, the end held by the forceps is tied by a piece of thread, and a length of about 3 inches is cut off, the fragment being carefully inserted into a drying-bottle, with the end of the thread hanging out. The cotton-wool plug is now replaced, which securely holds the thread, and the cord remains suspended in the middle of the bottle, hanging vertically over a layer of caustic potash, which has been previously placed at the bottom of the vessel. Next,

a second and a third portion of the cord is removed in a like manner, and placed each in a separate bottle, the small piece of cord which remains being used for the inoculation of a fresh rabbit, and a small portion is also taken and planted into a tube of broth in order to ascertain whether it is free from other organisms. The bottles are now labelled, and placed on a shelf in a dark-room, which is kept at a temperature of 25° C. The tube of broth above-mentioned is put on another shelf just above them, a microscopical specimen being taken from it, and examined, before the cord is allowed to be used for inoculation into

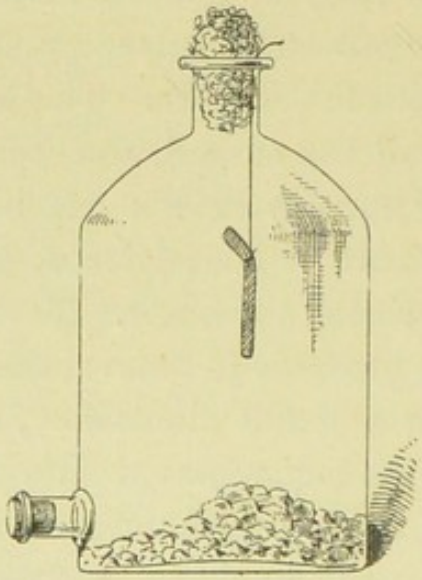


Fig. 20.—Drying Bottle.

human beings. The bottles which are used for the purpose of drying the cords would hold about 1 litre, and after being thoroughly washed have the two apertures closed with plugs of cotton-wool, and are then sterilised for twenty minutes at a temperature of 120° C. This done, the top plug is removed, and a handful of caustic potash broken up into small pieces is thrown in, filling the vessel to about the level of the lower hole. The plug is now replaced, and the bottle is ready for use. No cords of more than fourteen days old are used, as it has been found that after this time

they are inert and useless. When required for human inoculation, about 1 cm. of the dried cord is taken, and triturated as finely as possible with a glass rod, in a test-glass, then mixed with about 10 cm. of broth, adding it slowly until a complete emulsion is made. The paper cover is then again placed over the top of the glass, and the emulsion is ready for injection. The inoculation is made in a fold of the skin of the stomach, on account of the subcutaneous cellular tissues at this part being looser, and thus more rapidly absorbent. About 1 c.c. of the above emulsion would be an ordinary dose, but this varies according to the age and condition of the patient. The duration of treatment depends entirely upon the gravity of the bites received, mild cases being treated for from fifteen to sixteen days, whilst more severe ones receive inoculations for a longer period—extending up to twenty-one days, and during the first three or four days of such cases inoculations are often made twice daily. In all cases, however, the mode of procedure is the same, beginning with cords of thirteen or fourteen days, and gradually working down by degrees to those of one, two, or three days old, as considered necessary. After inoculation each patient has his wounds examined and dressed by an attendant. No special dieting is required, the patient being simply recommended to lead a steady and moderate life. The inoculation gives little or no pain, and is not attended by any swellings or redness as a rule. It is of course essential that all vessels, instruments, etc., which are used in the preparation of the cords and their subsequent inoculation, should be strictly sterile, and every possible aseptic precaution taken.

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

Antirabic Serum.—Valli, an Italian physician, early in the present century, showed that by administration through the stomach of progressive doses of hydrophobic virus, animals could be rendered immune against rabies. This important observation seemed to be lost sight of till Tizzoni and Centanni investigated this discovery. They also succeeded in immunising animals by the gradually increasing injection of rabic virus which they had attenuated by submitting it to peptic digestion. They furthermore found that the blood-serum of animals so immunised conferred passive immunity in other animals, and if injected into animals not more than fourteen days after infection, it prevented fatal effects, even if symptoms had already shown themselves. The serum would appear to contain similar antitoxic bodies such as are produced in the case of other diseases.

Owing to the want of statistical information in the case of persons bitten by really rabid dogs, it is impossible to form a true idea of the value of Pasteur's treatment, seeing we do not know the percentage of cases in which hydrophobia follows the bite of a rabid dog, and that in the majority of cases reported the animal was not rabid at all.

CHAPTER X.

MALARIA.

Not a disease of bacterial origin—Reasons for believing the *Plasmodium malariæ* to be the specific cause of the disease—Distribution and occurrence—Forms of the malarial parasite—Evolution of the organism—Flagellated and crescentic bodies—Mosquito theory—Varieties of the malarial parasite—Quartan, tertian, malignant tertian, and quotidian fevers—Morphological characters of varieties—Examination of the blood for the parasites—Stained blood preparations.

MALARIA is not a disease of bacterial origin, as was at first thought by many investigators, but the exciting cause is a protozoon, and is usually called the *Plasmodium malariæ*. It has also been known as the *Hæmatozoon* or *Hæm-amœba malaria*, because it is found in the blood. It was first shown by Laveran, in the year 1880, that certain pigmented organisms were to be found in the blood of malarial patients; and as one of the forms described by him was crescent-shaped, they received the name 'Laveran's sickles.' It has since been found that this particular organism described by him occurs chiefly after an attack of malarial fever, and is not necessarily 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.

The following are the reasons for believing the *Plasmodium malariæ* to be the specific cause of malaria:

1. The parasite may be found in every case of malaria.
2. It is only found in malaria.
3. It accounts for a peculiar feature of malaria, *i.e.*, melanæmia.
4. The cycle of the parasites corresponds with the cycle of malarial fever.
5. Quinine kills the fever-causing phase of the parasite and cures the fever. The crescent form of the parasite which is not pyrogenetic, is not influenced by quinine.
6. Malarial fever can be conveyed by injections of blood containing the parasite, and the parasite subsequently appears in the blood of the individual so inoculated.

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.

Malaria is endemic in certain localities, which are most often situated in hot climates, and they are usually, though not necessarily, low-lying and swampy. Moisture of the upper layers of the soil is an almost invariable condition in malarious districts. The tendency to malaria increases with the temperature, especially if the diurnal range is high. Clay soils are the worst, but chalk and sandy soils may become highly malarious if from any cause the ground water is high.

The malarial diseases reach their maximum of frequency and intensity in tropical and sub-tropical localities. They continue to be endemic for some distance into the temperate zone, but with diminishing severity and frequency as the higher latitudes are reached. As the intensity of the endemic or epidemic prevalence diminishes, individual cases assume more and more the intermittent type.

The malarial organism has not been cultivated by any artificial method yet devised. Undoubtedly the parasite has a home outside the body, but we do not know for certain what this is. It is not certain whether water may not be in some cases a medium for the conveyance of the disease. It is probable that the parasite passes through a metamorphosis in the body or bodies of animals other than man. But as yet little is known respecting the life-history of this organism outside the human body, and until we do but little can be done in the way of devising scientific preventative measures.

Forms of the Malarial Parasite.—From some external source the organism finds its way into the blood, and here it attacks the red blood corpuscles, each germ making its way into a red blood corpuscle. On examining the corpuscles, a certain proportion of them will be seen to contain a pale, nebulous, ill-defined body, which at first occupies but a small extent of the interior of the corpuscle (Fig. 21—1). Soon this amœboid body grows in size, becomes more defined, changes its shape, sometimes assuming a ring-like form, and begins to develop black pigment granules in its interior (Fig. 21—2). These pig-



Fig. 21.—Evolution of the Malarial Organism (intra-corpuscular bodies).

ment granules exhibit slow movements. As the pigmented body becomes larger the amœboid movements grow less, while the pigment tends to concentrate (Fig. 21—3). This pigment is no doubt derived from the hæmoglobin of the red corpuscles, the parasite growing at the expense of the latter.

Then the parasite undergoes segmentation, and we have developed from ten to twenty segments, arranged round the more or less central clump of pigment like a daisy or rosette (Fig. 21—4). Soon the bodies so formed break out of their corpuscular hosts and break up (Fig. 21—5). The spores, which are round or oval bodies about 1 μ in diameter, enter red blood corpuscles and again go through the same cycle of changes, repeating the growth and sporulation. In addition to these spores, and the intra-corpuscular bodies described, which appear to constitute the regular stages in the development, there are others, namely, the *flagellated bodies* and the *crescentic organisms*.

Flagellated Bodies.—If malarial blood be examined under the microscope for some time, it occasionally happens that certain more or less circular bodies with long flagella are seen. So far as is known, these flagellated bodies do not occur in the circulating blood, but only in the blood when it has got outside the body. They may be derived from the large intra-corpuscular pigmented spherical bodies, or from spherical bodies evolved from crescents. In either case the pigment granules become arranged in the form of a central ring, and afterwards show a peculiar vibratory movement which is apparently caused by the flagella which have formed within the sphere. At this stage the flagella shoot through the walls of the envelope of the sphere. The flagella, which are usually from three to six in number, are very delicate, actively moving filaments often having a bulbous swelling at their ends. They frequently break away and swim free in the blood.

The Crescentic Bodies. — If we examine the blood of a malarial patient who has been suffering for some time from one of the malignant forms of the infection—what has been incorrectly called æstivo-autumnal fever—in addition to those forms of the parasite already described as being

devoted to intra-corporeal reproduction, we may see what is known as the 'crescent body' (Fig. 22—7). This is a crescent-shaped parasite lying inside a blood corpuscle whose hæmoglobin it has evidently devoured. By careful looking one can make out as a delicate bow running between the horns of the crescent the shell, as it were, of the blood corpuscle, inside which the parasite developed

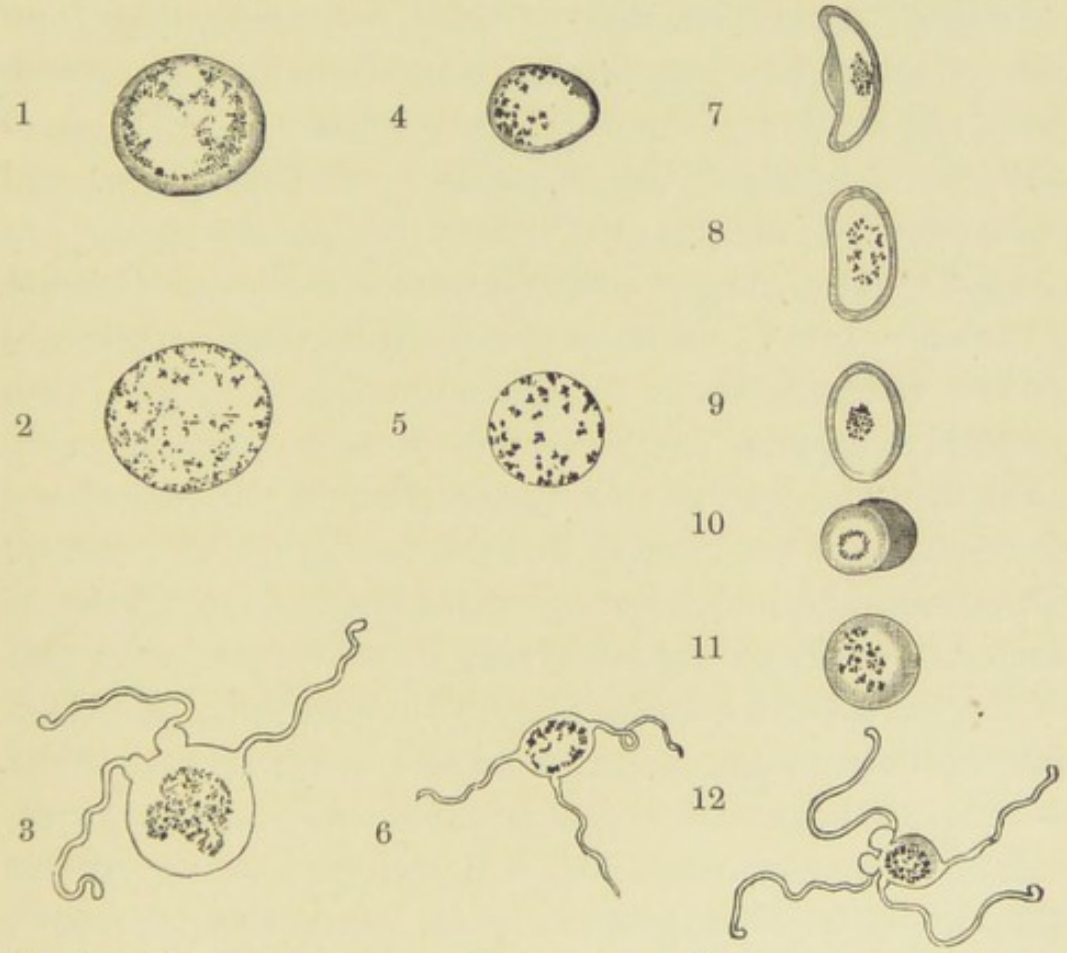


FIG. 22.—EVOLUTION OF THE FLAGELLATED BODY IN THE TERTIAN (1—3), QUARTAN (4—6), AND CRESCENT FORMING (7—12) PARASITES. (Compiled from Thayer and Hewetson.)

and is still lying. Presently the crescent slowly changes its form. First it becomes stouter, as it were, the ends rounding off more and the body becoming thicker (Fig. 22—8). Next it loses all appearance of being a crescent and becomes ellipsoid (Fig. 22—9). Then slowly the ellipsoid changes into a perfect sphere (Fig. 22—10). In the

crescent there is always a mass of motionless pigment granules about its centre. During the change of form described this mass of pigment still remains passive and central. The sphere once formed, however, a change occurs in this respect, the pigment granules beginning to move, at first slowly, but presently more actively, dancing about in the most energetic manner in the centre of the parasite. By-and-by, their agitation increasing, they burst through what Dr. Manson takes to be the delicate membrane hitherto enclosing them. The pigment now becomes diffused throughout the entire mass of the sphere, and continues to indulge in violent boiling-like movement (Fig. 22—11). At the same time the sphere itself becomes agitated—excited, as it were—changes form, writhes, is jerked violently about by some unseen force, and then suddenly flagella are projected from its circumference (Fig. 22—12), just as already described in the case of the free spheres of the quartan and the benign tertian parasite. That these ellipsoidal and spherical forms do not exist as such in the circulating blood may be proved, as in the case of the tertian and quartan plasmodium, by fixing the blood immediately on its removal from the body; on such slides —even although wet slides prepared simultaneously may exhibit crescents, ellipsoids, and spheres—we never find the two latter forms, but only crescents, and, of course, should fever be present or imminent, the usual intra-corpuscular forms of the plasmodium. This transformation of the crescent into the flagellate body is a very striking phenomenon, and must be referred to one of two things. Either it is a degenerative change in a dying or dead parasite, or it is a vital evolutionary change—a normal step in the life of the parasite. Dr. Manson's conviction is that it is the latter, and that the flagellated body is the first phase of the extra-corporeal plasmodium.

It is now generally considered that the crescentic bodies represent a sort of resting form for the life of the organism outside the body.

In the mosquito fed on crescent containing blood, crescent bodies are found plentifully. On spreading a drop of this 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.

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.

Varieties of the Malarial Parasite.—It has only been

recently established that the organisms causing the various types of malarial fever are distinct parasites, and are not, as was first thought by Laveran and others, due to one polymorphous organism. There is still a difference of opinion as to the exact number of varieties.

Undoubtedly the severity of malarial attacks is largely governed by the extent of the parasitism occurring in the blood-corpuscles. As already stated, malaria is characterized by a decided intermittency, periods of chill and fever, corresponding to the life cycle of the organism in the blood. The paroxysms of the disease, as represented by the chill, correspond with the escape of the spores from the mature organism into the blood serum. When these again invade the corpuscles, the fever diminishes, and during their growth until the next state of sporulation the patient has a respite from the more severe symptoms. The species of parasite may be arranged as follows :

(*a*) *Quartan.*—This is a benign variety, and depends on a parasite which takes seventy-two hours to pass through its cycle of development. The flagellated bodies, when they occur, are formed from spheres. The red corpuscles invaded by the parasite do not become decolourised or altered in shape.

(*b*) *Tertian.*—The cycle of development of this parasite takes forty-eight hours for completion. The organisms within the corpuscles show much greater movement than in the quartan type. Flagellated bodies are formed from spheres. Both the above varieties of fever are known as winter-spring fevers by Italian writers.

(*c*) *Malignant Tertian.*—This form requires about forty-eight hours for development. The organism is relatively minute, and is very actively amœboid when young, the parasite assuming a 'ring' form after a time. Its more advanced or sporulative stage is completed in the blood-

vessels of the deeper viscera. Its flagellated phase is developed from 'crescents.'

(*d*) *Malignant Quotidian.*—The cycle of development of this variety takes twenty-four hours. The parasite is always small, even in the adult state, and frequently assumes the 'ring' form in the corpuscle. The spores are generally formed by irregular segmentation. The flagellated phase is formed from crescents. This and the preceding produce fevers of a more or less irregular type.

Irregular types of fever may be produced by different generations of the same parasite, or by different varieties, resulting in mixed infections. It is quite possible, when the nature of the various tropical fevers has been more fully investigated, that other varieties of the parasite will be discovered.

According to various authors, there are five distinct forms of malarial parasite: quartan, benign tertian, malign tertian, pigmented quotidian and non-pigmented quotidian, whose characters may be arranged as follows:

Species.	Quartan.	Benign Tertian.	Malignant Tertian.	Pigmented Quotidian.	Non-pigmented Quotidian.
Cycle	72 hours	48 hours	48 hours	24 hours	24 hours.
Movement	Feeble; no movement when pigmented	Very active when young and pigmented	Very active	Active	Active.
Maximum Size	Less than a blood corpuscle	As large as a blood corpuscle or larger	½ to size of corpuscle	¼ to size of corpuscle	¼ to size of corpuscle.
Sporulating Form	Regular 'daisy' shaped	'Sunflower' pattern	Irregular heaped	Irregular or star-shaped	Irregular
Number of Spores	6 to 12	15 to 20	10 to 12	6 to 8	10 to 16.
Flagella formed from	Spheres	Spheres	Crescents	Crescents	Crescents.
Alteration in corpuscles	Does not alter in size or colour	Decolourises and increases the size	Atrophies	Shrinking and darkening	Shrinking and darkening.

The main distinction between the benign and malign species is that in the case of malign parasites flagella are produced from crescent bodies, while in the case of the benign parasites they are produced from simple spheres. Crescent bodies are always found in malign fevers, though not in the early stages; they continue in the blood for many days, and are not affected by quinine.

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 practical 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 free. In squeezing the blood from the finger, the same effect may be produced by the use of undue violence. The blood must be taken from patients who have not had quinine.

Dr. P. Manson recommends the following procedure for the examination of malarial blood (*British Medical Journal*, December 1, 1894):

Cleanse very carefully with alcohol or ether several slips and thin cover-glasses, and cover them up from the dust.

Wash one of the patient's finger-tips with soap and water, and afterwards with ether, and dry carefully. Ligature the end of the finger in the usual way, and prick the congested pad with a fine clean needle. Wipe off the first drop of blood which exudes, being careful to leave the skin quite dry, so that subsequent drops shall not 'run.' Squeezing the pricked finger-pad gently between finger and thumb, express a second and smaller droplet of blood from the puncture. This droplet ought not to exceed in size the head of a large pin. Touch the apex of the droplet with the centre of a cover-glass, and immediately lay this on a slip. The blood will now run out between slip and cover-glass in an exceedingly delicate film, in which, after a few minutes, the red corpuscles will be found to be each of them perfectly isolated, and lying flat on their sides. Prepare several such slides. Reject all slides in which the corpuscles in any considerable proportion are disposed in rouleaux, or are heaped up upon each other.

Perfect cleanliness of finger and slides, minuteness of the droplet of blood, thinness of cover-glass, and a certain quickness of manipulation, are the best guarantees for success in obtaining the flat disposition of the blood corpuscles *absolutely* indispensable.

Examine the slides so prepared with a twelfth immersion lens, and in not too bright an illumination. Scrutinise the interior of every corpuscle in the field, looking in them for specks of black pigment surrounded by a pale, hyaline, slightly or markedly amœboid substance; also for smaller, pale, unpigmented, hyaline, and more actively amœboid bodies in the same situation. These are the intra-corpuscular and commoner forms of the malaria parasite, and are always present in malarial fevers which have not been treated by quinine. The crescent and flagellated forms and the pigmented leucocyte—although the two

former are more rarely encountered, and only in certain cases—are much more easily recognised.

If no parasitic form be found in the first field, pass to a second, a third, and so on, devoting at least half an hour to the examination of the slide before pronouncing definitely in a negative sense on the presence of the parasite.

