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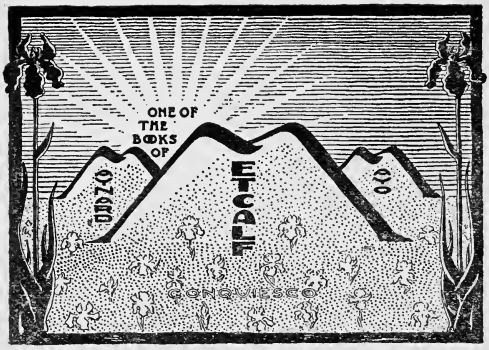
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BACTERIA AND THEIR PRODUCTS.

BACTERIA

AND THEIR PRODUCTS.

BY

GERMAN SIMS WOODHEAD, M.D. (EDIN.),

Director of the Laboratories of the Conjoint Board of the Royal Colleges of Physicians (Lond.) and Surgeons (Eng.); Formerly Research Scholar of the Honourable Grocers' Company.

WITH 20 PHOTO-MICROGRAPHS

AND AN APPENDIX GIVING A SHORT ACCOUNT OF BACTERIOLOGICAL METHODS, AND A DIAGNOSTIC DESCRIPTION OF THE COMMONER BACTERIA.

LONDON:

WALTER SCOTT,

24, WARWICK LANE, PATERNOSTER ROW.

1891

PREFACE.

IN the following pages an attempt is made to give some account of the main facts in Bacteriology, and of the life-history of Bacteria and closely allied organisms, and also to discuss the more important theories as to the part played by them in Nature's Economy ; especially in their relation to the commoner fermentative, putrefactive, and disease processes.

It may be held by some of my readers that too much prominence is given to certain questions, whilst others have been relegated to a comparatively obscure position, and that some points have been accentuated, perhaps at the expense of others ; but to such criticism it may be answered that whilst some facts carry conviction along with them, others can only be properly appreciated when seen by stage lights. On the other hand, many points have of set purpose been lightly touched upon because experience is constantly bringing home the fact that what is new to-day may be out of date to-morrow. I have, therefore, thought it better, in most instances, to confine myself to an exposition of well-accredited facts and to a discussion of some of the more stable theories.

Being privileged to hold a Sanitary Research Scholarship of the Honourable Grocers' Company for some years, I was enabled to devote considerable time to the study of the relations of Bacteria to Disease, especially in the case of tuberculosis, and many of the interpretations of facts here mentioned are based on observations then made ; the re-

mainder are drawn chiefly from the works enumerated at the end of each section. These lists, however, represent but a small part of the enormous mass of literature dealing with Bacteria and their Products, which has accumulated during the last decade.

To Professor Löffler's admirable work, "Vorlesungen über die geschichtliche Entwickelung der Lehre von den Bacterien," and to Mr. Watson Cheyne's "Antiseptic Surgery," I am specially indebted for much information and guidance in my search for facts and papers dealing with the earlier history of the subject.

It may be said of the Appendix that it is given, not with the object of supplying intending workers with every known method of research, and with full descriptions of every organism with which they may have to deal, but simply to enable them to *commence* work, and to recognize the commoner forms of Bacteria, of which about one hundred and forty are here described.

I take the opportunity of expressing my thanks to Dr. Cartwright Wood, who, whilst revising the proof sheets, has made several valuable suggestions; to my former assistant, Mr. Coghill, now of the Royal Veterinary College, and to Mr. Andrew Pringle, both of whom have made for me beautiful photo-micrographs, which, however, can be but imperfectly reproduced by any photo-mechanical process now available.

G. S. W.

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BACTERIA AND THEIR PRODUCTS.

CHAPTER I.

Introduction.

Growth of Subject—Place of Bacteria in Nature—Pure Cultures—Morphology—Physiology—Protoplasm governed by same laws whether in higher or lower Plants or Animals—Relation of Bacteria to everyday Processes—Fermentations, Butyric, Lactic, Colour, &c.—Brewing—Baking—Kephir making—Flax preparation—Digestion—Putrefaction—Nitrification, Mineralization—Relation of Bacteria to Water Supply—Filtration—Sewage—Modes of Transference of Pathogenic Bacteria from Patient to Patient, or from Water or Earth to Patient.

WITHIN the last decade bacteria have laid a very strong hold on the thought and imagination of the scientific world, and have come to be looked upon as playing a most important part, not only in the production of disease and in fermentation, but also in many everyday processes hitherto supposed to be dependent on very different causes. In consequence of this, bacteriology has been raised to the dignity of a science, and its ramifications have become so numerous and so wide-spreading that many of the other sciences, and even some of the arts, have been freely pressed into the service of one or other of its branches. The evolution of the science for long went on but slowly; the study of bacteria remained almost entirely in the hands of the botanists, although now and again scientific medical men, whose powers of observation and deduction were far superior to the methods of experimentation that had been at their command, made shrewd guesses at the causal relationship between the growth of certain bacteria

within and without the body and the etiology of certain infective diseases, and certain processes of putrefaction and fermentation. At first bacteria were claimed for both the animal and vegetable kingdoms by the workers in each, and the fortunes of war seemed for long to favour the zoologists; but after a sharp struggle the fission fungi, as they are called, were relegated to the domain of botany, and now for many years the morphology of these organisms has been an object of most careful study by scientific botanists. Bacteria were, however, so minute that it was impossible to study them very accurately with the older microscopes, and for long the group contained very few species, whilst in addition to the fallacies connected with actual observation of size, form, and methods of reproduction, there were others that resulted from the fact that it was impossible, until comparatively recent years, to obtain what are now known as pure cultivations; forms that were utterly dissimilar in morphological characters were supposed to be stages of the developmental cycle of the same organism, although they might be, and probably were, really nothing but different organisms that were capable of growing in a common medium. When at length more or less pure cultivations of certain organisms were obtained, botanists were able to study, in some cases very completely, the life-history and morphology of various forms of bacteria, and great advances in bacteriological science were made. Still no very accurate observations on the functions or physiological chemical processes associated with the life-history of bacteria and allied forms were made, and many of the biological questions, some of which had already been answered as regards animal and the higher vegetable protoplasm, still remained as in a sealed book so far as this lowly vegetable protoplasm was concerned.

In the last ten or twelve years, however, owing to the vast improvements that have been made in the methods of cultivation of these organisms, and especially of obtaining what are known as pure cultures, *i.e.*, cultures that contain a single species of organism only, most valuable data as to the functions and biological chemistry of these minute specks of vegetable protoplasm have been rapidly accumulated.

Pasteur's wonderful observations on yeasts first opened up the way for future workers. His practically pure cul-

tures were obtained by a kind of physiological separation, to obtain which he fed his yeasts on food specially suited to their nutrition—more suited to their wants, in fact, than to those of any micro-organisms with which they are usually found associated— and they were thus enabled to develop so luxuriantly that they prevented the growth of almost all other organisms, which might have found their way into the nutrient medium along with them. These cultures were comparatively pure, the presence of the small number of other organisms not being sufficient to vitiate the main general results.

Klebs (1873) tried to obtain pure cultures by his fractional method, which was simply the removal of those organisms that vegetated most luxuriantly in any special fluid to another flask, making special cultures of these, and so on until comparatively pure cultures were obtained. The difficulty, however, was that only the commoner forms could thus be separated and even then they could not be relied upon as being absolutely pure.

This method (the "maas" method) is even now sometimes employed to obtain pure cultivations of cholera bacillus. A drop of the discharge from a cholera patient containing a large number of organisms besides the specific cholera bacillus, when placed in broth which is so prepared as to be specially suited to the nourishment of the cholera organism, soon becomes almost a pure culture, as the cholera bacilli are able to multiply in an extraordinary degree at their "optimum" temperature and to far outnumber all the other organisms present, and it becomes a much easier matter to obtain from the broth culture a pure culture of the "comma" bacillus than directly from the cholera discharge itself. It may be said to correspond to the passing of a mixed culture through an animal. Certain organisms—those that are "pathogenic"—can grow luxuriantly whilst the others make a much weaker struggle for existence.

As soon as it became evident that no further steps of any very great importance could be taken unless pure cultivations were used, various workers set themselves to devise methods by which single organisms might be isolated, and from which a "pure-bred stock" might be raised. Then Roberts (1874) and Cohn (1876) obtained pure cultures of different forms of spore-bearing bacilli, especially the bacillus subtilis, by subjecting the fluid to the heat of 100° C., by which all non-spore-bearing forms were killed off. This method, however, has a very limited application, though combined with other methods, it enabled Kitasato to obtain

pure cultures of the tetanus bacillus. Salomonsen (1876) observing that the dark colour which makes its appearance in defibrinated ox blood always commences as minute points, concluded that each of these patches was due to the changes set up by micro-organisms growing at these points. He therefore drew such defibrinated blood into a capillary tube, and by carefully noting the appearances presented in the tube was able to follow the development of a variety of organisms which, when few in number, were separated by considerable intervals of bright red blood in which no organisms could be seen, though in time these gradually ran together. Lister (1878) and Naegeli (1879) obtained their pure cultivations by a kind of fractionating method, in which they distributed a number of organisms into large quantities of fluid by making greater and greater dilutions of their organisms in broth, until the dilution was such that a single drop did not contain more than a single organism, and a series of such drops introduced into a number of flasks containing sterilized broth gave a large proportion of pure cultivations. With the pure cultivations so obtained the first really accurate experiments in connection with the physiological chemistry of bacteria were carried on. This method, however, though a very great advance on any that had hitherto been devised, was somewhat complicated, and it was open to certain other objections (though it is still most useful in many investigations). It was not until Koch, perfecting Klebs' and Brefeld's gelatine method, was able to "fix" the organisms *in situ* as it were, in the nutrient medium, that the bacteria could be kept isolated, the resulting colonies studied and then removed to other media for further examination. The method was so simple and so reliable that it was eagerly seized upon by those who were interested in the solution, not of botanical problems merely, but of many questions connected with putrefaction, fermentation, and disease that had cropped up so frequently, but which had to be left without a satisfactory answer, and which, in consequence of Davaine's and Pasteur's researches, were again assuming such prominence.

In the first place, experimenters were able to put to the test the many observations that had been made on both the structure and the function of micro-organisms. Once a pure culture became available, it was an easy matter to determine

the conditions under which a special bacterium would grow. It could be transferred from medium to medium ; it could be placed under ærobic and anærobic conditions ; or it could be plied with antiseptic or other special reagents, and its behaviour under varying conditions or in the presence of these material could be accurately observed. There was no longer any danger of one form being mistaken for the developmental stage of a totally different organism, and much confusion as regards classification was gradually done away with, though the effects of the observations made on impure cultivations still make themselves felt in the obscurity that enshrouds certain groups which are still met with even in some of the better known classifications. As these pure cultivations were obtained it gradually became more apparent that, for practical purposes, it would be necessary to base our classification on certain specific characters of the organism ; and as in all processes in which bacteria play a part they are usually met with in a single and special form, it was originally found advisable to take these special forms as the diagnostic features in making such classification. The features of form and size, however, were merely superficial characters, and as a more careful study of bacteria was made, especially by the French school, it was found that the biological characters afforded a much more satisfactory basis of classification, especially, however, when taken in conjunction with the morphological features ; so that the media on which they grow, the products to which they give rise, the methods in which they cause the breaking down of dead or living protoplasm, the actions and reactions that take place between them and living animal and vegetable cells, and the effects upon them of organic and inorganic antiseptic substances, are now all taken into account in drawing up any classification. Thus we have yeasts that cause fermentation and yeasts that do not, whilst nearly related to them are ascomycetes that are essentially parasitic in their character ; we have micrococci that set up urea fermentation, micrococci that form pigment, others that give rise to suppuration or that apparently assist in the production of diphtheria ; and so on, throughout the whole of these low vegetable forms. The appearance under the microscope can, of course, give us no information on many of these points, and it is only by a most careful study of the

life-history of these organisms that any accurate information has been obtained.