Stained Blood Preparations.—Stained preparations are not only useful on account of the confirmation they offer of the appearances seen in the fresh blood, but the advantages they offer owing to their permanent character. The blood is collected as above upon a clean cover-glass, taking care that the drop is a small one; another clean cover-glass is very gently laid down upon the drop. As soon as the blood has spread out in a uniform layer, the two cover-glasses are drawn apart, and the two blood films allowed to dry spontaneously. The film is then fixed by immersion in absolute alcohol and subsequently stained with Löffler's methylene blue, or double staining may be effected by treating the dried blood first with a 1 per cent. aqueous solution of eosine, which stains the corpuscles pink, and then with Löffler's methylene blue, which stains the parasites and leucocytes blue.

The most satisfactory staining method for malarial blood is the use of Chezynsky's solution. This is made by mixing 20 parts of a 0·5 per cent. solution of eosine in 70 per cent. alcohol; 40 parts of a saturated aqueous solution of methylene blue, and 40 parts of water. The solution is filtered before use. The cover-glasses with the blood films, after fixing, should be floated face downwards upon this solution for about one hour. If the stain is warmed to blood-heat, or a trifle over, 20 minutes' staining will generally suffice. The cover-glasses are then drained, thoroughly washed in distilled water, dried and mounted in xylol balsam.

ACTINOMYCOSIS.

Organism first described by Bollinger—Commonly known as 'wooden tongue'—Morphology of the organism—Method of staining—Growths on artificial media—Occurrence and distribution—Pathogenesis—Appearance of the fungus in discharges—'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.

Morphology of the Organism.—The exact biological position of this organism is still a matter of dispute, but most authorities regard it as belonging to the class of higher bacteria known as cladothrix or streptothrix. Three types of the organism may be seen in the colonies as they grow in the tissues—namely, filaments, cocci and clubs. The filaments, which are best seen in cultures of the organism, are very thin, measuring about 0·5 μ thick, and are often of great length. The central protoplasm is enclosed in a sheath; the filaments, particularly in the centre of a colony, interlace, forming a network. In older filaments the protoplasm is seen to be broken up into coccoid bodies, giving rise to an appearance like a streptococcus. The cocci may break out from the sheath. These bodies are to be regarded as spores, or, if the organism is a streptothrix, as conidia. The club forms of the organism seen in sections will be referred to later.

Method of Staining.—The organism stains with the ordinary stains. To stain the actinomyces in sections, 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 an

unlimited time, on artificial media, glycerine agar and bread being the best. The cultures on bread, when fully developed, have a very peculiar and characteristic appearance, showing a dull-gray raised and wrinkled growth, of considerable thickness, of a bright-sulphur yellow or light chocolate colour, 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.

Occurrence and Distribution.—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.

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.

Pathogenesis.—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 characterised 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. 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' (the 'fungus foot' of India) is produced by the same or a similar organism.

YELLOW FEVER.

Organism discovered by Sanarelli probably the specific organism—Morphological characters of *Bacillus icteroides*—Growth on media—Diagnostic growth on agar—Pathogenicity for animals—Difficulty of isolation—Secondary infections.

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. 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.

Sanarelli has recently announced to the University of Monte Video the discovery of an organism which he has called the *Bacillus icteroides*, which he believes to be the specific organism of yellow fever. The organism is small, with rounded ends, and is often seen united in pairs. It develops on most of the ordinary media, the growth on agar being characteristic, according to the temperature of growth. It does not liquefy gelatine. In beef bouillon the bacillus grows quickly, without forming either pellicles or deposits. On solidified blood serum it grows in an almost imperceptible manner. Cultures on agar-agar represent for the *Bacillus icteroides* a means of diagnosis of the first order, but the demonstration by this means of diagnosis is efficacious only under certain determined conditions.

When the colonies on agar grow in the incubator, they present an appearance that does not differ from that of the

majority of the other species of microbes; they are rounded, of a slightly iridescent gray colour, transparent, even in surface, and regular in outline. If, instead of causing the colonies to grow in the incubator at a temperature of 37° C., they are allowed to evolve at a temperature of from 20°-22° C., they appear like drops of milk, and are completely distinct from those grown at incubation temperature. This peculiarity, which may be considered specific, may be made evident in less than twenty-four hours, serving thus to establish the bacteriological diagnosis of the *Bacillus icteroides.*

The *Bacillus icteroides* is a facultative anaerobe, and does not resist the Gram stain; it is unable to coagulate milk; it does not produce indol, and is very resistant to drying; it dies in water at 60° C., or after being exposed for seven hours to the solar rays, and lives for a long time in sea-water.

The microbe of yellow fever is pathogenic for the greater number of the domestic animals. Few microbes have a pathological dominion so extended and so varied. Birds are completely refractory, but all the mammiferous animals upon which Sanarelli has experimented have shown themselves more or less susceptible. But of all the animals, that which lends itself best to showing the close analogy between experimental yellow fever and human yellow fever is the dog. The organism is found in the blood and tissues, but never in the gastro-intestinal tract.

The isolation of the organism presents difficulties, due in part to the constant presence of secondary infections, and in part to the relative scarcity of the organism in the body.

These secondary infections, due almost always to certain species of microbes, as the colon bacillus, the streptococcus, the staphylococcus, the proteus, etc., may appear in the

organism long before the death of the patient, which is often attributable to their action rather than to that of the *Bacillus icteroides.*

ENGLISH CHOLERA (*Cholera nostras*)—AUTUMNAL AND INFANTILE DIARRHŒA.

English cholera or *Cholera nostras*—Occurrence—Similarity to true cholera—Exciting organisms appear to be *B. coli* and *Proteus vulgaris*, which appear to assume a specific character—Klein's researches—*Bacillus enteritidis sporogenes*—Diarrhœa as the result of meat poisoning—Infantile diarrhœa—Researches of Escherich, Macfadyen, Vaughan and others—Green diarrhœa.

EVERY year a certain number of cases of autumnal diarrhœa occur in various parts of the country, which are so severe in character as to be clinically indistinguishable from true Asiatic cholera. English cholera, or 'cholera nostras,' is the name usually given to these cases. The cases tend to occur sparsely, and as isolated cases spread over the country, instead of as in the case of true Asiatic cholera, in groups in particular localities.

A large number of cases of this character have been examined during the last two or three years by Dr. Klein, under the auspices of the Local Government Board (see *Local Government Board Report*, 1895-96). As the result of the examination of the dejecta of these cases, he has found the *B. coli communis* and the *Proteus vulgaris* profusely abundant. The former chiefly predominated, and sometimes it appeared in almost pure growth, and, what is more, the cover-glass specimens showed the bacilli in question distributed through the intestinal flakes in the 'fish-in-stream' arrangement which has been hitherto considered peculiar to Koch's comma, and so absolutely diagnostic of true cholera.

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 the *Proteus vulgaris* and *B. coli communis* were present in large quantities in the intestinal contents of the patients.

Although *B. coli* or the *Proteus vulgaris*, or both, were obtained from these cases, these organisms were sometimes so mixed up with others that it might be urged that no special significance could be attached to their presence in the bowel contents. In several of the cases Dr. Klein found these organisms present in the small intestine in enormous numbers. Under normal conditions these organisms are never present in the small intestine. The presence of these organisms in such enormous number cannot do otherwise than exert very serious pathogenic effects.

The metabolic products of the growth of both the *B. coli communis* and the *Proteus vulgaris*, which is *par excellence* the organism of putrefactive decomposition, when inoculated into the animal body, give rise to symptoms practically clinically identical with those of Asiatic cholera.

For the cultural characters of the *B. coli communis* and the *Proteus vulgaris*, see pp. 438 and 450 respectively.

Finkler and Prior isolated the organism which bears their name (see 'Vibrio Finkler-Prior,' p. 444), from a case of 'cholera nostras.' This organism, which morphologically resembles Koch's comma, has been assumed by many writers to be the specific organism of so-called English cholera ('cholera nostras'), but there is no evidence that

it has any relationship to this or any other disease-condition in man.

In February, 1896, a large number of persons suffered from symptoms of poisoning in the borough of Mansfield, and Dr. Buchanan was deputed by the Local Government Board to investigate the outbreak. He found that there had been about 218 cases, all traceable to potted meat supplied by one maker. The incubation period was from twelve to twenty-four hours. Diarrhœa was usually the first symptom, and the stools were very dark in colour and offensive. Colic was usually severe, and vomiting was a frequent occurrence in the early stages. No fatal case occurred. The majority of the patients were ill for a week or more. The meat used was not always of the freshest, and scrupulous cleanliness during the process of manufacture was not observed. Samples of the potted meat submitted to bacteriological examination by Dr. Klein were found to swarm with organisms, but no one organism which could be considered to possess specifically infective properties was detected amongst them. The bacillus proteus and coli were present in remarkable numbers.

During 1895 an epidemic of diarrhœa occurred at St. Bartholomew's Hospital. This outbreak was investigated by Dr. Klein (*Local Government Board Report*, 1895-96), who found in the dejecta of the sufferers, and also in certain samples of milk to which the outbreak was traced, an anaerobic spore-bearing bacillus having pathogenicity for rodents. This organism he called the *B. enteritidis sporogenes*. For cultural characters, see p. 441.

Dr. F. W. Andrewes (*Local Government Board Report*, 1896-97) has made a further investigation upon a number of cases of diarrhœa to ascertain if the above organism could be detected in the dejecta. He found that anaerobic bacteria are not commonly inhabitants of the normal

human intestine. Out of twenty cases examined of diarrhœa of different character, fourteen contained anaerobic bacilli, and twelve of these were the same morphologically and culturally, or differing only slightly with the *B. enteritidis sporogenes* of Klein.

Gärtner has obtained (1888) an organism (see p. 440) from the tissues of a cow which was killed in consequence of an attack characterised by severe diarrhœa, and also from the spleen of a man who died twelve hours after eating the flesh of this animal.

Infantile Diarrhœa. — Young children are particularly liable to attacks of diarrhœa, possibly due to the susceptible organism of the child predisposing to such intestinal disorders, which is in part largely due to the easily decomposible nature of their food, consisting largely of milk.

Escherich found that in the milk fæces two organisms predominated, viz., the *B. coli communis* and the *B. lactis aerogenes*. The investigations of Macfadyen, Nencki, and Sieber show that the bacteria of the small intestine primarily decompose carbo-hydrates, with the result that the contents of the small intestine have an acid reaction. This acidity will be a main factor in preventing the development of a putrefactive decomposition under normal conditions.

Escherich did not find in cases of infantile diarrhœa any organisms that might be called specific. He supposes that in the upper intestine a main factor in the causation of diarrhœa is abnormal acid formation by bacteria, and that in the lower intestine the decomposition is of proteid matter.

The action of the bacteria does not take place through a direct invasion of the organism, but through the absorption of poisons formed by them. It is probably through their

action on the milk and not on the body that the bacteria acquire their dangerous properties. In the child toxic effects may result from substances that produce little or no effect on the adult.

Baginsky examined forty-three cases of summer diarrhœa, but did not find any organisms of a specific character. The general conclusion he comes to is that several kinds of saprophytic bacteria may produce the disease under favourable conditions. The severe cases of diarrhœa seem to be due to poisons developed by bacteria from the proteid constituents of the food. Booker isolated altogether thirty-three forms of bacteria from cases of infantile diarrhœa. There was great variety, but no constancy in the types found.

Jeffries and Baginsky were not able to confirm Lesage's statement that the *green diarrhœa* of children is associated with the presence of a specific organism.

The researches of Vaughan upon stale and sour milk resulting in the isolation of tyrotoxicon have a great bearing upon infantile diarrhœa. The symptoms of tyrotoxicon poisoning resemble those of cholera infantum. Vaughan also obtained toxic bodies from cultures of Booker's bacteria, which produced vomiting, purging, and sometimes death in dogs.

DISEASES DUE TO PARASITIC FUNGI.

The following diseases, 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

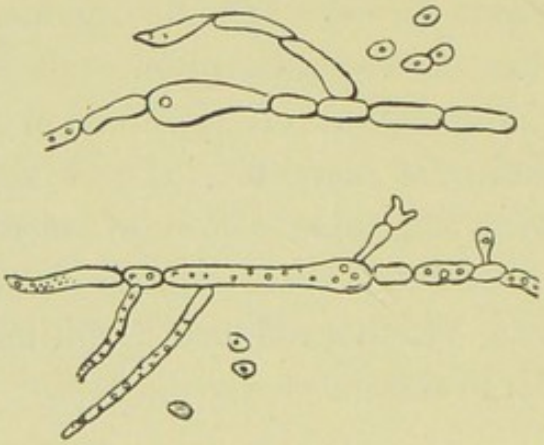


Fig. 23.—Mycoderma Albicans (Thrush).

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 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 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.

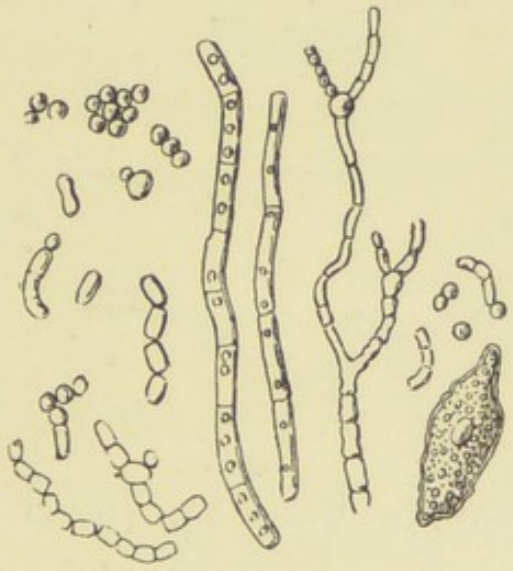


FIG. 24.—ACHORION SCHÖNLEINII. (Growth from a favus patch.)

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 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

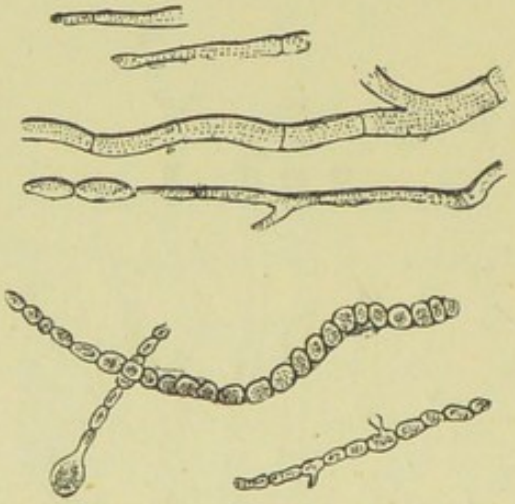


FIG. 25.—TRICHOPHYTON TONSURANS. (Mycelia and segmented filaments.)

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.

Adamson (*Glasgow Medical Journal*, xlvi., 236, after *British Journal of Dermatology*) recommends the following method for permanently staining *trichophyton tonsurans*: (1) Soak the hair in a 5 to 10 per cent. solution of caustic potash for ten to thirty minutes on a slide; (2) wash in 15 per cent. alcohol in water; (3) dry on a slide, and in the case of scales fix by passing through the flame; (4) stain in aniline gentian violet (made in the usual way, by adding a few drops of saturated alcoholic solution of gentian violet to aniline water) fifteen to sixty minutes; (5) one to five minutes in Gram's iodine solution; (6) decolourise in aniline oil two to three hours or longer; (7) remove superfluous aniline oil by blotting-paper, and mount in Canada balsam.

The disease affects man, dogs, cats, cattle, and many other animals.

PROTOZOA IN DISEASE.

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*, *Coccidii*, *Psorosperma*, *Amœba*, etc.

The parasite occurring in the blood in malaria, known as the *Plasmodium malariæ*, has already been described (see p. 273, *et seq.*).

Protozoa have been described by many observers as occurring in carcinoma, sarcoma, in the form of 'eczema' of the nipple known as Paget's disease; also in other pathological and morbid conditions of the human and animal subject.

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.

Coccidium oviforme.—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.

Surra.—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.

Protozoa in Fly Disease or Nagana.—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.

From Dr. Bruce's observations it would appear that the fly is viviparous, giving birth to adult larvæ, a most important fact hitherto unnoticed. The disease itself, he finds, is due to the presence in the blood of an inoculated animal of a flagellated hæmatozoon furnished with a membrane or 'fin,' running along one side of its body, with a flagellum at one end. The appearance of this hæmatozoon in the blood is signalised by a rise in temperature; the incubation period is from seven to twenty days, after which period the hæmatozoa may be found swimming actively among, and apparently 'worrying,' the corpuscles, the red blood corpuscles becoming very largely reduced in numbers. With the progress of the disease, the hæmatozoa increase in numbers. Dr. Bruce has demonstrated that it is possible repeatedly to feed tsetse on a healthy dog without producing disease in that animal, showing that the fly possesses no specific venom, but that if allowed to draw blood from a diseased animal, or the carcase of one, it will communicate nagana to healthy animals. The disease is invariably fatal in the horse, ass and dog, but perhaps not necessarily so in cattle, in which it runs a much slower course. From some preliminary experiments, arsenic appears to have a marked action on nagana, causing disappearance of the hæmatozoa, reduction in temperature, and maintenance of the red blood corpuscles.

Protozoa in Dysentery.—Certain organisms known as the *Amœba coli* have been described by Lösch, Grassi, Kartulis and others, as occurring in the intestines of persons suffering from dysentery. The amœbæ, when at rest, are round or slightly oblong bodies, consisting of an outer pale homo-

geneous substance enclosing a greenish refractive mass, which contain vacuoles of various sizes and a nucleus. Their movements consist first of a progressive motion, and secondly of a protrusion and withdrawal of pseudopodia. An alkaline condition is necessary to their growth. The organisms often contain foreign bodies, such as blood corpuscles, pus cells, bacilli, etc. They penetrate and undermine the tissues of the mucous membrane, producing their effects by liquefying the tissues, causing ulceration and necrosis. They frequently extend to the liver. Lösch communicated the disease to dogs by the administration of the fresh dejecta containing them. Kartulis succeeded in cultivating them in alkaline straw infusion, which he inoculated from the contents of a liver abscess which contained no bacteria. In twenty-four to forty-eight hours, at 35° to 38° C., a membrane forms upon the surface of the liquid consisting of the young organisms. Injection of the organisms into the rectum of cats resulted in swelling and erosion of the mucous membrane. No result followed when the amœbæ were administered by the mouth.

SOME DISEASES OF THE LOWER ANIMALS DUE TO MICRO-ORGANISMS.

Symptomatic Anthrax.

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.

Foot and Mouth Disease.

Foot and mouth disease, or *Eczema epizootica*, is an infectious disease of horned cattle, characterised by a vesicular eruption in the mouth and about the feet. It affects also sheep and pigs, and may also be communicated to man.

Up to the present time no satisfactory demonstration of the specific organism of this disease has been made. Schottelius has recently isolated an organism which he believed to be the specific organism, but inoculation experiments did not support this view, as the characteristic vesicles did not develop on subcutaneous inoculation into calves. The organisms described by this observer were streptococci, which only grew at blood heat, and were stained by Gram's process. The infection of this disease is conveyed by milk, and usually produces in the human subject an aphthous condition of the mouth.

Swine Fever.

Whether the organism of swine fever is identical or not with the bacillus of mouse-septicæmia, it is at any rate certain that it is a specific bacillus which can be cultured for many generations on artificial media, and is then capable of producing swine fever in a healthy animal. As, however, it is not readily distinguished by its size or shape, or its behaviour to stains, from other bacteria of common occurrence, the microscopic examination of affected tissues is not so useful for purposes of diagnosis as the naked eye appearance.

In the Report of the Board of Agriculture (The Diseases of Animals Act) for 1895 it is stated that many more animals recover from this disease than was formerly supposed, while in those that die an examination will reveal lesions of the alimentary canal that are so characteristic as to warrant a positive diagnosis. In the same report are a series of admirable plates showing the appearance of the characteristic lesions.

Cattle Malaria.

Celli and Santori (*Centralb. f Bakt.*, 1897, Nos. 15 and 16) report their investigations on a disease which attacks foreign cattle living in the Campagna, but spares those indigenous to the district. It is characterised by fever, great anæmia, an enlarged spleen, and bloody urine, and is very frequently fatal. The authors found in the red blood corpuscles of animals affected by and dead of the disease a small body assuming two types, according to whether its movements were amœboid or from place to place in the corpuscle. Culture experiments failed, but on one occasion they succeeded in inoculating a healthy calf with the disease, and in demonstrating the foreign bodies in the

blood during its continuance. They consider the bodies to be endocorpuscular parasites.