Within quite recent years it has been observed that very marked modifications of certain of the features may be obtained by subjecting the micro-organisms to conditions different from those under which they are ordinarily met with ; and as the outcome of these observations the way has been opened up for the study of the evolution, perhaps not of the micro-organisms themselves, but of their specific functional characteristics. It has been found, for instance, that under certain conditions, yeasts that ordinarily set up active alcoholic fermentation are no longer able to do so ; that by continued cultivation outside the body and in certain special media the anthrax bacillus loses much of its virulence ; whilst the tubercle bacillus, the cholera bacillus, the organisms met with in diphtheria and in other diseases, may, under certain conditions of cultivation, lose their disease-producing power in a most remarkable degree. On again being placed under conditions of a more favourable nature these organisms resume their pathogenetic or disease-producing properties. The same variations are met with in the colour-forming species of bacteria, according as the conditions under which they exist are favourable or unfavourable to the accentuation of certain functions of the protoplasm.

It will be evident from a consideration of all these facts that bacteria must be looked upon as being governed by much the same laws which govern other plants and animals ; that they are composed of protoplasm, the functions of which may be modified in various ways, and the forms of which may become more fully differentiated, and that where greater differentiation takes place the modification of function is always in the direction of greater specialization ; that the conditions suitable for the existence of the more lowly organized and functionally less specialized organisms need to be less specialized than do those that are necessary for the growth of the more highly developed fungi ; and that, consequently, the bacteria of disease and putrefaction are, with few exceptions, of a comparatively low form, although the functions of the protoplasm of these less specialized bacteria become more specialized as they dwell for a longer and longer period under any special set of conditions, one phase or power of the protoplasm being drawn out and developed in

excess and frequently at the expense of, some of the others, sometimes even at the expense of the living or resisting power itself of the organism. In all cases, however, it should be remembered that the differences in protoplasm are differences in degree rather than in kind, and that the laws which govern animal and other vegetable protoplasm govern the protoplasm of bacteria, and that the results of all investigations that have been carried on in connection with them must conform as strictly to physiological laws as any work that has hitherto been done in the domain of animal or vegetable biology ; and that where discrepancies of any kind, or apparent departures from ordinary physiological laws, are met with, the facts must be carefully revised, and if the discrepancies still continue, then so much the worse for either the facts or the laws. The "facts" are still incorrect or the laws must be revised.

There has grown, then, and still continues to grow, a science dealing with microbiology in all its morphological and physiological phases. Facts have gradually been accumulated, observations have succeeded observations, patient work and powerful concentration have played their part in elaborating our knowledge of the habits of micro-organisms without and within the higher plants and animals ; the conditions under which the modes of life of micro-organisms may be altered have been investigated ; the effects that they or their secretions exert on living and dead protoplasm have been most conscientiously examined ; the conditions that are essential in order that these organisms may stimulate the activity of living protoplasm, and the phases through which this protoplasm passes, from the stimulated condition to degeneration and death, have all been carefully studied. The organisms that have been found in certain diseases have been identified and classified, their modes of propagation and the channels by which they are conveyed from one host to another, sometimes through intermediate saprophytic or non-parasitic stages, have been determined, and the whole subject has been so prepared, that the great epoch-making minds of such men as Pasteur, Chauveau, Lister, and Koch, being brought to bear on these questions have found sufficient material at their disposal on which to generalize, and have placed before the world theories that appear to be more like fairy tales

than deductions made as the results of sober scientific investigation and thought. These men have also, however, been able to obtain many new facts, and to point out the gaps that still remain to be filled in before the theories so admirably expounded can be proved to demonstration. The French can boast of their Davaine and Pasteur, who, assisted by Chauveau and others, have given to us the germ theory of disease and the theories of fermentation and protective inoculation. In Germany, Klebs, Cohn, Koch, and their followers, have made marvellous contributions to the study of bacteria, and additions to our knowledge of their relation to disease. The Danes have furnished O. F. Müller, Warming, Panum, Salomonsen, Bang, and Chr. Hansen, who, seizing most of what was good in both the French and German schools, have succeeded in making most valuable contributions to various branches of the subject ; whilst, in this country, Burdon Sanderson, Greenfield, Klein, Watson Cheyne, and others, have all contributed their share, though Lister's name must always stand pre-eminent for the magnificent work which he has done in the domain of antiseptic surgery, by the evolution and perfecting of which he has done more, both directly and indirectly, to ameliorate the suffering, and to diminish the mortality in surgical cases, than has been achieved by the most brilliant operators the world has produced during the last century.

Many of the processes of everyday life are intimately associated with the specific activities of micro-organisms ; we are constantly meeting with these organisms, and it is now proved, beyond all dispute, that their presence is not merely accidental but is absolutely essential to the carrying on of, one might almost say, the most commonplace operations. Take, for example, those that are associated with the different processes of fermentation. Leaving for the moment the yeasts or saccharine ferments, I may refer first to the butyric acid ferment—Clostridium butyricum—which plays a most important part in interfering with the ordinary course of the saccharine fermentation. It decomposes starch and cellulose without requiring the presence of oxygen ; it, like other micro-organisms, appears to require for its nutrition, nitrogenous material, and, especially in the presence of the lactic acid ferment, it brings about the conversion of sugar into butyric acid ; it is, as might be anticipated, one of the

bacteria most frequently met with in some of the forms of putrefaction.