A rapid and certain diagnosis can, however, be made by the examination of the blood, whereby cases can also be detected which would otherwise escape observation. The disease appears to be identical with that described by Babès in Roumania as cattle hæmoglobinuria; by Smith and others as Texas fever; and by Sanfelice and Loi in Sardinia as hæmatinuria. When one considers the above-mentioned clinical characters, together with the characteristics of the parasite, the post-mortem appearances, the communicability from one animal to another of the same kind and race, and the further circumstances that the disease develops only in malarial neighbourhoods and seasons, and is most favourably influenced by quinine, its resemblance to human malaria is seen to be very marked.

Rinderpest.

This disease, which has recently caused such havoc amongst cattle in India and South Africa, has recently been investigated by Dr. Simpson, who has given the following particulars concerning it before a recent meeting of the Calcutta Microscopical Society.

The microbe is a diplobacillus, varying from 0·3 to 0·6 mm. in length, and about a third of this in breadth. Occasionally two diplobacteria are fixed end to end, and give the impression of a longer bacillus. The microbe is not unlike the bacillus found by Dr. Klein in ordinary calf vaccine. It is easily stained by the ordinary dyes, fuchsine and gentian violet. It grows aerobically and anaerobically, but gradually becomes attenuated in virulence by repeated growth in air, so much so that in the early part of 1895 two tubes rubbed into sores on the skin of an animal would kill the animal, whereas twelve tubes

now will only produce a slight illness. It is a motile bacillus, is sporeless, multiplies rapidly in bouillon with the formation of air-bubbles, and forms air-bubbles in stab-cultures of gelatine. It is destroyed at a temperature of 57° even when only exposed for a quarter of an hour.

By inoculation or intravenous injection in the calf it produces the characteristic symptoms of rinderpest, and in natural rinderpest the same microbe can be isolated, and will, when inoculated or injected, reproduce the disease in calves. Some progress has been made in the preparation of a vaccine and of an antitoxin, though the experiments are not sufficiently numerous or advanced to be more than encouraging.

In 1871, when the Indian Cattle Plague Commission investigated this disease, and came to the conclusion that it was the same disease as that which had caused so much destruction in cattle in England in 1866, the question of protecting animals by inoculating them with the crude virus, that is, with the fluids taken from a sick animal, was discussed, so also was the amount of protection produced by ordinary vaccination with vaccine lymph: both of these processes had been tried extensively in Russia and Austria, but not with very satisfactory results.

In inoculating with the crude virus, it was found that, though in many cases a mild disease was caused, very frequently a virulent type was produced, and that there was no real control over the disease. In the case of vaccinating with ordinary vaccine lymph, because of the view that rinderpest is allied to small-pox in man, the evidence as to protective effect was too conflicting to justify any practical action. With the microbe, however, now in our hands, I consider it to be merely a matter of time to prepare a vaccine which shall not only be protective, but which shall give us control over the disease.

Pleuro-Pneumonia.

This disease of cattle is at the present time practically non-existent in this country, owing to the vigour with which the stamping-out system has been carried out.

In a few cases pleuro-pneumonia was discovered in cattle imported from abroad, some of which were suspected before the animals were slaughtered. The lungs in an affected animal present a peculiar marbled appearance. Various organisms have been described in this disease, but at present no bacillus is admitted on all hands as the specific cause.

MICRO-ORGANISMS IN SOME PLANT DISEASES.

Dr. V. Peglion describes in *Malpighia* (1896, p. 556) a disease which attacks the stem of the hemp, causing disintegration of the tissues. It appears to be produced by an organism of the nature of a bacillus embedded in mucilage, and closely resembling *B. cuboniana*, a parasite of the mulberry. In Bulletin No. 12, for 1896, of the Division of Vegetable Physiology of the U.S. Department of Agriculture, Mr. E. F. Smith states that several species of solanaceæ—the potato, the tomato, and the egg-plant, *Solanum melongena*—are attacked by a disease which he calls 'brown-rot,' due to a hitherto undescribed parasite, which he names *Bacillus solanacearum*. It closely resembles *B. tracheiphilus* and the form known as 'Kramer's bacillus,' but differs in several characters from both. In the *Revue Mycologique* for 1896, M. E. Roze has described several bacteria which cause diseases in the cultivated potato, viz., *Micrococcus nuclei*, *M. imperatoris*, *M. pellucidus*, always found associated with the 'scab,' *M. albidus*, and *M. flavidus*.

According to C. Wehmer, the fungus which most commonly causes the rotting of fruits is *Penicillium glaucum*. In apples and pears this is accompanied by *Mucor pyriformis*, and in the case of medlars the latter is much the most common fungus. In lemons, oranges, and other tropical and sub-tropical fruits, *P. glaucum* is associated with two other closely allied species, *P. italicum* and *olivaceum*. In plums *Mucor racemosus* was also observed. In grapes *P. glaucum* and *Botrytis cinerea* are the most common fungi. It is the latter species which forms the gray tufts on walnuts.

CHAPTER XI.

MICRO-ORGANISMS OTHER THAN BACTERIA—FERMENTATION, ETC.

The yeasts, moulds and algæ: 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 ammoniacal fermentation of urea, the lactic and butyric acid ferments—Mixed fermentations—The unorganised ferments, or enzymes—The proteolytic, amylolytic, inversive and coagulative enzymes of bacterial origin—Putrefaction and oxidation—Action of water filter-beds—Nitrification of ammonia—Fixation of atmospheric nitrogen—'Nitragin'—Chromogenic bacteria and colouring matters—Colouring matter, *B. cyanogenus*, *B. prodigiosus*, *B. pyocyaneus*, etc.—Phosphorescent Bacteria—Other products of the metabolism of micro-organisms—The bacteriology of sewage.

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 sac-

charine 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 resembling 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

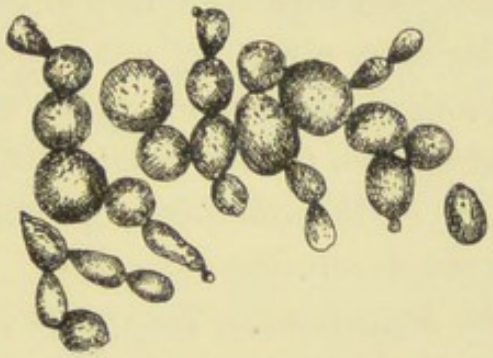


FIG. 26.—SACCHAROMYCES CEREVISIÆ.

brewery yeast. It grows as rounded or slightly ellipsoidal cells, which give off small cells by budding. The cells are

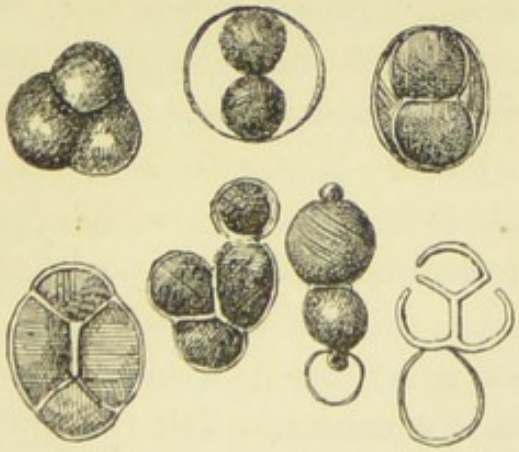


FIG. 27.—SACCHAROMYCES CEREVISIÆ. (Stages in the development of ascospores.)

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.

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.

Jörgensen has published an investigation on the origin of wine yeasts, from which it would seem that the *Sacch. ellipsoideus*, associated with wine fermentation, is a peculiar development, brought about by natural conditions, of the *Dematium* or *Chalara*-like moulds which are always present on grapes. Sorel also (*Comp. rend.*, 1895, December 16) has, by certain culture-methods, produced a yeast-like development from *Aspergillus orizæ*.

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 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.



FIG. 28.—SACCHAROMYCES PASTORIANUS I.

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 *Sacch. 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					
35° -	25 hours	—	—	—	—	None
33·5° -	23 hours	—	—	—	None	31 hours
31·5° -	—	None	—	—	36 hours	23 hours
30° -	20 hours	30 hours				
29° -	—	27 hours	None	None	23 hours	22 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.

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

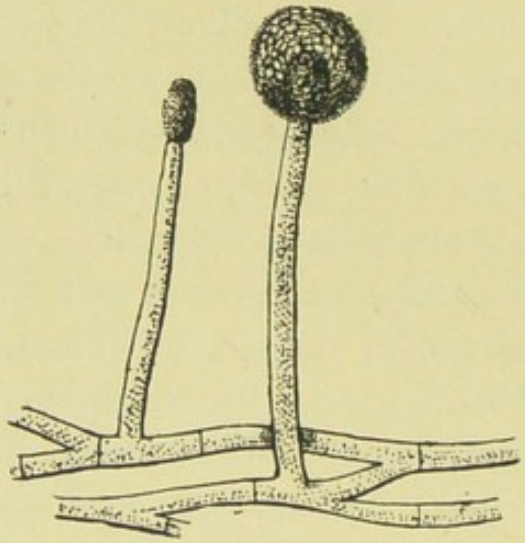


Fig. 29.—Mucor Mucedo.

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 bread, candied fruit, etc., on which are seen the stout swollen club-like fructifying hyphæ, upon which are

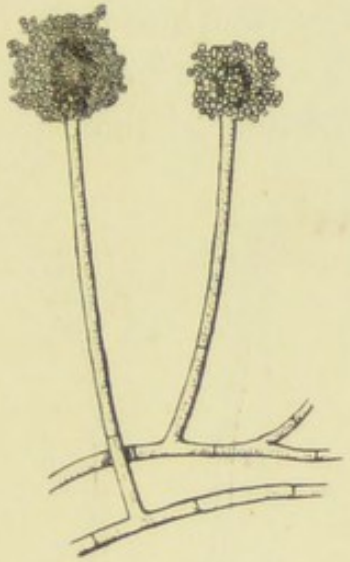


FIG. 30.—ASPERGILLUS GLAUCUS.

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 the 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 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

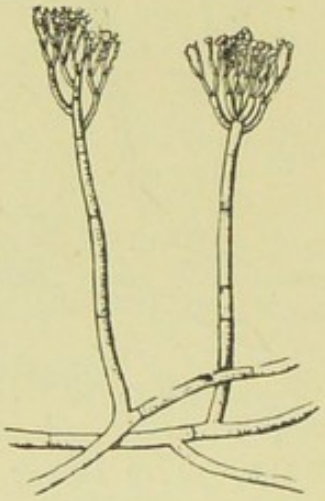


Fig. 31.—Penicillium Glaucum.

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 frequently found in sour milk and butter. The fibres of the mycelium grow upwards, become segmented, and

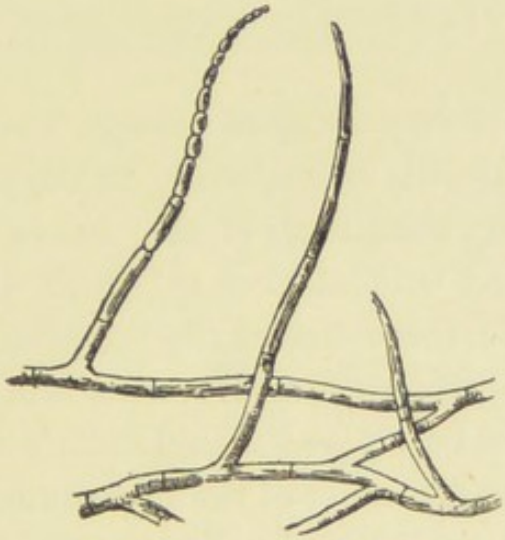


Fig. 32.—Oidium Lactis.

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 coverglass 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



Fig. 33.—Crenothrix Kuhniana.

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 zoogloea 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 zoogloea. 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 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 in-

fusions 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.

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:

$$\underset{\text{Glucose.}}{C_6H_{12}O_6} = \underset{\text{Alcohol.}}{2C_2H_5OH} + \underset{\text{Carbonic acid gas.}}{2CO_2}.$$

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 dispatched 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 im 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.

2. **Fermentation by Hydration.**—The most important fermentation 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 track 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 formation 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. The conversion of cellulose into carbonic acid and marsh gas (methane) by the putrefactive organisms is a case of simple hydrolysis, as will be seen from the following formula:

$$(C_6H_{10}O_5)_n + {}_nH_2O = 3_nCO_2 + 3_nCH_4$$

Cellulose. Water. Carbonic acid. Methane.

These 'soluble' ferments are divisible into a number of groups, the five most important of which are the following:

1. *Proteolytic.* — Those which change proteids into proteoses and peptones, as in the case of trypsin and pepsin.

2. *Amylolytic.*—Those which change amyloses (starch, glycogen) into sugars, such as diastase, ptyalin, and amylosin.

3. *Inversive.*—Those which convert the saccharoses, such as lactose, maltose, cane-sugar, etc., into glucose. Invertin, which occurs in the cells of yeasts and in the intestinal juice, is an example of this class.

4. *Coagulative.*—Those which convert soluble into insoluble proteids, such as rennet (chymosin), fibrin, and myosin ferment.

5. *Steatolytic.*—Those which split up fats into fatty acids and glycerine. Steapsin, which occurs in the pancreatic juice, is an example of this class.

Proteolytic Enzymes.—The proteolytic or albumen-dissolving ferments are a very common product of bacterial growth. The liquefaction of the gelatine, which is allied to albumen in chemical composition, which is caused by so many of the bacteria, is certain evidence of the presence of an enzyme of this class. The proteolytic ferment excreted by bacteria is more of the nature of trypsin than pepsin, since the liquefaction of gelatine takes place in a medium

which is alkaline or neutral in reaction, whereas pepsin is only effective in acid solution.

Amylolytic Enzymes.—A large number of the bacteria excrete ferments of the diastatic class. The organism of cholera, anthrax, glanders, and a number of non-pathogenic varieties possess the power of forming such ferments. They may be demonstrated by their action on starch paste. A thin starch paste containing 1 per cent. of thymol is combined with a culture of the organism, to which 1 to 2 per cent. of thymol has been added. The mixture is then incubated at blood heat for six to eight hours. A little Fehling's solution is then added, and the solution heated in a water-bath. If a reduction of the copper solution takes place, resulting in a reddish-yellow precipitate showing the presence of glucose, a diastatic ferment is present.

Inversive Enzymes.—The ferments of this class are very uncommon amongst the bacteria. Of a very large number examined only the following gave evidence of producing this ferment: *B. megatherium*, *B. fluorescens liquefaciens*, *Vibrio choleræ* and *Metschnikovii*. The enzyme may be demonstrated as follows: A 2 per cent. solution of cane-sugar containing 1 per cent. of phenol is mixed with a culture of the organism also containing 1 per cent. of phenol. After a few hours the solution is tested with Fehling's solution to ascertain if any glucose has been produced. Control tests are always necessary.

Coagulative Enzymes.—Ferments of the rennet class, which coagulate milk independently of the action of acids, are not wanting amongst the bacteria.

Duclaux (*Comptes Rendus*, 1891) and Hueppe (*Deutsche Mediz. Wochensch.*, pp. 48, 49) first pointed out that such ferments are conveyed by many different bacteria, which precipitate the casein in the presence of a weakly acid,

amphioteric, or even neutral reaction. The numerous tyrothrix bacilli isolated by Duclaux, the *Bacillus pyocyaneus*, yellow sarcina, and particularly the organisms described by Flügge (*Zeitsch. f. Hygiene*, xvii., p. 272), and characterised by their capacity to peptonise milk, belong to this class. Cohn (*Centralbl. f. Bacteriologie*, ix., p. 653) produced the precipitation even by means of bacteria of which the vegetative capacity had been completely destroyed with chloroform, thus showing that the fermentative action was due to a substance independent of the metabolic products of the organism. These substances have been isolated by Cohn and others ; they are destroyed in most cases at from 65° to 75° C. Some ferments, however, as, for example, that described by Gorini (*Hyg. Rundsch.*, 1893, p. 381), in association with the *Bacillus prodigiosus*, resist as much as an hour's exposure to 70° to 80°, and require at least half an hour's exposure to 100° C. for their destruction.

The amount of the ferment varies with the species and age of the culture and also with the temperature, much more coagulating ferment being obtained at 20° than at 37°. The ferment works, however, as does rennet, much more strongly at 37° than at lower temperatures. Its action is impeded by the presence of alkalies. When tryptic ferments are produced simultaneously, the coagulating ferments, which are developed more slowly, may fail to work, the casein being peptonised before the coagulating ferment has acted.

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 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.

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 waterworks 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.

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. 62.

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.

Mr. John Hunter, of Edinburgh, states in a communication to the authors that it is important to note that the size and form of the nitric bacteria are materially modified by environment, and it applies equally to the organism—or organisms—of the leguminosæ. From his inoculation experiments on sterilised soil, it would appear there is an absolute dependence of the one class of advantageous

organism upon the other, and that when the conditions which favour the abnormal development of one class be present, the destruction—to a great extent—of that class is certain. This is well illustrated by the application of nitrate of soda in considerable proportion — the nitric bacteria diminish most markedly.

The nitric bacteria appear to be incapable of working up *nitrogenous* organic matter themselves—they depend upon co-workers for the initial change, and while $CaSO_4$ can be used by the nitric organism, $CaCO_3$ is much more easily decomposed by them, and of course they must have the other essentials, viz., P_2O_5, K_2O, MgO, and a minute proportion of NaCl.

As an instance of the utilisation 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 lixiviated, 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.

Fixation of Atmospheric Nitrogen.—For more than a century the question has been debated as to whether atmospheric nitrogen was available as plant food. For many years the balance of opinion was against this idea, although it seems strange that scientists should have doubted that nitrogen was in some way assimilated, even though the means were not understood.

It was first definitely proved by M. Berthelot, in 1876, that some plants had the power of fixing free nitrogen; he also found that, apart from the growth of plants, some *soils* had the power of taking up nitrogen, which property has since been traced to particular special bacteria contained therein.

The discovery of these organisms was due to two German investigators—Messrs. Hellriegel and Wilfarth—who made a special study of the small, tubercle-like bodies which had long been observed on the roots of the lupin, peas, clover and other plants of the same class. A microscopic examination of these tubercles showed that they contained masses of very short bacteria. These bacteria were then isolated and cultivated, and it was definitely found it was the presence of these organisms that enabled the plants to directly absorb atmospheric nitrogen.

The absolute need for the presence of these organisms in soil intended for crops has been shown by the experiments of Professor Nobbe on plants which have been made to grow on soil that has been sterilized 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.

Artificial cultures of these organisms have recently been introduced commercially under the name 'Nitragin' in Germany, for the enriching of soils naturally poor in these organisms, with the result that the crops have been largely increased.

From what has been said respecting the utilisation due

to the agency of bacteria of free and fixed nitrogen by plants, it will be seen that these discoveries have revolutionised some of the older ideas respecting vegetable physiology, and certainly may be said to have marked an epoch in the history of scientific agriculture.

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 phenomenon 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 photo-

graphs 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.

For the cultural characters of these phosphorescent bacteria, see p. 448.

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.

The Bacteriology of Sewage.—Ordinary town sewage contains very large numbers of bacteria, often several million per cubic centimetre. The number will vary greatly with the freshness of the sewage, the season of the year (temperature), and may also be affected by the addition of various waste liquors from manufactories.

We are hardly yet in a position to specify precisely by name the bacteria that are constant inhabitants of sewage, but the following may be regarded as the most important:

Bacillus coli (several varieties).
Bacillus fluorescens liquefaciens.
Bacillus fluorescens non-liquefaciens.
Bacillus ramosus.
Bacillus subtilis.
Bacillus mycoides.
Bacillus subflavus.
Bacillus tholoeideum.
Bacillus cloacæ fluorescens.
Bacillus fluorescens stercoralis.
Proteus vulgaris.
Proteus mirabilis.
Proteus cloacinus.
Proteus Zenkeri.
Cladothrix dichotoma.
Beggiatoa alba.

Sewage begins to alter in composition almost immediately, and this alteration is due almost entirely to the action of bacteria, as if sewage is sterilised by heat and kept free from bacteria it will remain unchanged for any length of time. This rapid change in the composition of sewage necessitates the performance of any analytical operations on the spot or within the shortest possible time after the collection of the samples.

If it is desired to estimate the number of bacteria present, gelatine plate cultures should be made on the spot after diluting the sewage with sterile water. Besides plate cultures, anaerobic cultures should be made, as sewage will always contain some anaerobes which will not grow in plate cultures exposed to the air.

Such bacteria as the typhoid bacillus, the cholera bacillus, or the bacillus of diphtheria, might, of course, be identified in sewage taken immediately below a hospital, but researches distinctly point to the probability that no pathogenic bacteria can survive long in sewage, probably because they are crowded out by other organisms that are more readily able to flourish in that particular medium.

Laws and Andrews, for example, found that not merely did typhoid not flourish in sewage, but that it could not remain alive in it for anything like the length of time that it survives in ordinary drinking water, and that its disappearance from sewage is hastened by the presence of certain sarcinæ.