It must be remembered however that this is not the only organism that gives rise to the butyric acid fermentation; several other bacteria, differing most markedly in many of their features from the Clostridium butyricum, generally, however, like that organism, carrying on their work in the absence of free oxygen, have the power of setting up alone, or in conjunction with the Clostridium butyricum, the butyric fermentation. In ripened cheese, part of the flavour at any rate is due to the products formed in the ripening curd in the presence of this organism.

The lactic acid fermentation so frequently met with in milk, is also the result of the vital activity of several organisms, Pasteur and Lister both describing lactic acid bacteria and micrococci. Hueppe also describes a special bacterium which he says has the power of breaking up milk sugar and saccharose into lactic acid and carbonic acid; whilst from material taken from the mouth and teeth he succeeded in separating two micrococci, both of which had the power of converting sugar into lactic acid, and by a series of experiments he also proved that certain of the pigment-forming bacteria, such as Bacillus prodigiosus—the organism that gives rise to what is known as bleeding bread—supply so much lactic acid as a result of their metabolic processes, that in their presence milk is curdled, the casein being precipitated. Further, even a pathogenic form of micro-organism—micrococcus osteomyelitis—is said by Krause to set up the same reaction. Jörgensen mentions that "Delbruck found that in a mash prepared from dry mould and water, lactic acid was first formed at a temperature of 50° C., and from this he draws the conclusion that in this case the active lactic acid ferment has its maximum temperature at this degree of heat." This is an exceedingly interesting fact, for as Schöttelius and Wood have pointed out, as the temperature rises the Bacillus prodigiosus loses its power of forming a pigment, and if it is grown on potato or bread paste for example, in an incubator at blood heat instead of at the temperature of the room, the colour is gradually lost and the culture no longer smells of herring brine, but the power of forming lactic acid from milk sugar, with the accompanying precipitation

of the casein, is frequently considerably increased ; so that it would appear that the energy required for the building up of the pigment substance was in this case diverted into another channel, and lactic acid, and perhaps other substances, are produced in place of the usual pigment.

This example may afford some idea of the complexity of the problems that have to be grappled with by bacteriologists, and it may also help to explain how such different results have been obtained by different observers, and how it still may be possible to reconcile statements which at first sight appear to be in direct opposition.

It is a well-known fact that if the lactic acid fermentation once obtains a footing, in a solution of sugar for example, many of the other putrefactive bacteria find it impossible to develop. On the other hand, whilst the lactic acid organism cannot grow beyond a certain point, yeasts are perfectly able to develop where there is a certain quantity of acid present. This has been adduced as an explanation of the fact that comparatively pure yeast fermentation may go on even in the presence of the lactic acid organism, when it is unable to make further headway and when other putrefactive organisms are unable to grow at all.

Another fermentation, the results of the careful studies of which have been utilized in the commercial production of vinegar, is that due to the acetic acid ferments Mycoderma aceti and Mycoderma Pastorianum, by the action of either of which alcohol is converted into acetic acid.

In all the works on brewing, lists of micro-organisms that give rise to such conditions as bitterness, muddiness, various colorations — red, yellow, green, etc.—are given, and it is pointed out at the same time that all appear to give rise to some form of acid. It is supposed, for example, that certain species of sarcinæ are responsible for the sour and bitter tastes which are sometimes developed in beer ; a similar organism is also described as giving rise to the red colour which is sometimes developed in white beer, though it may be remarked that in this case a rod-shaped bacterium is also frequently present, and may be answerable for the presence of the substances that so seriously alter the taste of this beverage. A series of other organisms, which Lindner speaks of as Pediococci, all produce acid, and give rise to

unsoundness of beer, but as they are readily killed at a temperature of 60° C., they may easily be got rid of.

In the process of baking, as carried on in this country, there is a regular conversion of some of the starch of the flour into sugar by the yeast used — this sugar being in turn converted into alcohol and carbonic acid gas, to the setting free of which the "rising" of bread is due. The baking that follows serves three purposes; it kills the ferment, it fixes the remaining starchy matter in position, and it drives off the alcohol and the carbonic acid. In the baking of other kinds of bread certain other organisms are said to play a part; for instance, the Saccharomyces minor (Engel) was supposed to be the active fermenting agent in the manufacture of rye bread, but more recently this organism has been superseded, and the work of fermentation has been assigned to a bacillus—Bacillus Panificans (Laurent)—which in pure cultivations was found to be capable of setting up all the characteristic fermentation changes in the dough of black bread. This organism is made up of short motile rods, from which threads may be formed, these threads (in which spores are sometimes found) interlacing to form a film, especially when it is grown on the surface of nutrient liquids. Its spores are almost as resistant as those of the hay bacillus, and can only be killed by being subjected to boiling heat for a period of at least ten minutes.

Jörgensen, summing up Laurent's investigation, says: "The bacillus usually dissolves the gluten substance of the dough, grows in starch paste, and in mixtures of saccharose and mineral substances. It is found in an active state in large quantities in bread, and according to the author's researches, it can withstand for twenty hours the action of an artificial gastric juice. In the excreta it is found still more abundantly, and it appears to be generally distributed in plants and in various substances." More recent researches, however, have thrown some discredit even on the Bacillus Panificans; Dünnenberger maintaining that these bacteria are merely an impurity, and that in all cases the fermentation of bread is due to an alcoholic-forming organism—a saccharomyces in all cases proving the best agent for bringing about this fermentation.

Certain forms of unsoundness of bread are also due to micro-organisms. In addition to bleeding-bread (caused by

the growth of the Bacillus prodigiosus), which has served the purpose of the miracle-monger before to-day, sticky reddish-brown patches have been described as occurring in unsound bread, in which various bacilli, such as the ordinary potato bacillus, Bacillus liodermos, Bacillus mesentericus vulgatus, have been found, and on analysis of these patches dextrine, dextrose, starch, sugar, and even a small quantity of peptone, have been separated ; moulds of other fungi are also found in unsound bread.