The bacteriology of sewage is receiving attention at the present time on account of the recent development of so-called bacteriological methods of sewage treatment, which seem likely to supplant the earlier methods of sewage treatment in which chemical precipitation was relied on.

The intention of these processes is to bring about the

purification of sewage by natural or biological means without the addition of any chemicals whatsoever, and to render it fit to be run into rivers or streams without causing pollution.

It is only of recent years that the precise changes that occur in the passage of a water from pollution to subsequent purification have been correctly understood, and when these changes were still in obscurity it is not surprising that the methods intended to bring about purification were initially faulty, and did not aim in the right direction.

In this connection it will be of assistance if we first consider the general composition of town sewage, and it will be at once apparent that the earlier methods of sewage-disposal (precipitation) could at best only accomplish clarification, which, though an essential point, is nevertheless only a step in the successful purification of sewage. The constituents of ordinary sewage may be roughly classified as follows:

1. Inorganic matter in suspension.
2. Inorganic matter in solution.
3. Organic matter in suspension.
4. Organic matter in solution.

1. **The Inorganic Matter in Suspension** is chiefly sand and road grit, clay, etc., the greater part of which will settle out by simple subsidence if the sewage is allowed to remain at rest.

2. **Inorganic Matter in Solution.** — There is no great increase in the inorganic matter in solution as compared with unpolluted water, with the exception of a small amount of phosphates, which are, however, one of the chief causes of sewage fungus.

3. **Organic Matter in Suspension** (such as fæcal matter,

paper, vegetable débris, etc.).—These are almost entirely removable by any of the precipitation processes in use.

4. **Organic Matter in Solution.**—None of the chemical precipitation processes in use can have much effect on the removal of organic matter in solution, while some of them in which much lime is employed actually increase it.

It is the organic matter in solution that is the cause of many effluents, although bright and clear and comparatively free from smell, subsequently becoming putrid.

The natural purification of sewage may be regarded as taking place in two stages—though these may to some extent occur simultaneously—first, the digestion or liquefaction of the solid matters in suspension, and secondly, the oxidation of these with the organic matter originally in solution.

The study of these changes has now led to practical results in the introduction of new methods for the treatment of sewage, and we are now able to produce effluents of a far greater purity and uniformity than has been possible before.

It has been known for a considerable time that sewage from which the suspended matter has been removed may be very much improved by passing through land, but that to obtain good results the land must be prepared by underdraining, and that different soils vary very much in their power of producing purification.

The liability of land to become water-logged has led to the construction of artificial filter-beds of coke breeze, gravel, or furnace clinker, using the materials of such a size as to ensure speedy draining of the bed when the water is turned off.

Up to the present time the Local Government Board has insisted on the provision of land for the final purification of sewage effluents, but now that there is indisputable evidence

at hand to show the superiority of filter-beds, it is to be expected that their use will be recommended in preference.

The advantages of filter-beds (particularly such as are automatically filled and discharged) over land are very great :

1. Far less space is required.
2. There is no fear of the filters becoming foul, and consequently the danger of causing a nuisance is diminished.
3. The purification effected is probably greater, and is unquestionably more uniform.
4. The labour required is very slight ; and the non-dependence on the skill or judgment of an attendant renders the filters much more reliable than a farm.
5. Where filters are used the purification of the sewage is the sole object in view, so cannot be sacrificed to exigencies of cropping, as in the case of land.

In order that bacterial filters may continue to exert their oxidising and nitrifying effect, it is essential that they should be supplied with air, either artificially, by causing air to enter the filter-bed as in Ducat's system and Lowcock's system, or by working duplicate series of filter-beds so as to allow time for aeration, as in the Sutton filters, the septic tank process, and in Scott-Moncrieff's filter.

Sufficient analytical data have not yet been collected to enable the relative merits of these processes to be compared but evidence on the results of all of them will probably be available before long.

The action of such beds is to rapidly decrease the figures representing the 'albuminoid ammonia' and 'oxygen absorbed,' while the formation of nitrates in considerable quantities is also invariably to be expected. The presence of nitrates should indeed be insisted on in an effluent as

showing that true purification has begun, and that there is a store of oxygen that could be drawn on, as a guarantee against possible putrefaction.

It is impossible to judge of the quality of an effluent by any bacteriological means at present at our disposal, but various chemical standards have been proposed.

In a report on the septic tank process (Exeter) we have suggested such a chemical standard, laying stress on the necessity for insisting on the presence of nitrates in appreciable quantities.

It is at least safe to prophesy that in the near future all sewage works which do not discharge direct into the sea will be required to provide themselves with bacterial filters, and to produce an effluent conforming to some kind of standard.

CHAPTER XII.

DISINFECTION AND DISINFECTANTS.

Methods of disinfection of the body, discharges, clothes, home, hangings, bed-linen, etc.—Disinfection by sulphur and chlorine—Disinfection by formalin vapour—Equifex spray disinfection—Disinfection by heat—Disinfection by steam—Steam disinfectors—The construction of steam disinfectors—Lyon's disinfector—Equifex disinfector—Equifex low pressure disinfector—Thresh's disinfector—Reck's disinfector—Testing of steam disinfectors—Disinfectants—Bacteriological testing of disinfectants.

With regard to diseases generally, the 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.

The general preventive measures to the spread of disease have already been dealt with. The considerations involved in practical disinfection are of the greatest practical importance, 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 with an efficient disinfector are rendered perfectly sterile.

Excreta should be received into a 5 per cent. solution of carbolic acid made in a saturated (24 per cent.) solution of salt. If solutions of permanganates are employed, they will certainly part with their oxygen to oxidisable organic matter before the organised 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 mask or destroy effluvia will only lead to obscuring the ready perception of the inleakage 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 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. The steps to be taken depend on whether the room may be stripped of its paper or not.

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) thoroughly sprayed with formic aldehyde solution 5 per cent., 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.

Where it is not allowable to strip the wall-paper, the use of a disinfectant spray as described below is indicated.

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 organised 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 Formic Aldehyde (Formalin) Vapour.—Formic aldehyde is a gas possessing a high disinfectant power but practically no capacity for penetration. It is liable to change into a polymeric form possessing relatively little disinfectant power. It has been proposed to use it for disinfectant purposes in three principal ways; in the one by the generating it by the combustion of methylated spirit in a special lamp admitting a regulated quantity of air; in the second by the heating under a pressure of 3 to 4 atmospheres of a mixture of formaldehyde solution and calcium chloride (Trillat's autoclave); and by the volatilisation of paraform tablets. The best results have been obtained from the Trillat autoclave, the gas being conducted from it by a tube passing through the keyhole of the door into the room, which has to be carefully sealed,

and left exposed to the disinfectant atmosphere for many hours. The latest investigations (Abba and Rondelli, *Rivista d'Igiene*, July and August, 1897, and Piton, *Archives de Médecine Navale*, lxvii., 6), confirm the balance of experience up to date, and demonstrate that the use of formaldehyde vapour, even pushed to a strength considerably beyond what is usual, leaves organisms liable to escape disinfection, the feeble penetration of the gas being responsible for the failure.

Equifex Spray Disinfection.—On the whole, the most convenient and trustworthy method of disinfecting what cannot be exposed to steam lies in the use of the Equifex sprayer with a disinfectant solution. This instrument consists essentially of a strong reservoir, in which the disinfectant solution is either placed beforehand or pumped during the operation. Air is compressed by means of a pump, and part of it drives the solution through a flexible tube to a spraying-nozzle, in which, by a suitable contrivance, the velocity of the stream of liquid is cut down, while part passes under pressure through a parallel tube and catches up the stream of disinfectant, which it carries through the actual spray-producing nozzle. The relative proportions of air and disinfectant can be regulated at will by means of cocks; and thus a spray is projected on to the surface to be treated at a velocity sufficiently high to ensure penetration, and of a fineness which it is found in practice can be so adjusted as to leave the surface even of common wall-paper uninjured. This method is now coming into more general use in this country; in France it has been generally adopted for some years with good results. The practice is to spray vertically upwards, as it were in vertical stripes; and when a room is finished it is gone over a second time, the chance of the same spot being missed twice being thus reduced to a quantity which may be

neglected. The usual disinfectant has been mercuric perchloride 1 in 1,000 acidulated, or with salt added (3 to 5 per cent.). Of late formaldehyde solution has been used with excellent results.

This method is indicated with formaldehyde solution for cases where an offensive odour exists, the solution being a strong deodorant. It is also the best means available for disinfecting furs and leather articles, which cannot stand the action of steam.

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 and 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 disinfector, 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. It is quite inaccurate to speak of 'current steam' as contrasted with steam under pressure, as steam can be used as a current in either, with or without a pressure exceeding that of the atmosphere. 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. It has also been repeatedly shown that in the absence of pressure the temperature and disinfectant value of the steam depends largely on its velocity, and the rate of stoking will largely affect it —an objection which is serious because there is no convenient or trustworthy means of controlling either the velocity of the steam or the rate of stoking. What the temperature should be is still a matter of discussion. Many common bacteria, such as those of typhoid, diphtheria, and cholera, are with certainty destroyed 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 Lattraye) conclude that twenty minutes' exposure to a temperature of 110° C. should be allowed in all cases. It must be remembered, also, that in

practice thick blankets and similar objects oppose resistance to the penetration of steam, and this resistance is liable to vary with circumstances. It would be desirable in any case to have a margin of temperature; and the least, where it is possible to procure it, is an exposure for fifteen minutes to saturated steam at 115° C. Disinfectors giving a less temperature may, if properly designed to give saturated and not superheated steam, be useful in communities where the cost of a disinfector under the pressure which is desirable is utterly beyond the funds available. But it can give no certainty except for the organisms of diseases such as those named above, which have been shown to perish in all circumstances at temperatures well below 100° C. Disinfectors without pressure should, for the reasons named above, not be used at all.

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.

The Construction of Steam Disinfectors.—The chief principles involved have been stated above. To these must be added one of great importance—that the steam must be free from air. Koch in 1880 omitted this precaution, and found in consequence that steam at 212° acted more powerfully than at a higher temperature in a digester. Heydenreich and others showed what has been confirmed by all subsequent experiments, that this result was due solely to the presence of air in the vessel, and that when the air was allowed to escape, the steam acted more rapidly and effectively when under pressure than when used as a current without pressure.

The disinfectors principally known in this country are described below.

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 disinfection is effected by extracting some part of the vapour by means of the vacuum, and allowing the remainder 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 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 off; 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 air heated over coils at the bottom of the disinfection chamber 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,

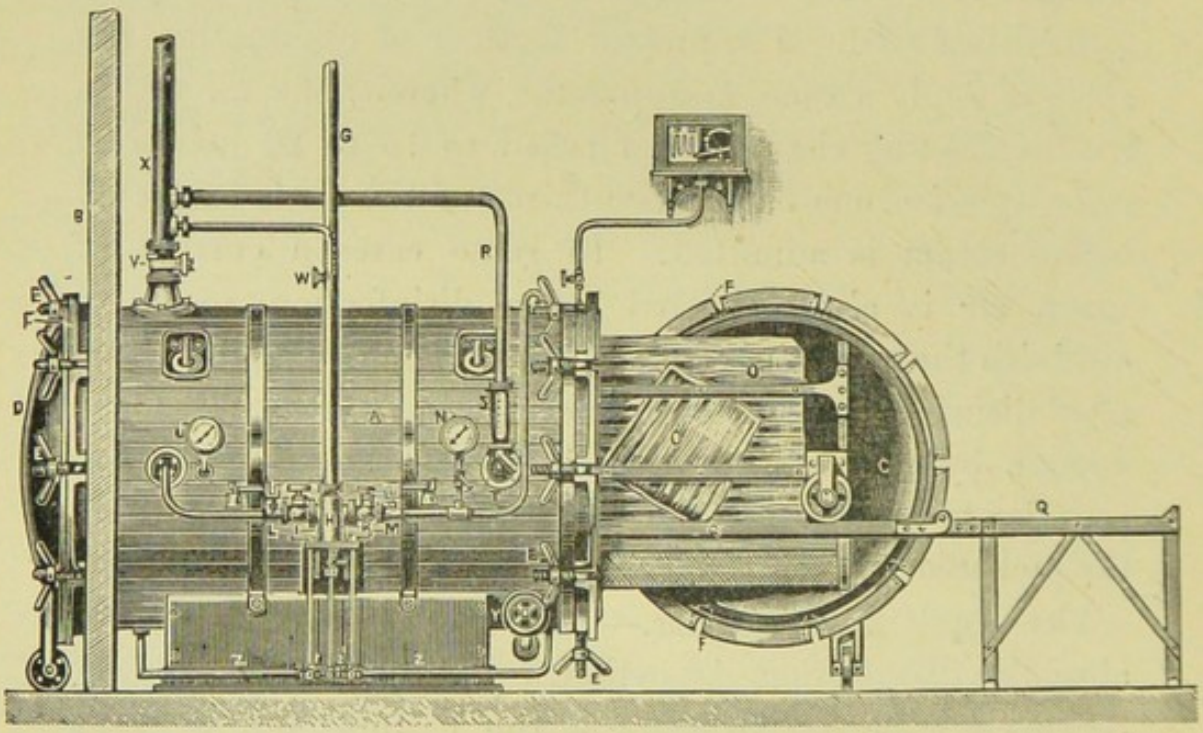


Fig. 34.—Equifex Disinfector.

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 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

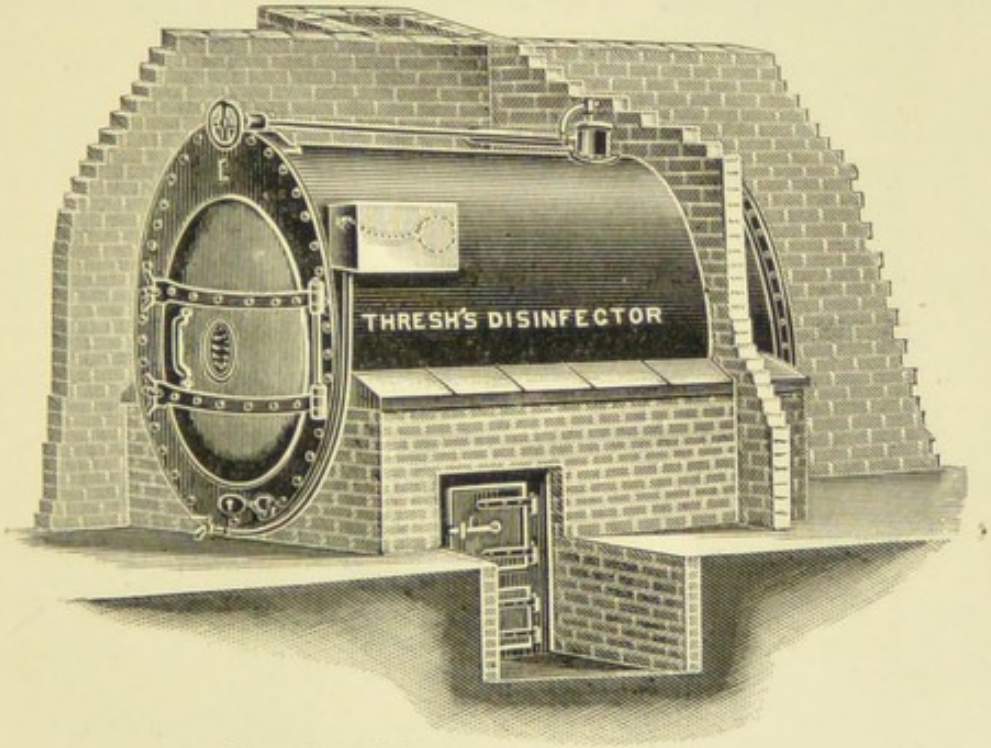


FIG. 35.—THRESH'S DISINFECTOR.

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.

The Testing of Steam Disinfectors.—Steam disinfectors may be examined either directly, by determining the exposure necessary for the destruction of test organisms, or indirectly, by measuring the temperature and other physical quantities. The direct method is unsatisfactory,

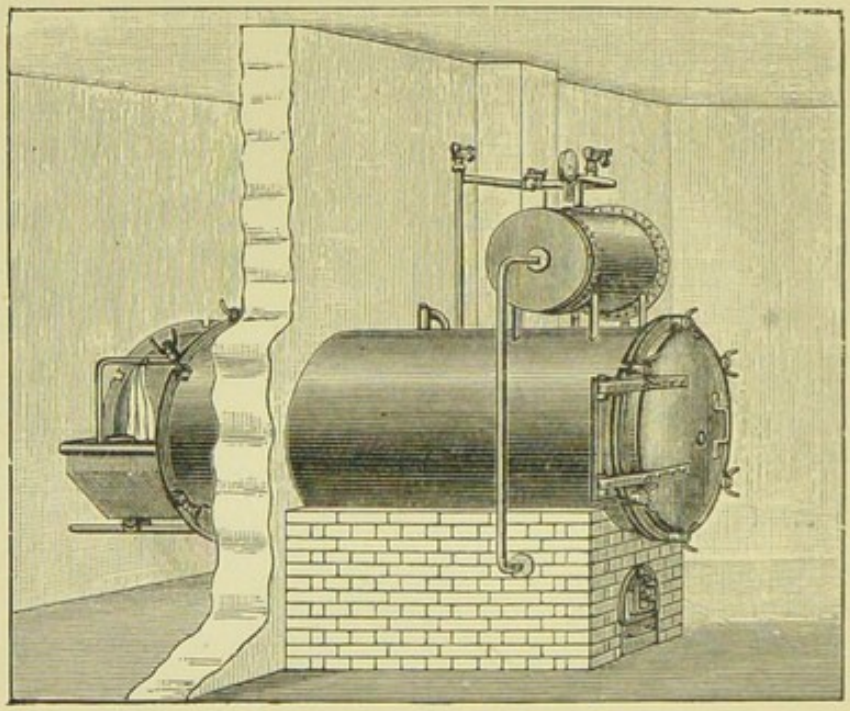


Fig. 36.—Reck's Disinfector.

owing to the wide variability which specimens of apparently the same organism may display through circumstances such as age of culture, presence of interfering media, etc., of which the variations cannot be measured, and of which even the qualitative effect cannot always be stated in general terms. In such an examination it is therefore impossible to take a given organism as comparable with the same organism used in previous experiments; and for it to have value, the inquiry must include

practically a redetermination of the thermal death-point of the organisms which may be considered to have a resistance equal to that of any which is likely to be treated by the disinfector in practice. It is therefore more satisfactory to take the temperature and exposure required as determined by the data to be found by the collation of the previous experiments, the effect of which has been stated above, and to determine the extent to which the disinfector provides the conditions which have been described as necessary for giving such an exposure to saturated steam free from air.

To obtain these data it is simplest to use electric and maximum thermometers, a pair being hung up inside the chamber, while another pair are under four folds of blanket, and a third pair are under eight folds.

The maximum thermometers should be of the Phillips type, in which a small portion of the column is separated from the remainder by a bubble of air.

They should be capable of registering up to 130° C., and must be tested against a standard thermometer in boiling water every day they are used.

The electric thermometers should be made with thin bulbs, but the quantity of mercury does not matter much. The bulb must not touch any portion of the wooden frame of the thermometer or the blankets; if it does erratic results will be obtained.

The platinum wires must be sealed in, or water will condense in the tube; it would be convenient if the tube could be left open so as to be able to set the wire so as to make contact at different temperatures, but this cannot be done conveniently on account of the water condensing in the tube. The platinum wire should be as thin as possible, or mercury will stick between it and the sides of the tube. The wires leading from the thermometers to the battery

and bell should be flexible, silk-covered wires, and it is convenient to have them of different colours.

The wires can be pinched tight between the door and the machine, so as to prevent escape of the steam without fear of cutting the wires. One battery and bell is sufficient for several thermometers, each thermometer being disconnected as the mercury rises and makes connection.

A good electric thermometer should ring in fifteen seconds on being plunged into boiling water. Suitable thermometers can be obtained from J. J. Hicks, of Hatton Garden.

In testing a high-temperature disinfector, using an electric and a maximum thermometer together within several folds of blankets, and turning off the steam directly the electric thermometer (set to ring at 100° C.) rang, it would be expected that the maximum thermometer would also register 100° C., or only a little above. This is not the case, as the maximum thermometer may be found registering several degrees higher. A satisfactory explanation of this is not at present forthcoming.

The presence or absence of superheat is tested by comparing the pressure in the disinfecting chamber as measured by a pressure-gauge with the temperature obtained in various parts of it. In the case of non-pressure disinfectors the barometric pressure is the pressure in question. Regnault's tables, stating the temperature of saturated steam at various pressures, will enable the results to be interpreted. The presence or absence of air at any epoch can be tested by condensing the exhaust steam in a tube provided with a cock on the side next to the disinfector and dipping into water. If it is free from air, the tube will fill completely with water; if not, the residual air will collect at the top.

A steam disinfector should be examined for uniformity of temperature in various parts of the disinfecting chamber.

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 Bacteriological Testing of Disinfectants.—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 this 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 dry. 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, however, 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 boiling 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 examination 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, prefer-

ably 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. Defries and others have found that the results of this method are not necessarily the same as those obtained by the method of mixtures, even when the operations are conducted simultaneously on the same cultures and solutions. This method is also 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, and thereafter preferably also with water, so as to avoid such antiseptic effect as the compound resulting from the previous treatment may possess. Irregular penetration, however, of the disinfectant makes it quite possible that some portions may not be reached by this process. These objections to the method are to a considerable degree removed when threads of slag-wool are used instead of silk. This substitution does not, however, affect the liability of the threads to be contaminated in one or other of the successive manipulations; and a new difficulty is introduced in the liability of the organisms to be mechanically washed off the smooth surface of the glass. Defries found this to

occur fairly often, and substituted for the glass threads small test-tubes, on the bottom of which small measured drops of culture were allowed to fall and dry, the subsequent addition of disinfectant, antidote, water, and broth, being performed in the same tube. In this way the risk both of pollution and of loss from washing during the various manipulations is reduced, especially when a little gelatine is added to the original culture, which is kept in a burette at melting-point and allowed to solidify in a very thin layer at the bottom of the tube.

It must be remembered that in these, as in all bacteriological examinations, every operation has to be controlled. Thus, for instance, when organisms are prepared for test by drying, a portion of the untreated organisms must be inoculated into broth to make sure that at least they have survived. The nature of the control varies in different cases; but no experiment is complete until a corresponding inoculation has been made, embodying, as far as possible, all the test conditions, except the presence of the substance under examination.

Similarly it is necessary to examine the cultures microscopically before application of the disinfectant, and, in the event of growths, to identify them both in this way and by subcultures.

It is found that the action of a disinfectant is enhanced in most cases by its application at a temperature higher than the optimum, even when that temperature is below what would 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. In some cases the addition of a substance possessing by itself little or no disinfectant action, may greatly enhance the efficiency of a disinfectant. Thus, carbolic acid solution, saturated with common salt, is

much more effective than when used by itself, though salt has practically no disinfectant capacity. 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, such as powders, 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. The chief danger to which they give rise is, that persons who are sufficiently credulous to use them are liable to regard them as justifying neglect of those more effective precautions of cleanliness and of true disinfection which are of real service.

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—Regulations of Imperial German Health Department—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—Stoddart's method—Isolation of the cholera bacillus from water—Examination of filters—Examination of milk—Number of bacteria found in milk—Milk diseases—Blue, red, yellow, bitter, stringy, soapy milk, etc.—The organisms producing these diseased conditions—Necessity for improved sanitary control of dairies—The sterilisation and pasteurisation of milk—Detection of the tubercle bacillus—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 depths 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

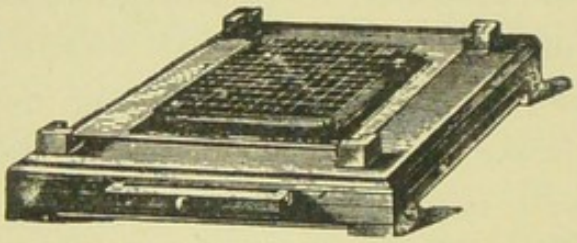


Fig. 37.—Wolffhügel's Apparatus.

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.

The following procedure is recommended in the notification of the Imperial German Health Department, issued in regard to the filtration of surface waters used for public water supplies:

'In order to secure uniformity of method, the following is recommended as the standard method for bacterial examination:

'The nutrient medium consists of 10 per cent. meat

extract gelatine with peptone, 10 c.c. of which is used for each experiment.

'Two samples of the water under examination are to be taken, one of 1 c.c. and one of $\frac{1}{2}$ c.c. The gelatine is melted at a temperature of 30° to 35° C., and mixed with the water as thoroughly as possible in the test-tube by tipping backwards and forwards, and is then poured upon a sterile glass plate. The plates are put under a bell-jar, which stands upon a piece of blotting-paper saturated with water, and in a room in which the temperature is about 20° C.

'The resulting colonies are counted after forty-eight hours, and with the aid of a lens.

'If the temperature of the room in which the plates are kept is lower than the above, the development of the colonies is slower, and the counting must be correspondingly postponed.

'If the number of colonies in 1 c.c. of the water is greater than about 100, the counting must be done with the help of Wolffhügel's apparatus.'

In those cases where there are no previous records showing the possibilities of the works and the influence of the local conditions, especially the character of the raw water, and until such information is obtained, it is to be taken as the rule that a satisfactory filtration shall never yield an effluent with more than about 100 bacteria per cubic centimetre.

The filtrate must be as clear as possible, and the colour, taste, temperature, and chemical composition, must be in every way satisfactory.

To allow of a complete and constant control of the bacterial efficiency of filtration, the filtrate from each single filter must be examined daily. Any sudden increase in the number of bacteria should cause a suspicion of some

unusual disturbance in the filter, and should make the superintendent more attentive to the possible causes of it.

Every city in the German empire using sand-filtered water is required to make a quarterly report of its working results, especially of the bacterial character of the water before and after filtration, to the Imperial Board of Health.

Detection of Sewage Pollution.—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 the 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 results of experiments made by one of us on waters of known origin shows 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	over 1,000,000	over 1,000,000		

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 colon bacillus was formerly regarded 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 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.

If in a sample of water two or more organisms characteristic of excremental dejecta are found in association with the bacillus coli—say, for instance, the *Proteus vulgaris* and the sewage variety of *Proteus Zenkeri*—this should be regarded as almost positive evidence of pollution with sewage.

It is necessary to point out, when investigating the characters of organisms in connection with sewage pollution, that there exist in sewage and in water, particularly when the latter is polluted with manure or with sewage, in addition to the aerobic organisms, certain others which in the ordinary bacteriological analysis of water, sewage, and other materials are generally overlooked; these are organisms which grow only anaerobically, and which, therefore, do not make their appearance in the ordinary plate cultures. Although as a rule they are neglected in water and sewage analysis, they are, nevertheless, by their numbers and by their functions not without importance. The anaerobic bacilli are endowed with the power of more or less rapidly peptonising and decomposing gelatine, of rapid multiplication in grape-sugar gelatine or grape-sugar agar in the depth, and of forming gas. Their spores can be heated to 80° C., for half an hour or an hour, without interfering with their subsequent power of germination into the bacilli.

In this connection, as has already been mentioned elsewhere, Klein traced an outbreak of severe diarrhœa to a sporogeneous virulent anaerobic bacillus, *B. enteritidis sporogenes*, which in morphological and cultural respects has some points in common with the bacillus butyricus.

Furthermore, showing the importance of these organisms, it was stated by Klein (Harben Lectures, 1896) that he had recently occasion to examine a sample of sewage effluent which had been subjected to a certain

treatment. This treatment affected the aerobic bacilli to such a degree that it reduced them from over 3,000,000 per cubic centimetre in the untreated effluent to 160 per 1 c.c. in the treated effluent; but when examining the effluent on the above lines for the presence of anaerobic spores, it was found that these latter, as also other spores, had remained unaffected by the treatment. This Klein considers is sufficient proof to show the importance of the analysis embracing also anaerobic cultures; the same applies to the bacteriological analysis of water, in which the detection of aerobic sewage organisms may be difficult owing to their small number, but may nevertheless contain the spores of some definite anaerobic sewage organisms.

The Isolation of the Typhoid Bacillus from Water.—There is very great difficulty in isolating the *B. typhosus* from water that has been very copiously contaminated with specifically polluted sewage; there is even greater difficulty in detecting it when the specific pollution has been small in amount. 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, 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 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 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

* *Gazette des Hôpitaux*, 1887, p. 202. † *Ibid.*, p. 384.

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.

The great objection to the use of phenolated broth is that when cultivated at blood-heat the colon bacillus and its allies multiply at from two to five times as quick as the typhoid bacillus, even if this is not suppressed altogether. This objection also applies, but in a less degree, to phenolated plates, the surface of which may be covered by the expanded colonies of the colon bacillus.

It must be remembered that the preliminary concentration does not improve matters numerically, and, as has been pointed out by Stoddart, it is not unreasonable to suppose that the somewhat violent treatment may have a more injurious action upon the typhoid bacilli than upon the hardier forms. Since by no method at present known can the ratio of the typhoid to the colon bacillus and the common water saprophytes be increased, and the general tendency is for them rather to decrease, the difficulty is best met by increasing the area of the plates rather than by any method of concentration. This numerical difficulty has, perhaps, not been fully appreciated by many workers on this subject, but it has been forcibly presented by Laws and Andrewes in their recent report ('Report upon the Micro-organisms of Sewage,' presented to the London County Council,' 1894). In the case, for instance, of a moderately polluted water containing 50,000 microbes per cubic centimetre: of these, possibly 90 per cent. may be suppressed

by the addition of phenol, leaving 5,000 to be dealt with by plating out. There would obviously be no advantage in concentrating such a water, since it is impossible to deal satisfactorily with a plate of ordinary size containing 1,000 colonies. It would then be necessary to subculture every one of these colonies, for the naked-eye appearances are not to be relied upon.

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 less 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, 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

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

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.

Stoddart's Method.—A very promising method for the separation of the typhoid from the colon bacillus has recently been introduced by F. W. Stoddart (*The Analyst*, May, 1897). This process is based upon the difference in the behaviour of the typhoid and coli bacilli when grown upon solid media incubated near their melting-points. The most satisfactory procedure is as follows: An agar-gelatine medium containing 0·5 per cent. of agar and 5 per cent. of gelatine, with the usual proportions of peptone and salt, is made, with all precautions to avoid loss of moisture during preparation. The reaction of the medium must be distinctly alkaline. The agar-gelatine is poured into flat-bottomed flasks or dishes to a depth of about 5 m.m., sterilised, and allowed to cool slowly in the steriliser, so as to avoid the exudation of moisture on the surface. The plates or flasks, which should not be more than a few days old, are then inoculated with a charged needle, and are

incubated for twenty-four hours. In use the centre of the medium is touched with a platinum loop charged with the material to be examined, the flask or dish is enclosed in a much larger one in order to prevent condensation, and the whole is placed in an incubator kept at 35° C. for twenty-four hours. Pure cultures of typhoid so treated produce an opalescence occupying about two-thirds of the medium ; *B. coli* gives a flat plate somewhat thicker and moister than the usual form. If the inoculation is made with both organisms, either from separate cultures or from a mixed culture, we get a flat plate of *B. coli* in the centre, with an opalescent halo of pure typhoid. Plates of this medium, inoculated direct from typhoid stools, gave without difficulty the same pure culture of typhoid bacilli; and it is anticipated that this will become a valuable diagnostic test, as easy of application, though not quite as rapid, as the serum test. It is best applied by putting two or three loops of stool into a little sterile broth, shaking and inoculating as described. Tap-water, also inoculated with a trace of a broth culture of typhoid, or typhoid and coli mixed, readily yielded pure cultures of typhoid.

It was found, however, that on applying it to polluted waters, that in many cases there were present organisms, of which more than a dozen were isolated, which responded to all the accepted tests for the *B. coli*, but differed from it in growing like typhoid on the agar-gelatine medium. Moreover, when mixed cultures of typhoid and one or more of these coli-like forms were inoculated on to this medium, the typhoid was suppressed, and a pure culture of the non-pathogenic organism obtained.

The same inhibition of typhoid also resulted when a mixed culture in broth was attempted, though the true *B. coli* and typhoid grow normally together. As these organisms retain their vitality in media so highly car-

bolised as to totally inhibit typhoid, there appears at present no means of separating typhoid from them, unless the former is present in such abundance as to be detectable by plate culture, either in the usual form, or as modified by Elsner.

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, in the present state of our knowledge,

* 'Report on the Results of the Investigations on the Micro-Organisms of Sewage,' presented to the London County Council, December, 1894.

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 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 speci-

men 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 microbial 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 is a frequent source of infection of such diseases as tuberculosis, typhoid, diphtheria, and scarlet-fever. This source of conveyance of disease has already been discussed under their respective headings.

Fifteen epidemics of diphtheria, thirty-two epidemics of scarlet-fever, and forty-eight epidemics of typhoid-fever, have been recorded since 1881 as directly due to contaminated milk-supplies, by Dr. E. Hart, in the pages of the *British Medical Journal*. See the reprinted 'Report on the Influence of Milk in the Spreading of Zymotic Diseases' for the detailed reports of the individual outbreaks.

As already shown, the typhoid bacillus and the cholera spirillum are capable of rapid multiplication in milk, with-

out 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.

The peptonising organisms were minutely investigated and classified by Flügge (*Zeitschr. f. Hyg.*, xvii., pp. 272, 342). In general, they require many hours' boiling for their destruction; boiling for an hour, for instance, being sufficient to destroy the lactic acid and butyric acid bacteria, but not the peptonising organism to which those of bitter milk belong. Their development is greatly favoured by warmth, so that they multiply with great rapidity at room-temperature in summer time. It is not easy to detect their action on milk with the eye, the commencement of the peptonising giving at the utmost a thin, clear layer under the cream. Experimentally, several of these organisms have been shown to produce serious intoxication, and it is some organisms of this group which are responsible for infantile diarrhœa. Their significance is probably even greater than this fact implies, because, if not sufficient to produce acute specific disease in adults, they or their decomposition products in milk are liable to set up digestive derangements which render the consumer more accessible to other diseases of intestinal origin.

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* or *Bacterium syncyaneum* of Ehrenberg.

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. The organism is described at length by Hueppe (*Mitth. a.d.k. Gesundheitsamt*, ii., p. 335), and by Heim (*Arbeiten a.d.k. Gesundheitsamt*, v., p. 518), and figured in Lehmann and Neumann's Atlas. It gives an alkaline reaction, and produces neither coagulation nor acidity. It usually yields two pigments, one of the ordinary fluorescent type, and the other of a bluish to grayish colour, which becomes more strongly blue up to azure in unsterilised milk with an acid reaction. The addition, for instance, of *Bacillus acidi lactici* a day or two after syncyaneus has been introduced into the milk shows this very clearly. The addition of soda or potash produces a pink coloration. Numerous other organisms are also capable of turning milk blue. Scholl (*Fortschr. d. Med.*, 1889) isolated six bacilli which had this capacity.

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*), which is described in detail by Grotenfeldt (*Fortschr. d. Med.*, 1889, ii., p. 41). It gives milk a red coloration, which is developed best when the medium is slightly alkaline and kept in the dark, and is checked by acidity and light. On standing, the cream rises

as a yellowish layer, and the casein is precipitated, though the reaction remains alkaline and the clear serum is pink.

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* (red yeast) of Demme (Festschrift, Hirschwald, Berlin, 1890). The latter is liable to cause infantile diarrhœa. 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.

Bitter milk is recognisable by the taste, which is very often also mouldy and accompanied by coagulation, though the latter phenomenon may not occur until the milk is warmed. Several organisms have been isolated which have the capacity of producing bitterness in milk, notably

by Hueppe and Löffler. The bacillus of bitter milk proper is that of Bleisch (*Zeitschr. f. Hyg.*, xiii., p. 81)—a facultative anaerobic organism, consisting of stout rods with bundles of flagella rapidly liquefying gelatine, producing a thin, flat, grayish growth on agar and potato.

In milk it will after a week produce transparent yellow streaks below the cream, the milk itself coagulating, and the coagulum being subsequently, earlier or later, almost completely dissolved. The bitter taste arises after the second week; there is no smell; the reaction is acid. At higher temperatures the milk becomes bitter, and gives the biuret reaction after twenty-four hours, while spores are produced which resist boiling for six hours. The micrococcus of bitter milk is that of Cohn (*Centralbl. f. Bakt.*, ix., p. 653), which coagulates milk, and then forms it into a slimy solution, with a slightly sour and very bitter taste.

Ropy or 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. Adametz (*Milch. Zeitung*, 1889, p. 48) found in two streams large numbers of a bacillus which had the property of producing a high degree of ropiness in sterilised milk; so that under microscopical examination no trace of the structure of the fat corpuscles could be observed, although the fat had not been decomposed. This water may either have been used to wash out the utensils, or grass moistened with it may have been conveyed into the stables as hay, and the

organisms have found their way into the milk through the dust. This explanation is not inconsistent with the fact that the trouble of ropy milk has been known to disappear on the substitution of a good for a bad fodder. The recognition of ropy milk can be readily made by taking a few drops between the fingers, when it will be found capable of being drawn out into threads. Amongst the 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. Soapy milk may be produced by the *Bacillus lactis saponacei* of Weigmann and Ziron (*Centralbl. f. Bakt.*, xv., p. 464). It does not coagulate milk, but makes it slimy and slightly ropy, with a faint soapy taste. It grows best at 10° C. Weigmann has also discovered this organism in the straw used as litter, from which it appears that the milk becomes infected when the litter is changed at milking-time.

Slimy Milk.—Slimy milk is attributable to various organisms. The *Micrococcus viscosus* of Schmidt-Mühlheim (*Archiv. f. Physiol.*, xxvii., p. 490) is of 1 μ diameter, often occurs in wreathed chains of fifteen or more cells, and gives a slime analogous to that of plants, and derived from the milk-sugar. The process seems to differ from that of slime-production in wine in that it forms no mannite and no carbonic acid. Hueppe also isolated a coccus, and numerous bacilli have been discovered, all possessing the property of making milk slimy. In the case of Guillebeau's *Bacillus Hessii* (*Ann. de Microg.*, iv., p. 225) the slimy character disappears after two days' exposure to 35° C. The *Bacillus lactis viscosus* of Adametz mentioned above is notable for the length of time during which it operates, and the completeness with which it attacks the milk. Its effect is apparent after four or five days, and is continued for four weeks, by which time the milk corpuscles have practically disappeared and the milk is transparent. The casein is not precipitated; no acceleration of the process occurs on a rise of temperature, and there is no special smell. The *Bacillus lactis pituitosi* of Löffler (*Berlin Klin. Wochenschr.*, 1887, p. 631), on the other hand, gives a specific smell, and renders the milk slimy and slightly acid, especially at the lower part. Whether the viscous substance is derived from the milk-sugar or the casein has not been determined.

In the space at command, it has only been possible to enumerate a small number of those organisms which have been known to produce obscure changes and abnormal appearances in milk, and it cannot be said that in the case of all of them a pathogenic character has been demonstrated; but it is unquestionably the case in regard to a large number, and none of the appearances in question are either natural in milk or proper to it. They cannot, of course, be classed as adulterations; but on the same

principle as that laid down by Lehmann, which regards as adulteration anything which does not normally belong to the food in question, it is proper that milk having these appearances should be rejected, and steps taken to prevent them. A rough-and-ready, but very useful and effective test, is the rate at which milk goes sour. For practical purposes, it may be said that any milk which goes sour rapidly is a bad milk, although the converse is not necessarily true. According to Schatzmann, if a sample of milk be kept for twelve hours at 40° C., and within that time coagulates, it is to some extent defective; and in nine hours no change whatever should appear to have occurred. The presence of colostrum is a ground for the immediate condemnation of milk; it can usually be detected by the presence of long elastic yellowish threads.

The remedy for almost all of these diseases lies above all in cleanliness, both as to the udders and body of the cow, the stable, and the hands of those employed in milking. Clean milk-pans, pure water, and a cool, odourless and clean store-room, are absolutely indispensable. The milk-cans or other utensils are best made of earthenware or well-tinned copper or iron, and they should be thoroughly scalded and cleaned between each change of milk.

In addition to the faults which arise from specific bacterial causes, milk may be watery in the case of ill-kept and ill-fed cows. Salty milk may be watery (1027 to 1029 specific gravity). Salty milk is stated to occur only in connection with inflammation of the udder. It is to be detected not only by its taste and its high percentage of ash, but by its low percentage of milk-sugar. According to Klenze, 2·4 per cent. of small deposits of calcium carbonate in the milk glands may give rise to sandy milk.

The faults mentioned so far are those which arise for the most part through causes external to the cow, and are

diseases of the milk itself. Milk may, however, be no less defective through disease in the cow. A large number of diseases are known to be capable of being conveyed in this way; in many cases the milk itself is affected unfavourably, so that it would be rejected for its unpleasant smell or taste; but this is far from being invariable. Cows suffering from pulmonary tuberculosis give, for instance, an unpleasant and unsavoury milk, which carries its own condemnation with it; those, however, which suffer from generalized tuberculosis may yield a milk which only in advanced stages gives the slight yellow coloration, the reduction of cream, fat and milk-sugar, and the increase of albumin which has been attributed to such milk. Nevertheless, it has been shown that cows suffering from generalized tuberculosis, whether it has or has not affected the udders, are capable of conveying the bacterial infection into the milk, which constitutes, therefore, a serious danger. Scheurlen (*Arbeiten a.d.k. Ges.-Amt.*, vii., 1891) investigated the extent to which milk can be freed from bacteria suspended in it by the operation of a centrifugal machine; and his results, although not obtained with that object, are of considerable assistance in arranging the examination of milk. He found the curious result that, while of the large majority of the bacteria contained in milk three-fourths went into the cream on being centrifugalized, and the rest stayed in the separated milk, and the same result was obtained by merely leaving the milk to stand, and that these results held good not only for the ordinary milk bacteria, but also for anthrax, typhoid and cholera organisms, the tubercle bacillus only remained to a small extent in either the milk or the cream, and the large majority was ejected under the centrifugal influence.