In 1882, Kern described the peculiar ferment known as *kephir* grains, by means of which the Caucasians set up a double alcoholic and acid fermentation in milk.

These kephir grains, says De Bary, in the fresh living state are "white bodies, usually of irregular roundish form, equal to or exceeding a walnut in size. They have their surface roughened with blunted projections, and furrowed like a cauliflower ; they are of a firm, tough, gelatinous consistence, becoming gradually cartilaginous, and are of a yellow colour when dried ; they are chiefly composed of a rod-shaped bacterium," many of these being united to form long threads, arranged in a kind of felt or network, the meshes of which are filled with a tough gelatinous membrane, which binds the organisms together into a kind of zoogloea mass. This rod-shaped organism is known as Dispora caucasica as at the end of each rod is a rounded spore.

Along with these may usually be found a small proportion of a yeast-like fungus which, however, is merely entangled in the gelatinous mass, although it certainly undergoes development by sprouting. There is also present the ordinary Bacterium lactis which, with a number of other impurities, adheres to the kephir grains ; this also occurs in the milk itself. To prepare the specially fermented milk, one volume of these kephir grains is moistened and added to about six or seven volumes of fresh milk, the whole is protected from the dust, but is exposed to the air for about twenty-four hours at the ordinary temperature of the room, and is frequently shaken ; the milk is then poured off and a fresh quantity added ; the milk that is poured off is mixed with double its quantity of fresh milk, put into bottles, well corked, and frequently shaken. This bottled sour milk soon becomes sparkling and effervescent, and is ready for use after it has been bottled for a day or two. It then con-

tains lactic acid, a considerable quantity of carbonic acid, which varies "according to the temperature and the duration of the fermentation, but is sometimes sufficient to burst the bottle or drive out the corks." The liquid contains about one per cent. of alcohol.

We have already seen that the kephir grains and the sour milk, together, contain (1) a yeast fungus which is capable of bringing about the fermentation of grape sugar ; (2) the bacillus of lactic acid ; and (3) the rod-shaped bacteria that predominate in the gelatinous mass of the tough grains. Now, though this yeast fungus can set up the fermentation of inverted milk sugar, it cannot affect the milk sugar itself, so that we must look elsewhere for the inverting power.

This appears to be contained in an enzyme, or ferment, that is produced by a large number of bacteria, amongst others by the rod-shaped organisms already referred to, and by the lactic acid bacillus, and these were supposed to prepare the milk sugar for the action of the yeast fungus. This appeared to be a perfectly satisfactory explanation, until De Bary found that, by violent agitation of the souring milk to which no kephir grains have been added, alcoholic fermentation may still be induced ; so that it would appear that by freely oxygenating the milk during the process of lactic acid fermentation, when in fact the molecules are being re-arranged, oxygen is taken up into chemical combination, and alcohol and carbonic acid are generated ; water, in all probability, being formed as this goes on. This is adduced as one of the processes of fermentation by free oxidation, and is an example of a chemical fermentation giving results similar to those yielded by biological fermentation. Until, however, it can be demonstrated that De Bary was working with material in which all impurities were excluded, these results can scarcely be accepted as absolutely reliable.

In the kephir, which is a perfectly fluid mass, we have a quantity of lactic acid. Now, it is a well-known fact that, under ordinary circumstances, when milk turns sour, there is a precipitation of the curd which forms a more or less solid coagulated mass. What has become of this coagulum in the kephir ? If a piece of meat be exposed to the action of putrefactive germs, it will be found that after a time it is reduced to a soft, pulpy, almost liquid mass. In the same

way, a number of the nitrogenous foods taken into the stomach are softened and otherwise very considerably altered.

These materials have in both cases undergone what is known as a process of peptonization : from coagulable or colloid albumen they have been converted into a much more soluble and diffusible albuminoid, and are thus prepared for further changes as they are acted on directly by living animal or vegetable protoplasm. This peptonization appears, however, to be also brought about by an enzyme which is elaborated by certain bacteria, especially by such as are associated with putrefaction and with the digestive processes. In the case of the kephir it was thought that the peptonization took place as a result of the action of an enzyme, formed by the rod-shaped organisms of the zooglœa mass of the kephir grains, and it was argued that this must be a soluble ferment which could diffuse from the gelatinous mass in which the grains were embedded into the milk ; it could there act on the casein as it was gradually precipitated, or possibly even before precipitation took place ; for it was observed that these rod-shaped organisms were never found outside the gelatinous masses, and therefore could not be acting directly on the casein.

This is well enough in theory, but as equally good kephir can be produced by the oxidation effected by free movement of the sour milk, the peptonization cannot be solely due to these organisms. It may be urged that other organisms may, in the presence of a large quantity of oxygen, supply the peptonizing function in an acid fluid in which, under ordinary conditions, they would not be able to exist, the large amount of free oxygen driven into the milk enabling them to obtain a supply of energy, to live and carry on their function even in the presence of the special lactic acid bacteria, which seize with avidity the whole or a part of the oxygen contained in the milk, according to the quantity present, in this instance a part only, as there is such a large supply under the above conditions.[1]

It has already been mentioned that the Clostridium

[1] For the further description of the kephir grains the reader is referred to E. Kern, "Bot. Ztg.," 1882, p. 264; Alexander Levy, "Deutsche Medicinal Zeitung," 1886, p. 783 ; De Bary, "Lectures on Bacteria," translation, Oxford, 1887.

butyricum or Bacillus amylobacter plays an important part in determining the butyric acid fermentation of the vegetable acids, and that it plays an active part in the ripening of cheese. Because of its action on cellulose, and then of its further action on dextrine and glucose, it also has much to do with the decomposition and destruction of the cellulose of fleshy and juicy plant tissues, and its aid is requisitioned in the separation of these parts from the tougher fibre of hemp, flax, and similar materials, as in the process of maceration the enzyme converts the cellulose into butyric acid.