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, Stilton, 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 by Rowland 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 p astered 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.

Sterilisation and 'Pasteurisation' of Milk.—These two processes are now coming into extensive use for the purpose of preserving this article of food almost indefinitely, or with a view of increasing its keeping powers. The sterilisation of milk, that is to say, to render it definitely free from organisms, is by no means an easy matter, owing to the very resistant nature of some of the bacteria that it always contains. A sterilisation of milk to be absolute would require six hours' heating at boiling-point, or an exposure for a shorter time to steam under pressure. Fractional sterilisation is too long and too cumbrous a process for general use, whilst the continuous action of steam affects the lactose, rendering the milk brown, and further, by con-

verting the soluble lime salts into insoluble ones, interferes with the coagulation of the milk by rennet, and causes the fat globules to partially coalesce.

A large number of special appliances have been invented for the sterilisation of milk. They may be divided into two classes: those that are arranged for dealing with the milk in bulk, and those that are adapted for dealing with small quantities of it in bottles. As regards the former the most effective, and at the same time the most economical, forms of apparatus are those in which the milk is treated with steam under pressure, the storage vessels being filled and closed in such a way as to make it impossible for any micro-organisms to get in during the process. For household purposes it is sufficient to have an inner vessel to contain the milk, and an outer one in which water is placed. If there be a free outlet for the steam, and if the inner vessel be not allowed to touch the bottom of the outer one, the milk will not actually boil, since its boiling-point is about 1·5° F. higher than that of water.

The 'pasteurisation' process consists in heating the milk to 65° to 70° C. for a short time, and then rapidly cooling it so as to prevent the rapid increase of the bacteria that remain, and to preserve the fresh flavour of the milk. This process simply destroys the non-sporing organisms, and where it is only necessary to preserve the milk for a limited time this process may be employed with advantage.

Various forms of apparatus have been invented for the pasteurisation of milk in large quantities. These are chiefly of two types: in the one such as Thiel recommends the milk is made to flow over a heated corrugated surface; and in the other, the vessels containing the milk are surrounded by water, the temperature of which can be kept at a definite point for a definite period. If pasteurisation is to be effectual the milk must not only be heated up to a

temperature of 68° C., but must be maintained at this temperature for twenty minutes. Bitter has invented an apparatus for efficient pasteurisation, in which the milk is kept at a temperature of from 70° to 72° C., for thirty minutes, after which it is rapidly cooled.

A very simple apparatus is sufficient for household use, the essential parts being: an easily cleansed bottle with a cotton-wool plug, to contain the milk; a metal vessel provided with a wire stand at the bottom to support the bottle; and a thermometer, the stem of which passes through the lid of the outer vessel so that the temperature can be ascertained without the cover being removed. The temperature of the water should be slowly raised to 70° C., after which the vessel should be taken off the fire, and should be kept for thirty minutes under a thick cosy. At the end of this period the bottle should be taken out of the water, and put in a cool place so that the temperature of the milk may be lowered as rapidly as possible.

By this method of procedure as much as 99·9 per cent. of the organisms in the milk may be destroyed.

A very important point in connection with the pasteurisation of milk is to determine if this process is as effectual in destroying pathogenic organisms as the sterilisation process.

Forster, from his own and other investigations conducted in his laboratory, states that milk kept for half an hour at 65° C. is freed from the organism of tubercle, typhoid, cholera, etc. Flügge, from his own researches and those of others in his laboratory, states that half an hour at 70° C. is necessary. As regards the bacillus of diphtheria, Löffler states that half an hour at 60° C. is sufficient to destroy it. The results of these experiments have been confirmed by other workers; and it may safely be accepted that milk

kept for half an hour at 70° C., and kept from subsequent contamination, is free from these organisms. Macfadyen and Hewlett (*Trans. Brit. Institute Preven. Medicine* (first series) have, however, recently proved that heating in milk for thirty seconds at 70° C. is sufficient to kill the following pathogenic organisms : *B. diphtheriæ, B. typhosus, B. tuberculosis, Staph. pyogenes aureus.*

There are a number of objections to the use of sterilised milk which do not apply to pasteurised milk. Undoubtedly the chief disadvantage in the case of sterilised milk is that the caseine is very much less digestible than in the case of raw milk. In the case of children with weak digestions, this may give rise to rickets, due in great part to the fact that the children undergo a process of slow starvation, due to an insufficiency of carbohydrates and fats.

A second, though less important objection, is the fact that the taste and smell of the milk are altered ; and although this rarely matters in the case of young infants, it sometimes causes difficulty with older children. Another disadvantage is, that the greater part of the carbonic acid gas in the milk is driven off, thus inducing an alteration in the composition of the phosphates, and a precipitation of calcium and magnesium carbonates. Fourthly, some of the fat globules coalesce, the result being that the emulsification is not quite so perfect as in raw milk. Fifthly, the lact-albumin is coagulated, and gives rise to the albuminous skin which forms upon the surface as the milk cools, even if it has not been boiled, and contains entangled in its meshes a considerable quantity of fat, thus rendering the milk correspondingly poor in this most important ingredient.

The use of pasteurised milk is, however, strongly to be recommended, particularly in the case of children, for the following reasons: First, the digestibility of the casein is

only diminished to a slight extent; secondly, the taste and smell of the milk are not permanently altered; thirdly, less carbon dioxide is driven off; fourthly, the condition of the fat remains practically unchanged; fifthly, the lact-albumin is not coagulated.

Tyrotoxicon in Milk.—The presence and detection of this poisonous ptomainic body in milk and milk products has already been dealt with (see p. 118).

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. 140).

Scheurlen's method for demonstrating the tubercle bacillus in milk is to steep it for twenty-four hours in absolute alcohol, digest in ether for another twenty-four hours in order to remove the fat, and stain according to Ziehl's method. Ilkavitch (*Munchener med. Wochenschr.*, 1892, p. 5) described a convenient method of applying this result: 20 c.c. of milk are coagulated with citric acid, filtered and dissolved in saturated aqueous solution of Na_3PO_4. The solution is treated with 6 c.c. of ether, the fat which rises to the surface is decanted, and the remainder, after the addition of one or two drops of acetic acid, is centrifugalized in a copper tube with a screwed

bottom. The deposit is separated from the fluid by means of a fairly well-fitting ball, which is let into the tube on a stalk. The bottom of the tube is then scraped, the deposit divided between two cover-glasses and stained for the tubercle bacillus.

Undoubtedly the most satisfactory method of examining milk for the tubercle bacillus, if time is no object, is by the intraperitoneal or subcutaneous injection into guinea-pigs.

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 made 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, are 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. The air supplied to the Houses of Parliament is filtered through cotton-wool.

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 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

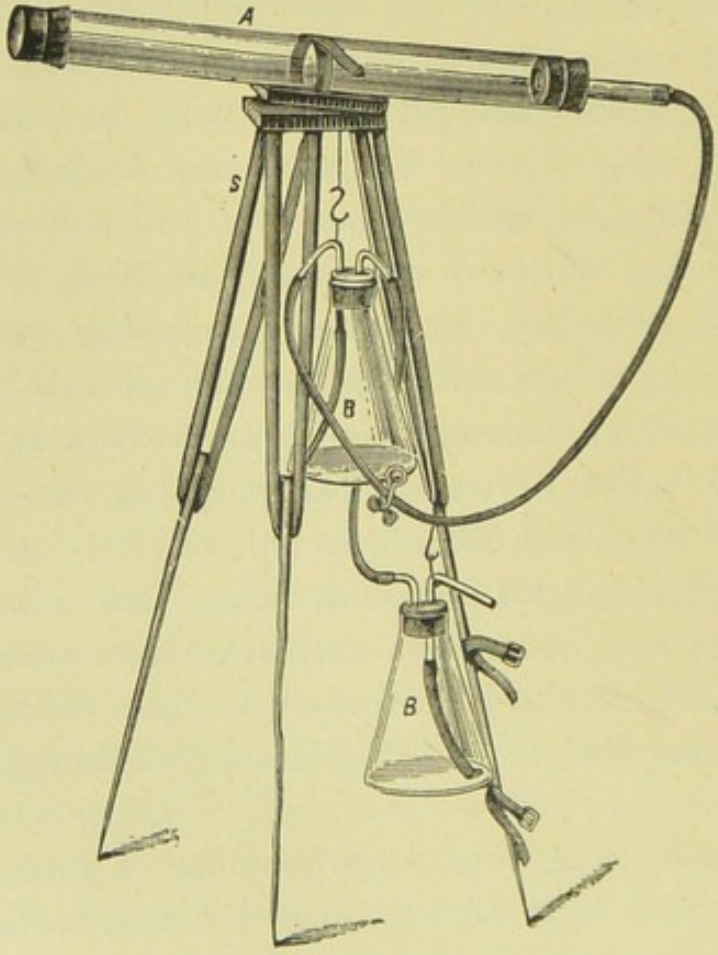


Fig. 38.—Hesse's Apparatus.

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 FULLY DESCRIBED IN THE PREVIOUS PAGES.

Micrococcus aerogenes—M. agilis—Bacillus aquatilis—B. arborescens —Black torula—*B. coli communis—B. enteritidis* (Gartner)—*B. enteritidis sporogenes* (Klein)—*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—Proteus Zenkeri—Proteus Zenkeri* (sewage variety of Klein)—*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 Streak.—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 Streak.—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 Streak.—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.

Bacillus Coli Communis.—This colon bacillus, which is identical with the *B. Neapolitanus* of Emmerich, was discovered by Escherich in 1885, who obtained it from the normal stools of children. It has since been found to be widely distributed and a normal inhabitant of the intestinal tract of man and animals.

Bacillus coli is a motile, aerobic (facultative anaerobic), non-sporing, non-liquefying rod. It is killed by thorough drying, and by a temperature of 66° C. in five minutes. The length of the individuals varies between 0·8 μ and 1·5-3 μ, though in later stages in culture both longer and shorter filaments are met with; its thickness is about 0·4-0·5 μ. When examined in the living state from the intestinal contents in health and disease, only a minority of the microbes are as a rule found to be possessed of motility, though in some cases motility may be observed in many individuals. The same holds good for artificial cultures; as a rule, only a minority show motility, while in old cultures the motile individuals are rare.

Cultural characters of typical *B. coli communis*:

Gelatine Plates.—Bacillus coli forms typical colonies on the surface of gelatine at 20° C.; after twenty-four hours they are recognisable as flat, translucent, grayish, roundish patches, slightly thickened in the middle part or near one margin; after forty-eight hours the patches are considerably enlarged, angular, thin and filmy, and translucent in the marginal, thicker and less translucent in the middle part. The whole patch is dry, whitish in reflected light, and under a magnifying glass appears fairly homogeneous, though after several days it commences to show some kind

of concentric differentiation. The colonies in the depth of the gelatine appear as spherical dots, white in reflected, brownish in transmitted, light.

Gelatine Streak Culture.—After twenty-four hours it is a grayish band, thicker in the line of inoculation, gray, filmy, knobbed or crenated in the marginal part. After forty-eight hours it has spread considerably in breadth; but has retained the above aspect, except that the middle part is more thickened, and the whole growth appears more white in reflected light. After three or four days the band has spread over the greater part of the surface of the gelatine, but it is still dry, filmy, crenate, and irregular in the marginal part, and the whole part, examined under a glass, appears more or less homogeneous.

Gelatine Shake Culture.—Copious gas formation after from twenty-four to thirty-six hours.

Agar-Agar Streak.—Forms a grayish-white moist expansion. Grows abundantly, producing a dirty-white, faintly-shining expansion.

Potatoes.—An abundant soft shining layer is produced of light brownish-yellow colour. The growth upon potatoes differs considerably according to the age of the potatoes.

Milk.—Renders it acid at 37° C., and coagulates it in from twenty-four to forty-eight hours.

Broth.—Renders it turbid. After from three to five days' incubation at blood-heat, if a few drops of a dilute solution of potassium nitrite solution and then a little sulphuric acid is added, a pink coloration (indol reaction) is obtained.

While these are in general the characters of the typical bacillus, such as can be isolated from stools normal and pathological, there occur in various other substances—dust, water, sewage, etc.—bacilli which, examined as regards all the above points, coincide in some, but differ in others. These, owing to their general morphological similarity,

such as size and shape of the rods, and flagella, two to eight, and further, owing to their non-liquefaction of gelatine and the power to grow well in phenolated gelatine and broth, and to the practical identity of their appearance and rapidity of growth in gelatine plates, and in gelatine streak, potato, and broth cultures, must, for the present, be considered as bacillus coli, though on account of their differing from the typical bacillus coli, in respect either of gas-production in gelatine shake culture, or of clotting of milk, or of indol-reaction, they must be considered as varieties of that microbe.

Pathogenesis.—Comparatively small amounts of a pure culture of the colon bacillus injected into the circulation of a guinea-pig usually cause the death of the animal in from one to three days, and the bacillus is found in considerable numbers in the blood. But when injected subcutaneously or into the peritoneal cavity of rabbits, a fatal termination depends largely upon the quantity injected. Klein states (Report of the Local Government Board, 1895-96) that it forms toxic substances which, when injected into the animal body in sufficient amount, cause intoxication. This intoxication is known as sapræmia, and it is characterised by fall of temperature, vomiting, purging, muscular twitchings, collapse, and death—post mortem there is found severe congestion of the intestine, with watery contents. Thus, if a broth culture of *B. coli*, after incubation at blood-heat for four or five days, be sterilised (so that the enormous mass of the colon bacillus that have developed in the medium are killed), and 2 to 3 c.c. of it be injected subcutaneously into a guinea-pig of about 250 to 300 grams weight, it will produce the above symptoms and cause the death of the experimental animal.

Bacillus Enteritidis (Gartner).—This organism was first obtained by Gartner, in 1888, from the tissues of a cow

which was killed in consequence of an attack characterised by a mucous diarrhœa, and also from the spleen of a man who died twelve hours after eating the flesh of this animal. It is a short bacillus, about twice as long as broad, frequently united in pairs; chains of four to six elements are sometimes seen. It is aerobic, non-liquefying, and motile. Spore formation not determined. Stains with the usual dyes, and presents the peculiarity of polar staining.

Cultural characters:

Gelatine Plates. — Pale-gray, superficial colonies are formed at the end of twenty-four hours; under low powers these are seen to be coarsely granular and transparent; the central portion usually presents a greenish colour.

Gelatine Streak.—A thick, grayish-white layer is formed, which after a time becomes much wrinkled. The gelatine is not liquefied.

Agar Streak.—At the end of from eighteen to twenty hours at blood-heat a grayish-yellow layer is formed.

Potatoes. — A moist, shining, yellowish-gray layer is developed.

Pathogenesis.—Mice usually die in from one to three days when fed with a pure culture of this bacillus; rabbits and guinea-pigs die in from two to five days after subcutaneous injection; dogs, cats, chickens, and sparrows appear to be immune; a goat died in twenty hours after receiving an intravenous injection of 2 cubic centimetres of a culture in blood serum. The organism gives rise to a severe inflammation of the intestinal mucous membrane. The bacilli are found in the heart's blood.

Bacillus Enteritidis Sporogenes (Klein).—As already stated, this organism was discovered to be the cause of an outbreak of diarrhœa, and was subsequently discovered by Andrewes in a number of cases of severe diarrhœa. Forms

long straight bacilli, often forming long jointed threads. Thickness, ·8 μ; average length, 4 to 8 μ, but very variable; threads of 20 to 30 μ may be seen. No spontaneous movements seen. No flagella demonstrable.

Spore formation: Very scanty and difficult to obtain in cultures; but it must occur, since in all cases the organism was obtained from material which had been heated to 80° C. for ten minutes.

This organism, which is a *strict anaerobe*, has the following cultural characters:

Formated Agar. — Surface growth on agar-agar, containing 0·5 per cent. of sodium formate, in an oxygen-free atmosphere; in one to two days there is a delicate, semi-transparent, grayish growth of confluent colonies, with smooth or wavy edges, which later show fern-like and dentate outgrowths.

Grape-Sugar Gelatine.—Stab cultures show scanty dotted growth in twenty-four hours, extending to within $\frac{3}{4}$ inch from the surface. In forty-eight hours the growth is confluent, white, opaque, and somewhat filamentous in texture. Growth proceeds slowly, and liquefaction appears from the second to the fourth day. The gelatine is usually only partially liquefied even after several weeks. Gas formation does not appear, as a rule, till the fourth or fifth day; later, the gas extends up the needle track, and commonly bursts laterally through the gelatine to the side of the tube, about $\frac{1}{2}$ inch from the surface, and so up to the surface. Along this track the growth rapidly passes to the surface, where it appears as a very viscid, grayish material, full of gas-bubbles. Old cultures show varying liquefaction of the gelatine, which after a time becomes clear, with a dense white deposit of bacilli. The latter remain unaltered for a long time, and do not rapidly break up. Spores are, however, hardly ever seen in them. Cultures

retain their vitality for six weeks or more. Shake cultures in grape-sugar gelatine show similar appearances, but liquefy rather less slowly. The earliest colonies are visible in two days, and they become somewhat irregular in shape.

Growth in Milk.—Milk cultures incubated at 37° C., in an atmosphere free from oxygen, coagulate energetically in from one to four days, usually in two. A firm white curd separates from a clear whey, and undergoes no further breaking up or digestion. A strongly acid reaction is produced, and the cultures have a faint sour smell, sometimes, but not always, distinctly like butyric acid. Tested volumetrically, the amount of acid produced is not large.

Pathogenesis.—Subcutaneous inoculation of guinea-pigs with recent milk cultures produces in some cases intense spreading hæmorrhagic œdema and necrosis, and death in twenty-four hours. In other cases it is not pathogenic.

Black Torula (*Saccharomyces niger*).—This yeast is frequently met with in the air. Cultural characters:

Gelatine Plates and Streaks.—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 Streak.—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 Streak.—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 temperature. 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 Streak.—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. Houston (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. They are short rods, which frequently occur in chains. The first two organisms liquefy gelatine very rapidly, but the last does not. This last organism—the *Photo-bacillus Flüggeri* 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, and 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, with bristling edges, which afterwards throw out irregular branches. In the depth characteristic zooglœa 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.

Proteus Zenkeri.—This is a common putrefactive organism. The bacilli vary greatly in length, average about 1·5 μ long by about 0·4 μ in breadth. It is aerobic and facultatively anaerobic, non-motile and non-liquefying. Spore formation not observed. Cultural characters:

Gelatine Plates.—At the end of twelve hours superficial colonies are seen of 2 to 3 mm. in diameter, which under a low power appear laminated and of a brownish colour.

Gelatine Streak.—A rather thick grayish-white opaque layer is formed, which soon covers the entire surface of the gelatine, and is easily detached from it. This species is distinguished from the two preceding ones by the fact that it does not liquefy gelatine or blood serum, and does not give off a decided putrefactive odour when cultivated on these media.

Pathogenesis.—The above three species of protei give rise to local abscesses and to symptoms of toxæmia when injected into small animals.

Proteus Zenkeri (*Granular or Sewage Variety of Klein*).—This organism, which was discovered by Klein, and called by him the *granular or sewage variety of Proteus Zenkeri*, is a non-liquefying, non-sporing, aerobic, non-motile bacillus,

and occurs in the form of short and long chains and filaments. Klein found it to occur in a small percentage of intestinal discharges, and in about 15 per cent. of sewage samples. Klein has not met with it in any other material, and owing to its occurrence in sewage, and its far more restricted distribution than the colon bacillus, Klein is inclined to think that if this species be found in water, such water has most probably been polluted with sewage. Cultural characters :

Gelatine Plates.—Forms flat, dry, translucent, patch-like colonies, very similar to the colon bacillus, but growth is much more rapid.

Gelatine Streak.—Forms a translucent band, with filmy, irregular, or crenate edges, very similar to the *B. coli*. Like the colon bacillus, it grows well in phenolated gelatine and in phenolated broth.

Gelatine Shake Culture.—Does not form gas.

Milk.—Does not coagulate.

Broth.—Does not give the indol reaction after three days' growth at blood-heat.

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 con-

tents of the tube then becomes liquid, a tough pellicle forming on the surface. It grows on carbolated media.

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. Jacinthus*).—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.

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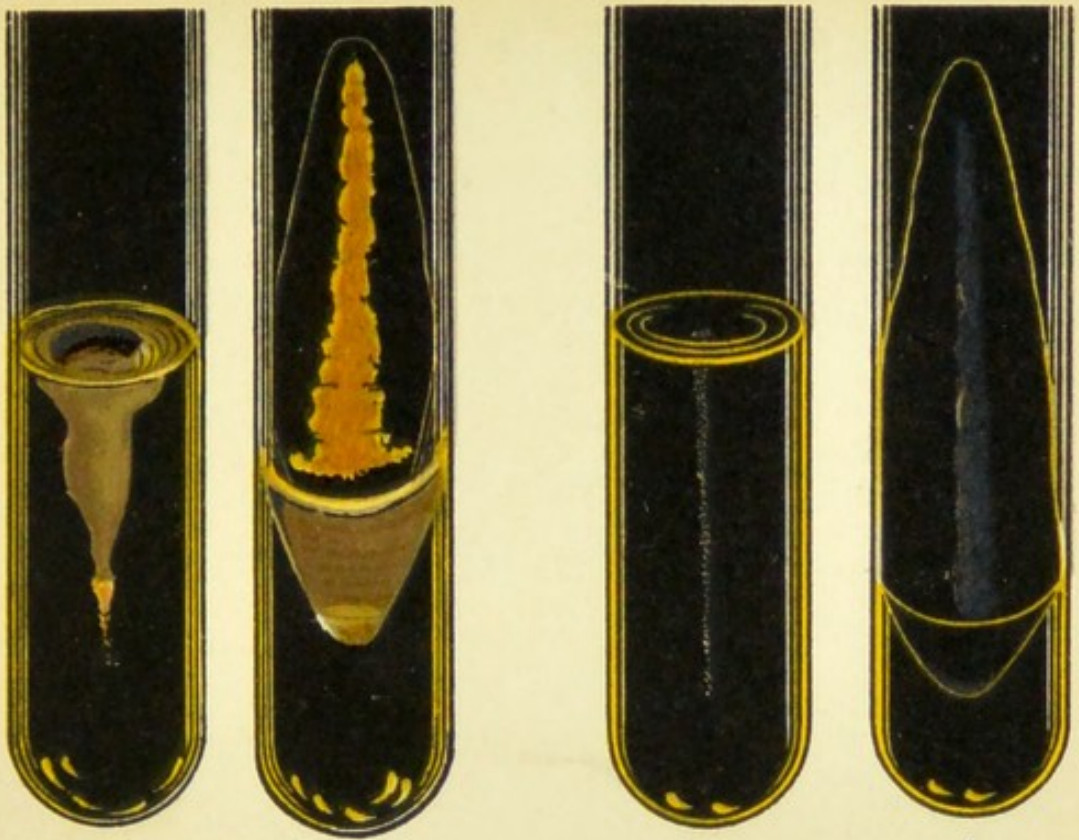
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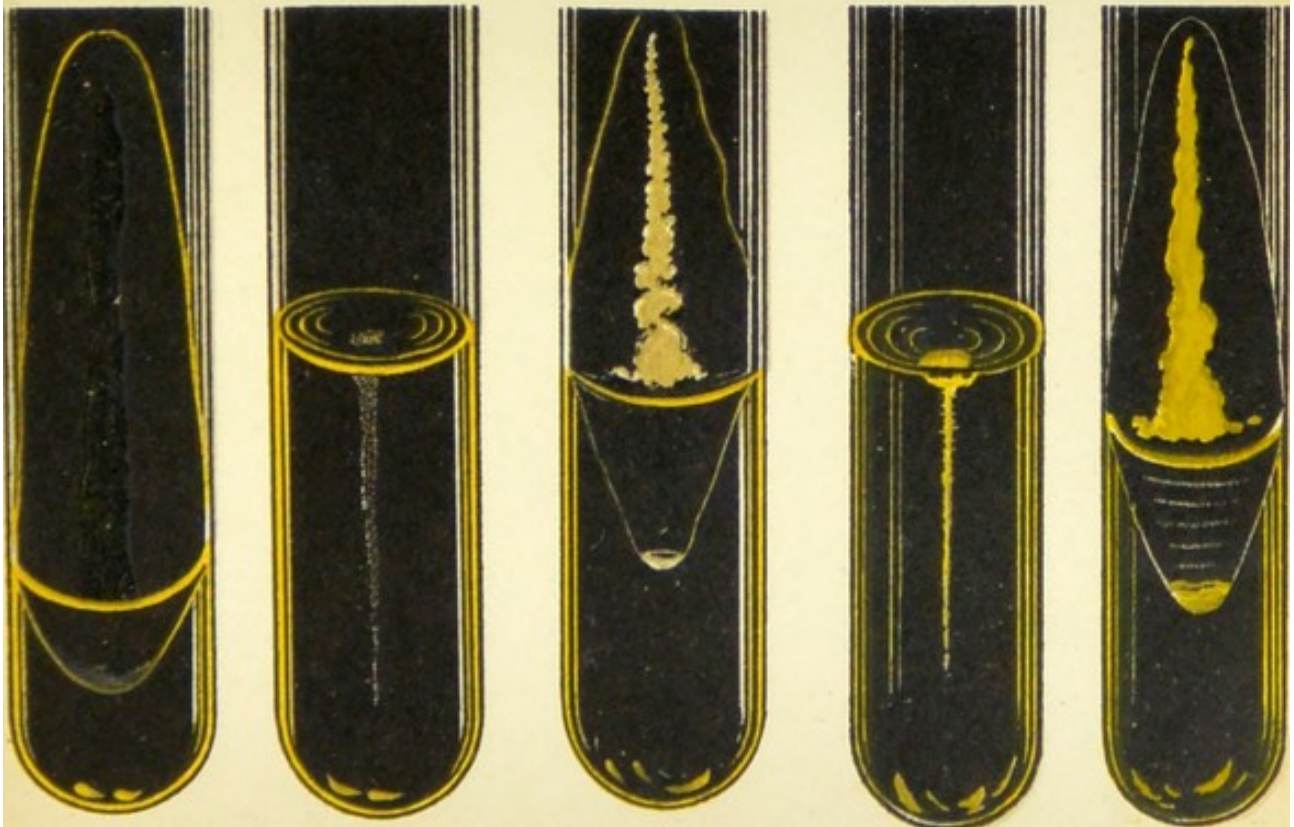
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COLOURED PLATES

Plate 1.



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PLATE II.

CULTURES OF ORGANISMS.

Fig. 10.—Bacillus pneumoniæ (Friedländer)—Streak culture on agar.

Fig. 11.—Bacillus pneumoniæ (Friedländer)—Stab culture in gelatine.

Fig. 12.—Bacillus coli communis—Stab culture in gelatine.

Fig. 13.—Bacillus coli communis—Streak culture on gelatine.

Fig. 14.—Bacillus typhosus—Stab culture in gelatine.

Fig. 15.—Bacillus typhosus—Streak culture on gelatine.

Fig. 16.—Bacillus diphtheriæ—Stab culture in gelatine.

Fig. 17.—Bacillus diphtheriæ—Streak culture on glycerine-agar.

Fig. 18.—Bacillus fluorescens non-liquefaciens—Stab culture in gelatine.

Fig. 19.—Bacillus prodigiosus—Stab culture in gelatine.



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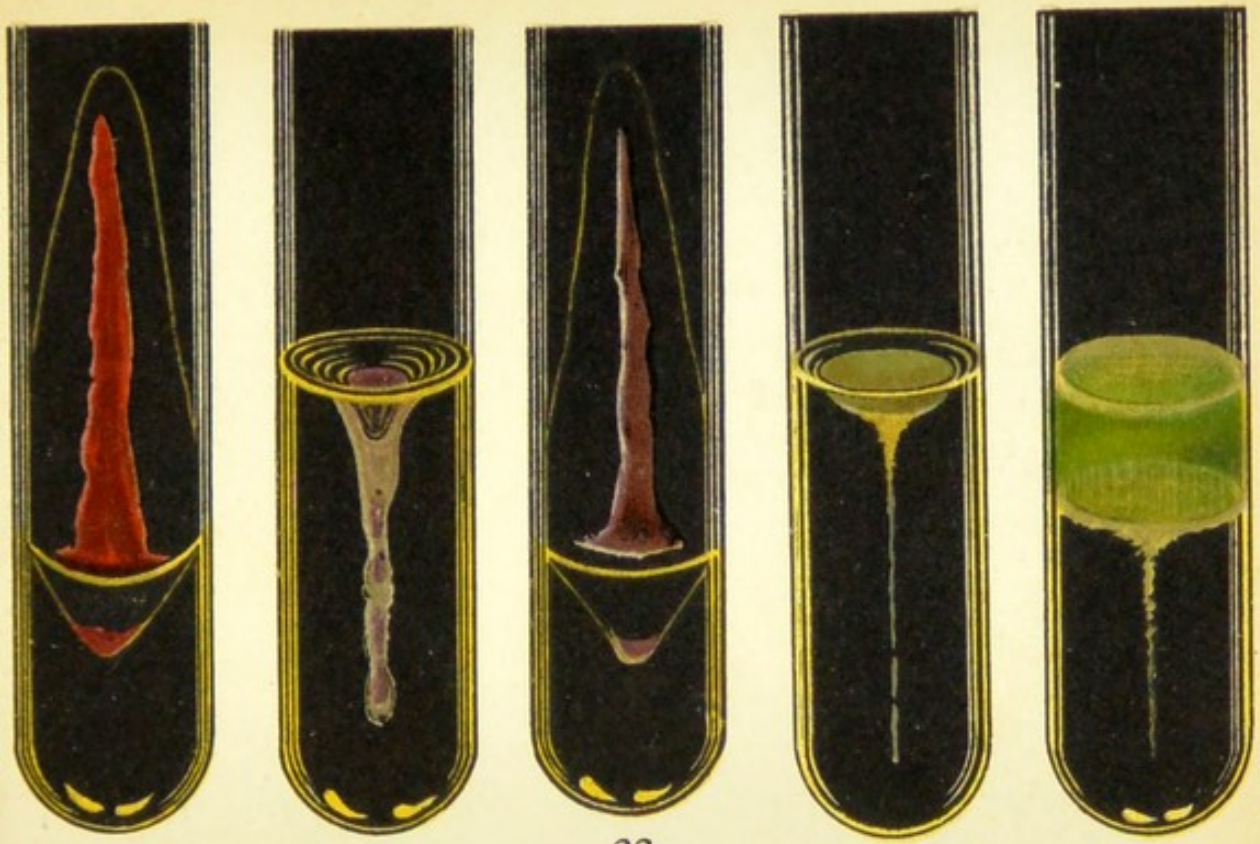
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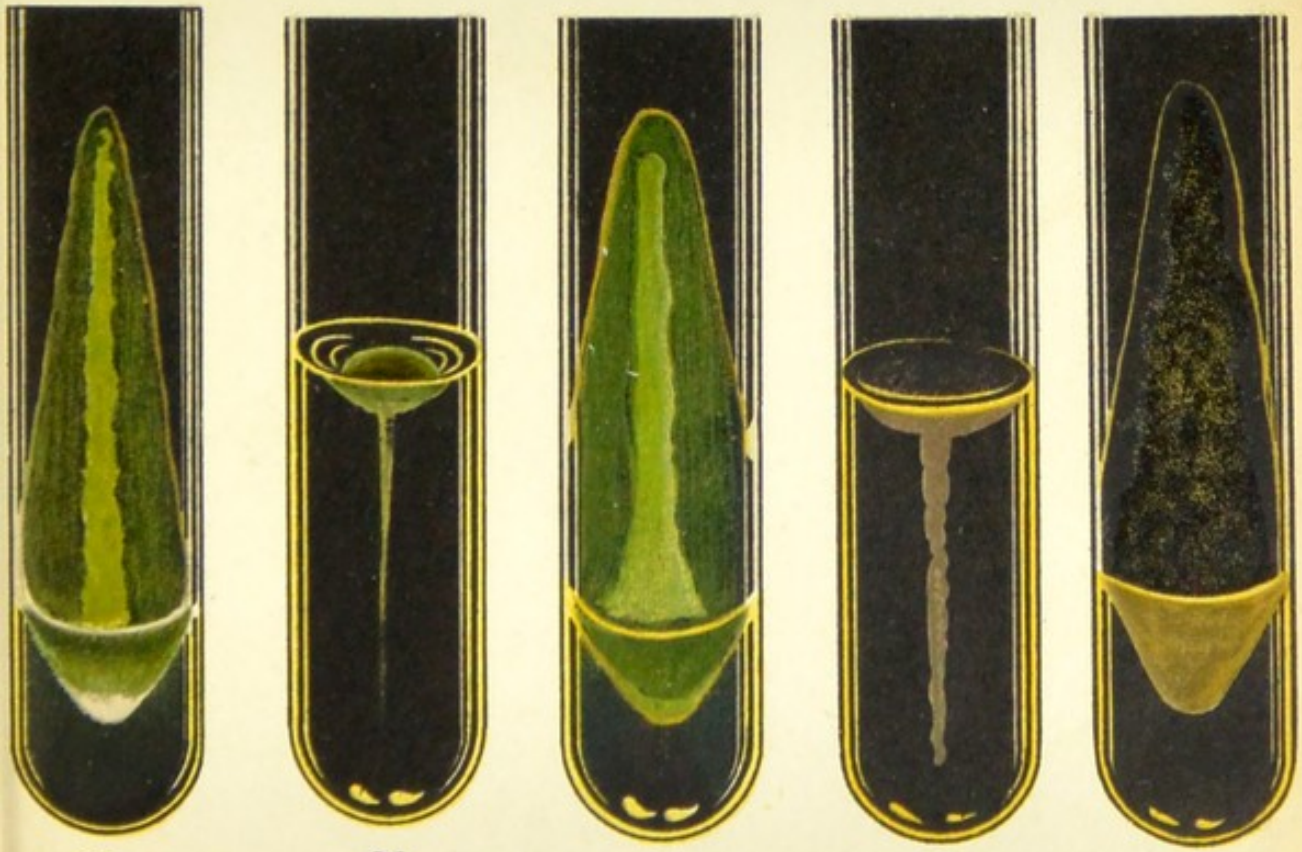
PLATE III.

CULTURES OF ORGANISMS.

Fig. 20.—Bacillus prodigiosus—Streak culture on agar.
Fig. 21.—Bacillus violaceus—Stab culture in gelatine.
Fig. 22.—Bacillus violaceus—Streak culture on agar.
Fig. 23.—Bacillus fluorescens liquefaciens—Stab culture in gelatine.
Fig. 24.—Bacillus fluorescens liquefaciens—Stab culture in gelatine, 5 days old.
Fig. 25.—Bacillus fluorescens liquefaciens—Streak culture on agar.
Fig. 26.—Bacillus pyocyaneus—Stab culture in gelatine.
Fig. 27.—Bacillus pyocyaneus—Streak culture on agar.
Fig. 28.—Proteus vulgaris—Stab culture in agar.
Fig. 29.—Proteus vulgaris—Streak culture on agar.



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PLATE IV.

CULTURES OF ORGANISMS.

Fig. 30.—Bacillus murisepticus (mouse septicæmia)—Stab culture in gelatine.

Fig. 31.—Bacillus murisepticus (mouse septicæmia)—Streak culture on agar.

Fig. 32.—Bacillus anthracis—Stab culture in gelatine.

Fig. 33.—Bacillus anthracis—Stab culture in gelatine.

Fig. 34.—Bacillus anthracis—Streak culture on agar.

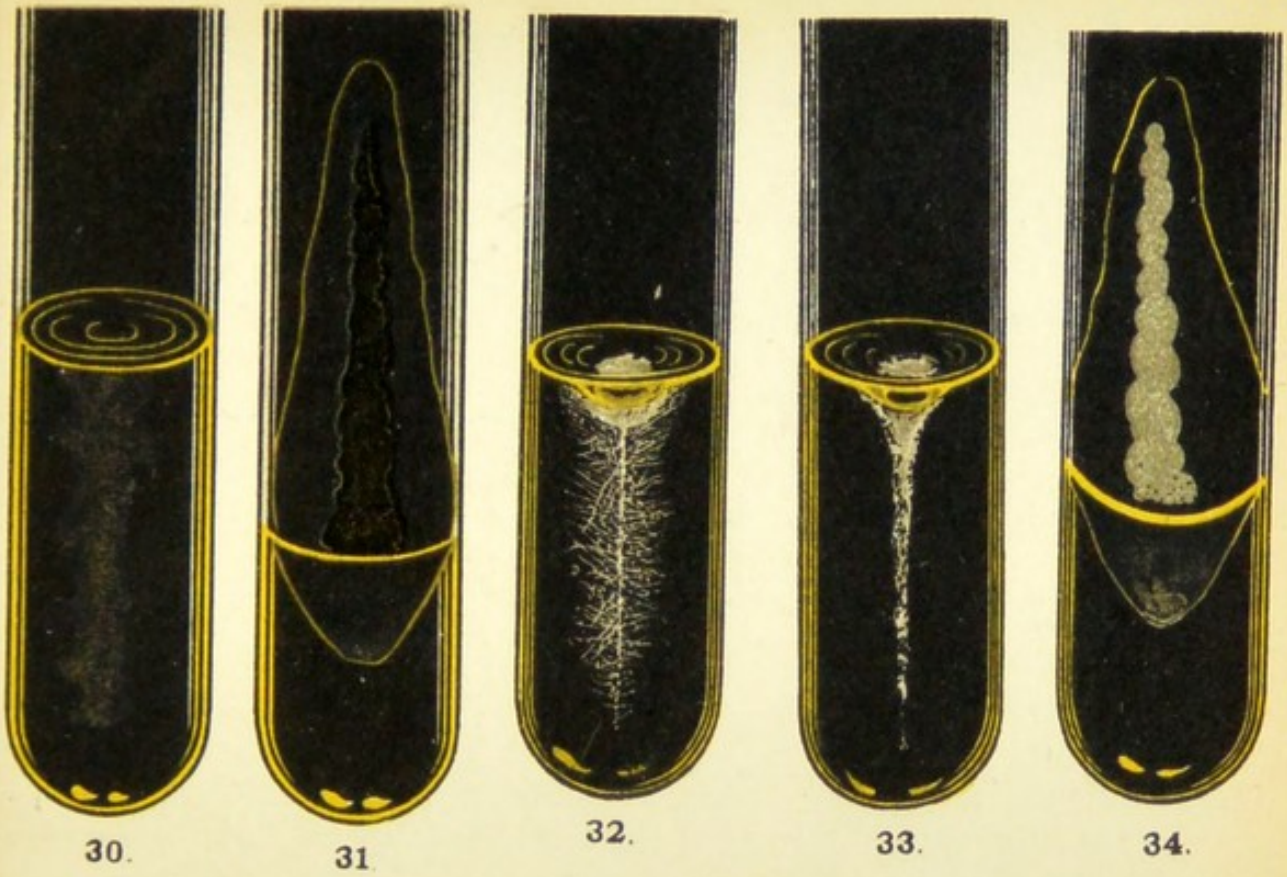
Fig. 35.—Bacillus tetani—Stab culture in glucose-agar.

Fig. 36.—Bacillus tetani—Stab culture in glucose-agar.