Van Tieghem holds that much the same processes go on in the stomachs of ruminant animals, and that the Bacillus amylobacter, which is usually found there, thus does a very large part of the work which, otherwise, would have to be performed by the epithelial cells of the stomach itself. This bacillus, however, acts not only upon cellulose and starch paste, but it also exerts a most important action on nitrogenous substances. Fitz and Hueppe have both pointed out that the casein of milk is first coagulated in the presence of this organism, is then peptonized and liquefied by the action of its enzyme, and that the products thus formed are afterwards converted into certain lower compounds, such as leucine, tyrosine, and even ammonia, all of which are constantly met with during the processes of both digestion and decomposition. Duclaux also showed by experiment that an organism, which resembles the bacterium amylobacter very closely in many respects, sets up a series of similar changes in the casein of milk, and in casein that has been converted into cheese; and he showed that the process of "ripening" brought about in the presence of the Tyrothrix bacillus, is due to the peptonization and liquefaction of some of the substances of which unripe cheese is composed; certain secondary or ultimate products similar to those above mentioned being found during the ripening process.

That bacteria are general scavengers is now generally acknowledged, and almost innumerable observations have been made with the object of proving that the presence of certain definite organisms is essential for the perfect breaking down of dead or effete animal and vegetable matter. It has already been mentioned that some species have the power of breaking up cellulose and of converting it into much simpler substances, both externally and in the alimentary canal.

Bienstock went so far as to describe a particular bacillus which, he considered, was able by itself to produce the whole series of changes that occur in the contents of the intestine and in putrefying albumen or fibrine. This organism he describes as being somewhat smaller than the Bacillus subtilis; it is rod-shaped, but at one end there is usually a small enlargement, in the centre of which a clear round spore may be seen. It is from this feature that the organism derives its name of "Drum-stick" bacillus. Cultivated on fibrine it disintegrates it and gives rise to the formation of leucine, tyrosine, carbonic acid, water, and ammonia, and of traces of other putrefactive products. The process does not however stop at this point; the bacillus still continues to act on the leucine and tyrosine, and decomposes them into still simpler compounds; whilst, if it be introduced at once into a prepared solution of one of these earlier decomposition products, tyrosine for example, it continues the breaking-down process just as if it were still acting on the tyrosine which had been formed during the process of ordinary putrefaction. As De Bary points out, however, this bacillus cannot claim a monopoly of the work connected with putrefaction. If any putrefying fluid be examined, countless organisms will be found, and amongst these very different species may be observed. There are rods of different sizes both as regards thickness and length, spirals of different forms, micrococci of different sizes and arranged in different groupings, one or other organism predominating according to the nature of the putrefaction process, of the material that is being broken down and the stage at which the breaking-down process has arrived. It would appear in fact as though there were developed special organisms for the setting up of special fermentations, and also that after the breaking down has been carried a certain length by one organism, the aid of another is invoked to complete the process more thoroughly and more expeditiously. We have in this, as in the case of the process of digestion, an exemplification of the fact that nature economizes her resources as much as possible; she does not call on the animal cells of the alimentary tract to do work that can be equally well done by micro-organisms, nor does she demand the exercise of more than one or two functions from each of the simple protoplasmic specks that we call bacteria. To each one is assigned

its special work and, though it is possible that many of them started with certain powers in common, it seems that through the exercise of some of these common powers under special conditions they have become gradually so differentiated functionally, that, as amongst organisms more highly developed, each is able to carry on its own work best at those special stages of the putrefactive process at which it is found. It might at first sight appear that all this can have but little bearing on any practical work in which we are engaged, or in which we take an interest, but on more careful consideration it will be found that these putrefactive organisms really keep up the circulation of matter, utilizing the excretions of living beings and the carcases of dead animals and plants, after breaking them down into their simplest constituents, to supply those elements that are necessary for the nutrition of plants, allowing them to present themselves in their most assimilable forms, and in the proportions most suitable for the nutrition of the growing, highly organized vegetable protoplasm. Bacteria in fact serve to transform inert organic matter into inorganic substances. This transformation, or "*Mineralization*," in most cases, commences only after protoplasm has lost its vitality, and most micro-organisms are capable of attacking this dead protoplasm only ; though, as we shall find later, a certain number of bacteria have acquired the faculty of being able to attack even living protoplasm. The processes of decomposition may be divided into two kinds : first, those going on as the result of the activity of organisms that are capable of taking up their oxygen from the air, and, second, those the result of the activity of organisms that so break up and rearrange the organic molecules containing oxygen, that not only do they, the bacteria, take up oxygen themselves but they allow of its being handed on to the products to which in their processes of metabolism they give rise. It is probable that here we have to do, not only with nascent oxygen, but that we have certain products set free during the process of decomposition which seize upon oxygen with very great avidity. This decomposition or rearrangement is spoken of as a process of nitrification, or a conversion of the nitrogenous elements into ammonia, nitrous and nitric acids, carbonic acid and water, or, speaking more generally, it may be said to be a process of mineralization of the organic