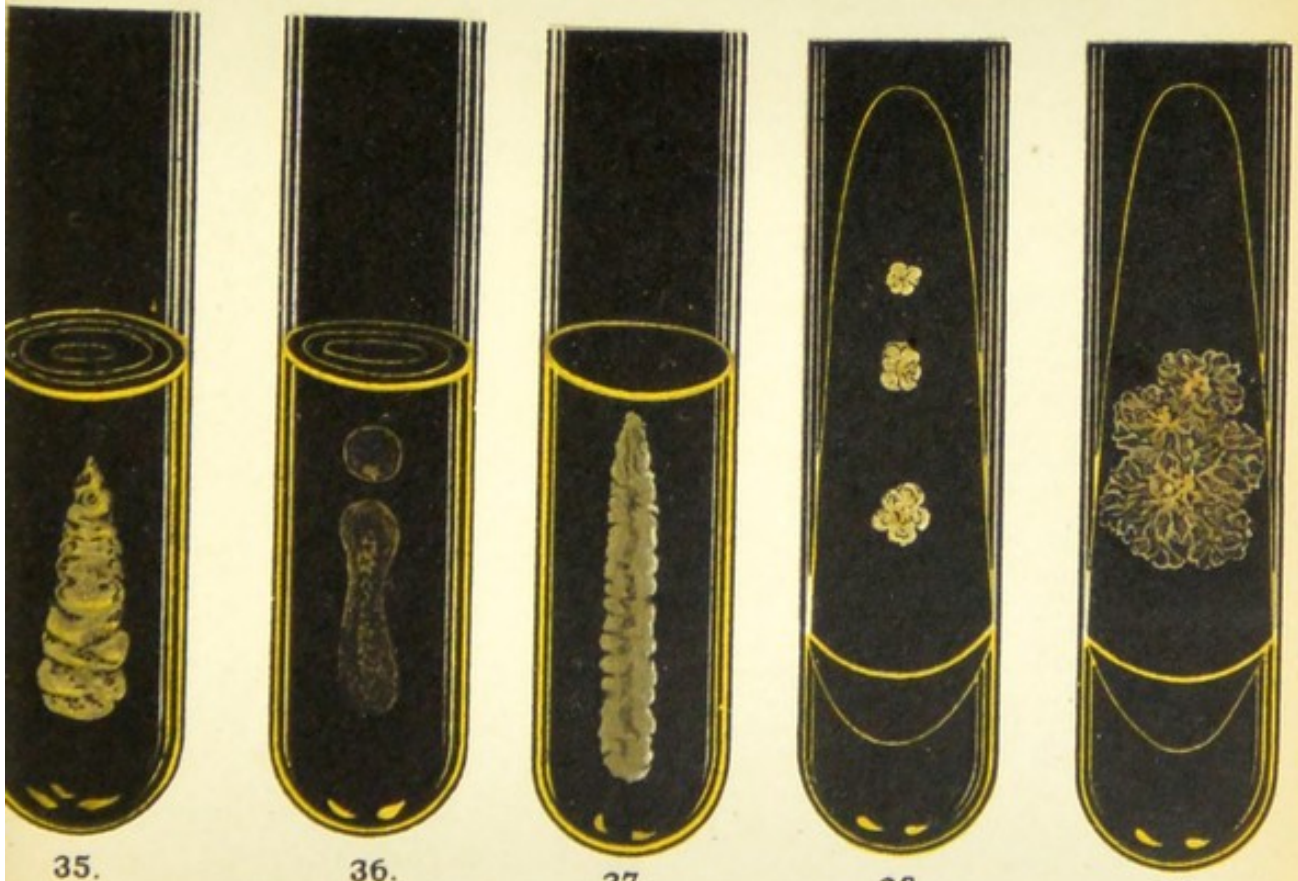
Fig. 37.—Bacillus œdematis maligni—Stab culture in glucose-agar.

Fig. 38.—Bacillus tuberculosis—Streak culture on glycerine-agar.

Fig. 39.—Bacillus tuberculosis—Streak culture on glycerine-agar.



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PLATE V.

CULTURES OF ORGANISMS.

FIG. 40.—Spirillum choleræ Asiaticæ — Stab culture in gelatine, two days old.

FIG. 41.—Spirillum choleræ Asiaticæ — Stab culture in gelatine, three days old.

FIG. 42.—Spirillum choleræ Asiaticæ — Stab culture in gelatine, five days old.

FIG. 43.—Spirillum choleræ Asiaticæ — Stab culture in gelatine, seven days old.

FIG. 44.—Spirillum choleræ Asiaticæ—Streak culture on agar.

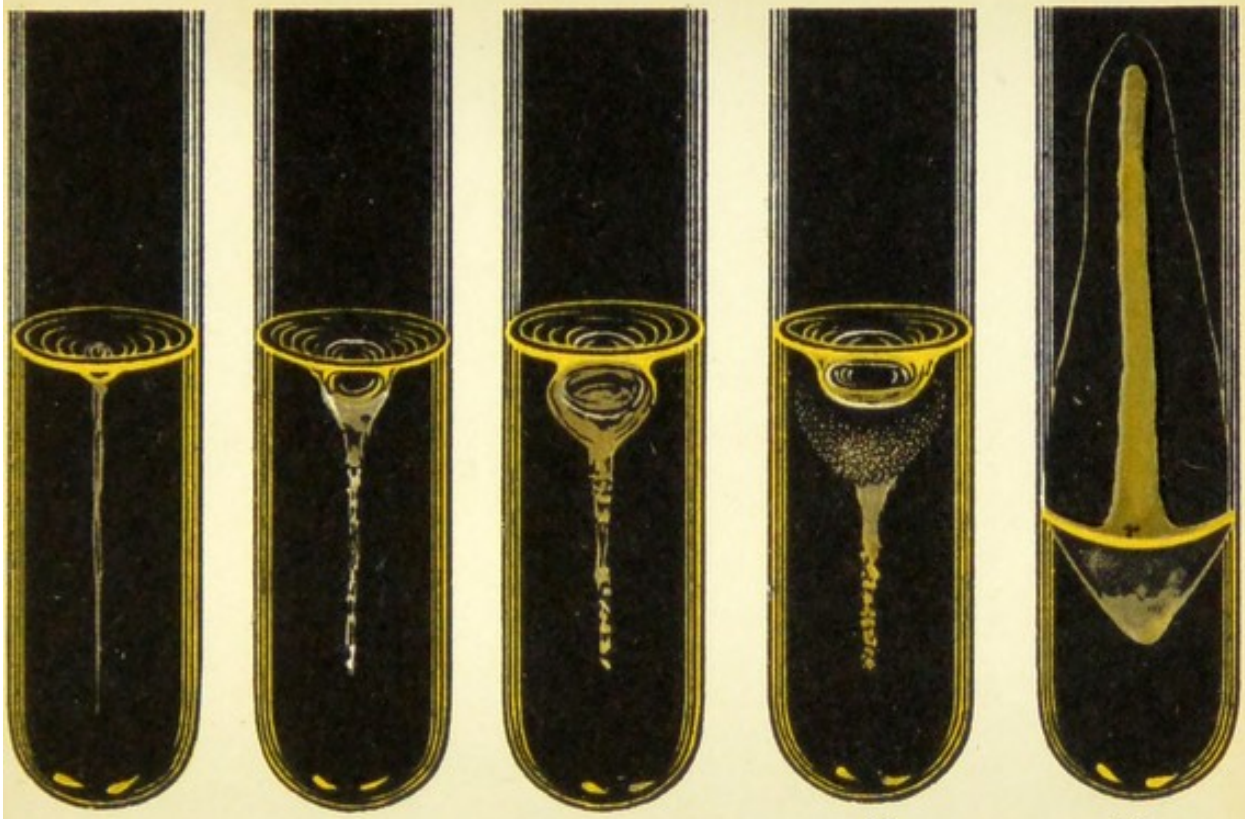
FIG. 45.—Spirillum choleræ Asiaticæ—Indol reaction.

FIG. 46.—Spirillum Finkler-Prior—Stab culture in gelatine, two days old.

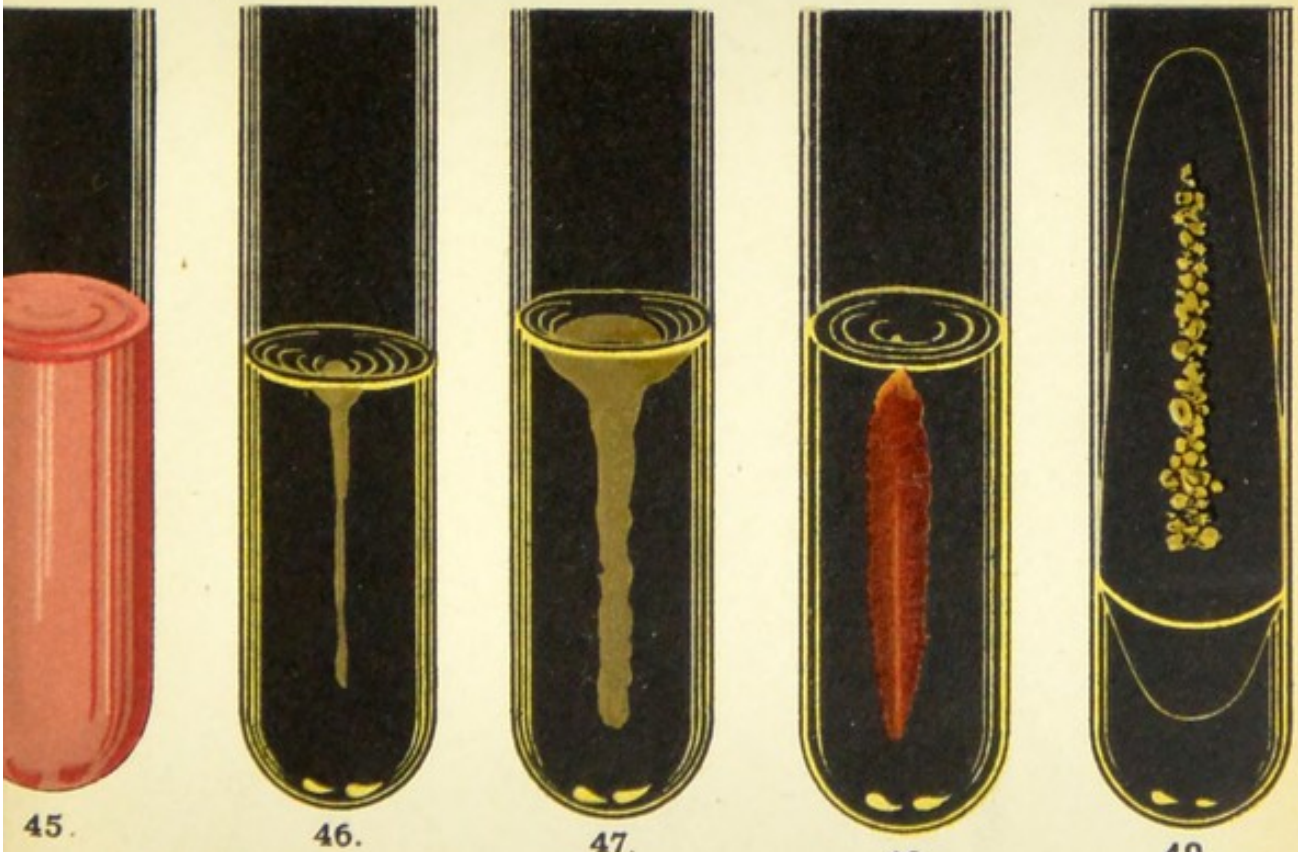
FIG. 47.—Spirillum Finkler-Prior—Stab culture in gelatine, four days old.

FIG. 48.—Spirillum rubrum—Stab culture in gelatine.

FIG. 49.—Actinomycosis — Streak culture on glycerine-agar.



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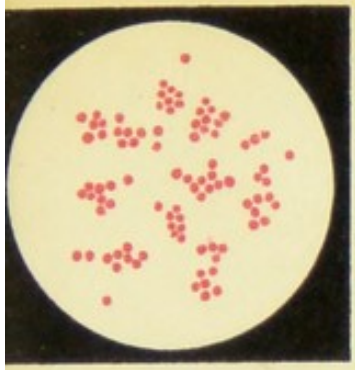
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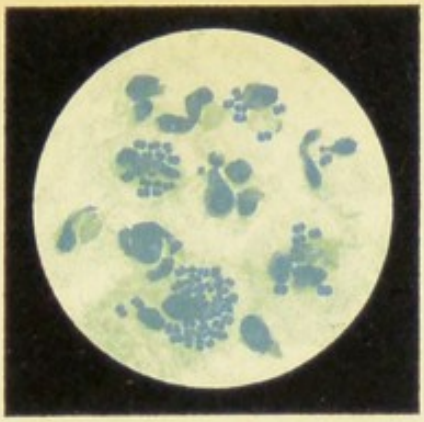
PLATE VI.

COVER-GLASS PREPARATIONS.

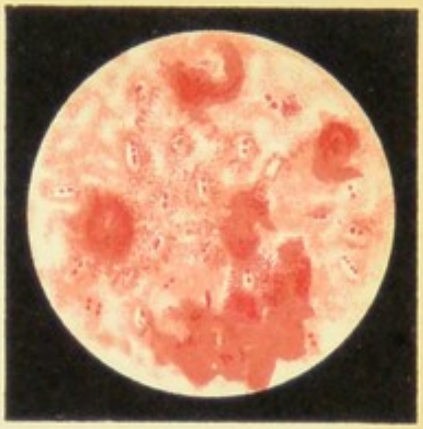
Fig. 50.—Staphylococcus pyogenes aureus.
Fig. 51.—Micrococcus gonorrhœæ in pus.
Fig. 52.—Diplococcus pneumoniæ in sputum.
Fig. 53.—Streptococcus pyogenes.
Fig. 54.—Micrococcus tetragenus.
Fig. 55.—Sarcina lutea.
Fig. 56.—Bacillus typhosus.
Fig. 57.—Bacillus typhosus, showing 'leptrothrix' forms.
Fig. 58.—Bacillus typhosus, showing flagella, stained by Löffler's method.
Fig. 59.—Bacillus diphtheriæ.
Fig. 60.—Bacillus diphtheriæ—Involution forms.
Fig. 61.—Proteus vulgaris, showing the lengthened filaments.



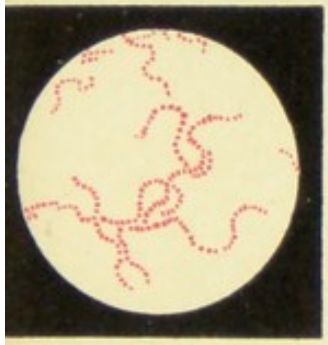
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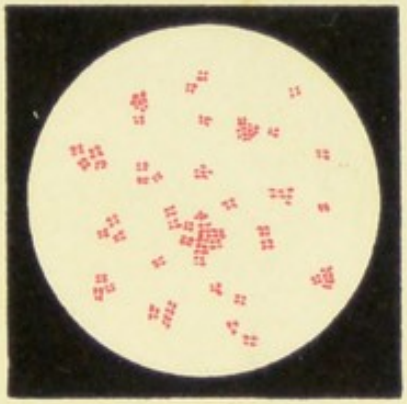
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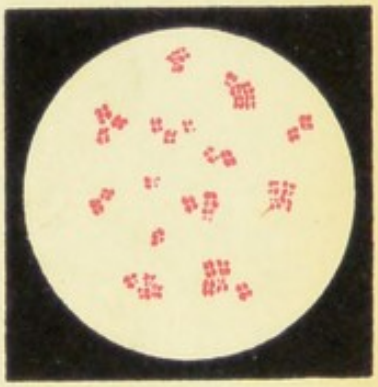
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PLATE VII.

COVER-GLASS PREPARATIONS.

FIG. 62.—Bacillus anthracis in a section of the spleen of a mouse.

FIG. 63.—Bacillus anthracis (pure culture).

FIG. 64.—Bacillus anthracis, unstained, showing spores.

FIG. 65.—Bacillus anthracis, double stained preparation showing spores.

FIG. 66.—Bacillus tetani, with spores.

FIG. 67.—Bacillus œdematis maligni, showing flagella (Löffler's method).

FIG. 68.—Bacillus œdematis maligni, showing spores.

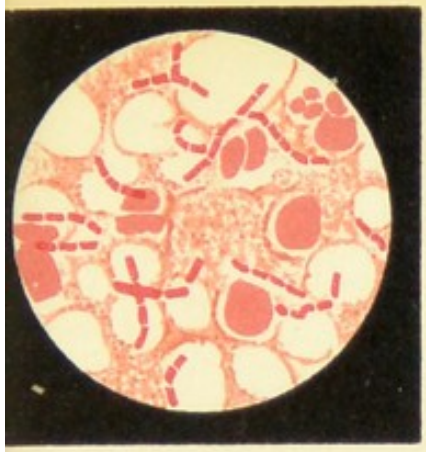
FIG. 69.—Bacillus tuberculosis—Impression preparation.

FIG. 70.—Bacillus tuberculosis—Section through a 'giant' cell.

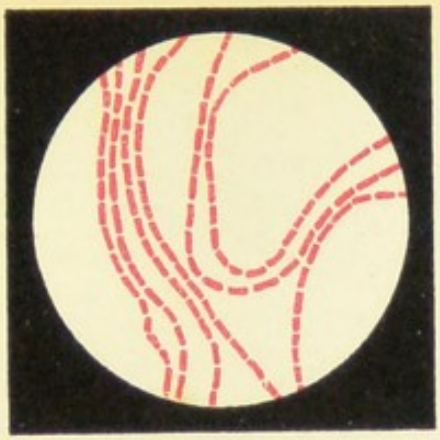
FIG. 71.—Bacillus tuberculosis in sputum.

FIG. 72.—Spirillum choleræ Asiaticæ.

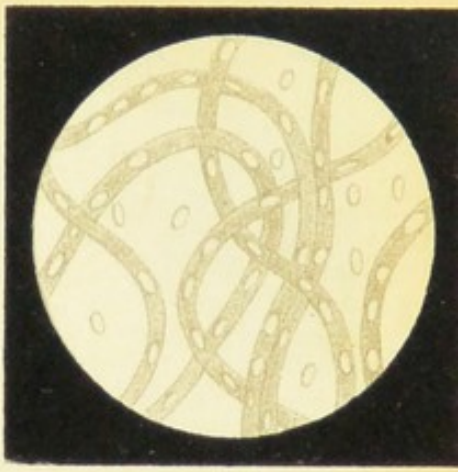
FIG. 73.—Spirillum choleræ Asiaticæ with flagella (Löffler's method).



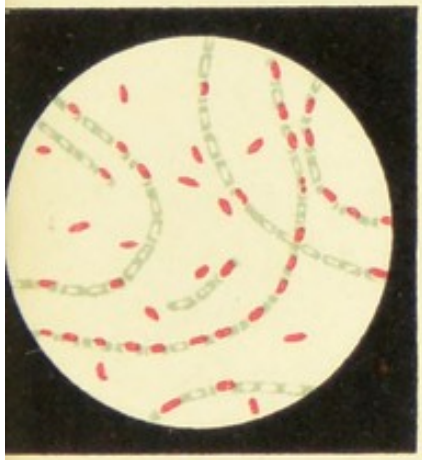
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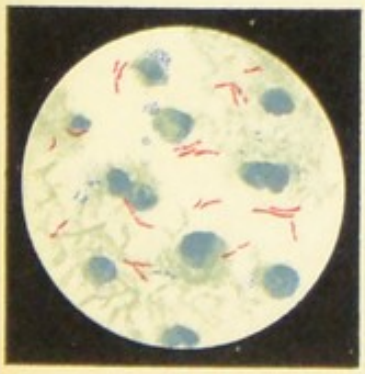
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PLATE VIII.

COVER-GLASS PREPARATIONS.

Fig. 74.—Spirillum choleræ Asiaticæ

Fig. 75.—Spirillum rubrum.

Fig. 76.—Spirillum undula, showing the flagella (Löffler's method).

Fig. 77.—Spirillum Obermeieri in blood.

Fig. 78.—Cladothrix dichotoma.

Fig. 79.—Actinomycosis (pure culture).

Fig. 80.—Bacillus lepræ in a section of skin.

Fig. 81.—Bacillus of influenza in blood.



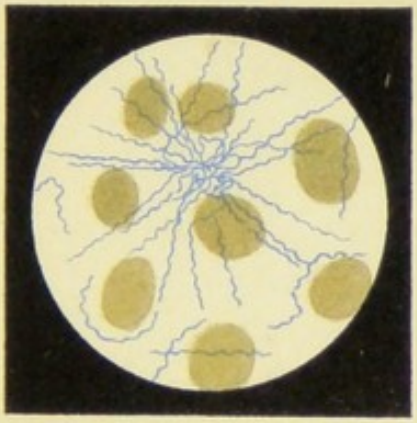
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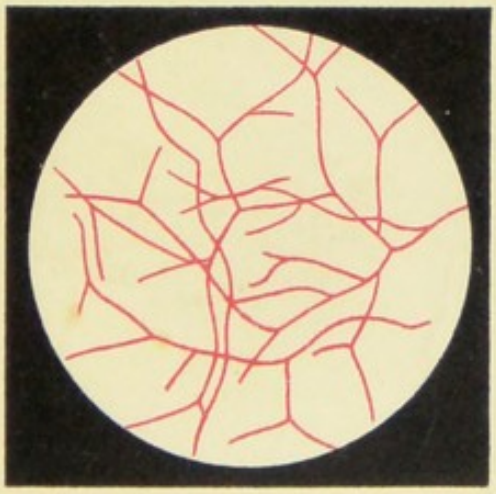
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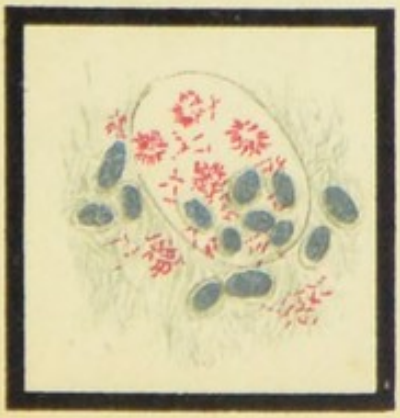
77.



78.



79.



80.



81.

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A.

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B.

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