forms of nitrogen, phosphorus, carbon, and hydrogen, during which they become finally oxidized or mineralized to nitric acid (HNO_3), phosphoric acid (H_3PO_4), carbonic acid, CO_2, and water, H_2O. In nature this process goes on in the superficial layers of the earth or in the presence of the atmosphere. That it takes place much more readily near the surface of the ground and in porous earth can easily be understood if what takes place in the oxidation that goes on in spongy platinum is borne in mind. In the earth we have a spongy material, the upper surface of which is well supplied with air, and usually also with moisture; there is also a certain amount of organic matter present on which micro-organisms feed, and any additional organic matter brought to this spongy mass is rapidly seized upon by the micro-organisms, is oxidized, and thus prepared for the nutrition of the plants that are growing in this soil. So necessary is this whole process of oxidation by micro-organisms that Duclaux insists that soil rendered sterile (as regards micro-organisms), and supplied only with sterilized water and air, is incapable of supplying sufficient nutrient material to plants to enable them to flourish even moderately well. All the organisms found in these superficial layers under ordinary conditions are ærobes; in the deeper layers of the soil are micro-organisms that give rise to the second kind of decompositions. These bacteria, which are anærobic (that is, they can flourish without being supplied with free oxygen) in character, have a special power of taking up the oxygen that is contained or combined in the products which have filtered down from the surface where the decompositions by direct oxidation are going on. Living away from the atmosphere and being unable to obtain or to utilize free oxygen, these organisms have developed the faculty of being able to wrest oxygen, by force, as it were, from the oxygen-containing bodies that come down to them from nearer the surface, sometimes however using part only of the combined oxygen and setting free another part by which further oxidation may go on; in doing this they carry the process of decomposition a stage further, and after the altered organic materials have been attacked by these anærobic organisms they appear to contain little that will provide nutrition for micro-organisms of any kind; so that, after we come to a certain depth, bacteria are not

to be found in the soil. This depth, usually reckoned at about twelve feet, varies of course according to the nature of the soil, its moisture, porosity and temperature, and also according to the amount of organic matter that lies on the surface; cultivated ground always containing more organisms and to a greater depth than fallow ground having the same geological characters.

The relation of all this to our water supply is obviously one of paramount importance. If water be taken from near the surface of soil in which there is a large quantity of organic matter present, there must necessarily be a large number of putrefactive organisms in it, especially those of an ærobic nature; if however we take water directly from the deeper layer, these putrefactive organisms are usually absent, but a number of "water" organisms are now present, which under special conditions, especially if the water be kept perfectly quiet and unoxygenated, and a high temperature be maintained, can develop in the water, from which they may in turn be cultivated by certain well-known methods. If water be taken from a much deeper layer, micro-organisms are found to be almost or altogether absent, and not only micro-organisms, but organic matter, although in some cases in which micro-organisms are almost entirely absent, organic matter may still be present in appreciable quantities. The superficial layers of earth in this case act, not only as a mechanical, but also as a biological, filter; the water, with its contained organic matter, passes through the successive layers in which bacteria can grow, and gradually percolates to those layers of earth where there are no organisms. It has been demonstrated that even deep natural water contains facultative ærobic organisms, unless it is obtained at once after it has undergone natural filtration—that is water that has not stood in an underground basin—when it may be almost germfree. The organisms cannot go down with the water; first, because they are held back mechanically, the soil acting as a porous filter, by which solid particles, extremely minute as they are, are kept back; but, in addition, and quite apart from this purely mechanical effect, the bacteria (many of which are unable to develop without oxygen) cannot leave the surface with impunity, and such as are carried down by the action of the water die as their supply of oxygen is gradually cut off; for, in consequence

of the rapid oxidation that is going on at the surface, very little free oxygen is left for the use of bacteria at even a short distance from the surface. It will, of course, be objected that this does not apply to the anærobic bacteria, but in their case it must be remembered that only a certain definite proportion of oxidized material can reach them from above, most of the organic matter having already been converted into inorganic material and used up by growing plants ; the supply is very rapidly cut off, the reduction of what remains after the plants are satisfied being completed, and the bacteria cannot continue to live, because they can no longer obtain any material for their nutrition.

All the knowledge that has been gained concerning the process of putrefaction has not been collected in a day, and it was only by a careful marshalling of facts as they were accumulated, and by filling in, sometimes merely with suggestions, gaps that still remained, that the theory of the filtration and biological and chemical purification of water has been built up. But to the practical hygienist it is not sufficient to know that water coming from springs deep down in the earth is free from organisms, and that it may be drawn and consumed with impunity as it rises from the ground. He has something more to consider ; he has to consider under what conditions it can be kept fit for drinking purposes, and by what means it can be most readily and safely distributed to those who use it. He has to remember that water at rest containing organic matter, and exposed to the hot rays of the sun, soon teems with organisms that may or may not be perfectly harmless ; he has to bear in mind the nature of the ground from which water is collected, whether it comes from cultivated areas or from regions in which there is sewage of any kind ; for upon these factors depend the absence or presence of bacteria from the water supply. Further, he may be faced with the problem of how to transform a supply of biologically impure water into a supply fit for drinking purposes.

The mere chemical analysis of water will not give sufficient information to guide a sanitary engineer on these points, though it will indicate to him the lines on which he will have to work. For example, it is quite possible to have a water containing a considerable quantity of organic material which, through its treatment by sand filters, or by

some similar process, may contain no micro-organisms, and may be perfectly fit to drink so long as it is fresh, although, if it were allowed to stand in a warm place and exposed to dust and germs of various kinds, there would soon develop in it such an enormous number of organisms that it would be looked upon as totally unfit for drinking purposes. On the other hand, water containing little or no organic matter might be so contaminated by certain disease germs that it would be absolutely dangerous to health, and even life, if used for domestic purposes.

The relation of bacteria to sewage need not be insisted upon, as it is evident that with such a large quantity of organic matter, in which putrefactive changes must be rapidly going on, bacteria of many kinds must necessarily appear, and it is easy to conceive that an enormous number of different species might be present, especially where the sewage is diluted as it flows into comparatively pure water.

Let us observe how a disease-producing organism may find its way into water that is used for drinking or other purposes, and thence may attack a comparatively healthy individual. In typhoid fever, a germ, which will afterwards be described, has been found. It has special characteristics, and may be separated as a pure culture. A patient has typhoid fever ; the bacilli which we find specially in the walls of the alimentary tract pass out along with the excreta, and, under ordinary circumstances, would be killed by the addition of disinfectant fluid to the stool ; but, as frequently happens, some of the excreta without the addition of a disinfecting fluid by chance finds its way into the drains, or, worse still, on to the surface of the soil near a well or some other source of water supply. The typhoid bacillus continues to multiply in the water or in the organic matter of the sewage, from which it ultimately finds its way into the water, and although such water may appear pure enough (it is often beautifully clear and sparkling), as soon as it is taken into a slightly disordered stomach and intestinal canal, the bacillus gains a foothold, and another patient is attacked with typhoid fever. This may happen simply through the rinsing out of a milk pail. A patient partaking of milk (a most admirable nutrient medium for the growth of the typhoid bacillus) from such a pail is struck down with "Typhoid." Another example : there

is a small drum-stick shaped bacillus, similar to the putrefactive bacillus described by Bienstock, which, on making its way into a surgical wound, sets up a series of changes in the tissues, accompanied by the production of a most virulent poison, which, acting apparently on the nervous system, gives rise to reflex spasms and convulsions, and a condition known as lock-jaw or tetanus is set up. This organism is found on the manure heap, in cultivated soil, and even in water that comes from such soil ; it is also found in the dust of hay, straw, and even in the harness and cloths used for equipping the horse. If water containing these organisms be used for the purpose of washing a contused wound, or if any of the above dirt or dust should obtain access to such a wound—often merely a most insignificant bruise—tetanus or lock-jaw is set up with terrible certainty, and the patient very frequently succumbs to the disease.

Innumerable other instances might be given in proof of the statement that the knowledge, first, of the forms, and, secondly, of the biological and physiological characteristics of the various micro-organisms, is now absolutely necessary for a thorough understanding of even many everyday processes. The colour formed in "blue milk" is due to the action of a micro-organism ; as also are the phenomena of bleeding bread, the Cape meal orange ferment, and the characteristic appearance of green cheese. As a result of the knowledge gained through the study of the life history of septic organisms, thousands of valuable lives have been saved in our surgical wards alone ; large industries have, through Pasteur's indefatigable exertions, been preserved from almost absolute ruin ; as a result of the observations of numerous investigators, our knowledge of certain classes of diseases is gradually becoming more precise and accurate, and the time has now arrived when we may look forward to a system of medicine in which, by preventive and curative inoculation, we shall be able to grapple successfully with some of the deadliest forms of disease with which we have at present helplessly and almost hopelessly to contend.

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CHAPTER II.

What are Bacteria?

Structure — Myco-protein — Limiting Membrane — Gelatinous Capsule—Special Cell Contents—Oxide of Iron—Sulphur—Colouring Material—Flagella—Modes of Multiplication and Development—Division—Rate of Vegetative Multiplication — Endospores — Arthrospores — Classifications of Cohn, Van Tieghem, Zopf, Winter and Rabenhorst, De Bary and Hueppe, Flügge Baumgarten, &c.

Under the general term Bacteria may be included those minute, rounded, ellipsoid, rod-shaped, thread-like, or spiral forms of vegetable organisms sometimes spoken of as belonging to the class of lower fungi. They are also known as Fission fungi (the Schizomycetes of the Germans); a term applied to them because of their multiplication by a process of division, transverse to the longitudinal axis in the case of the rod-like forms, but varying somewhat in the case of the rounded organisms. Each single organism consists of a small speck of protoplasm or vegetable albumen, to which may be given the name of cell, as although these specks are so minute that they can be seen and studied only with the aid of the very best optical appliances at our command, it is found that they are by no means simple in structure, nor are they in all cases even similar. This protoplasmic speck is differentiated into certain well-defined parts.

The round cells or micrococci, the simplest of all forms, are seldom more than 1-25,000th part of an inch in diameter; the elongated cells have, on an average, the same diameter, or they may be a little more, and are from 1-12,000th part to 1-6,000th part of an inch in length, though there are marked deviations from these dimensions in certain forms. Accepting the above figures as being accurate, it would require 25,000 of these spherical cells, placed in a row, or the same number of the longer ones, placed side by side, to make up a chain or band one inch in length. The vegetable

albuminoid or protoplasmic substance of bacteria was first analysed and described by Nencki, who, because of the nitrogen contained in it, and because of the similarity of its structure to animal and vegetable protoplasm, looked upon it as a proteid material ; he gave to it the name of myco-protein or fungus protein.[1] During certain periods of the existence of these micro-organisms, especially in certain species, this white of egg or jelly-like substance, which invariably occupies the central part of the cell, is perfectly transparent and is slightly more refractile than water ; at other periods, or in other species, it may be finely or coarsely granular ; under certain conditions still more marked and characteristic changes may occur, vacuoles or clear spaces making their appearance. It is usually extremely resistant to the action of acids and even of alkalies.

That this myco-protein cannot always be of the same composition is evident from the fact that minute granules of chlorophyll or of fat may be made out lying in the substance of the protoplasm in special species, whilst in others small starch or sulphur granules, or particles of different kinds of pigment may be observed. This speck of vegetable albumen is really the active part of the cell, and it is in this that we obtain those histo-chemical colour or staining reactions that are so characteristic of the protoplasm of the higher vegetable and animal cells which, as is well known, seem to have a peculiar affinity for certain dyes or stains. Carmine, for example, is taken up most voraciously by the nucleus or central portion of the cell which then assumes a brilliant carmine hue, not by any means equal throughout ; in consequence of this inequality the minute structure of the nucleus may be readily and even accurately studied. The surrounding protoplasm, which appears to be less active, is much less vividly stained, whilst the cell wall, which is the least active part of the cell and serves as little more than a boundary wall of formed material, if present at all, remains, in the majority of cases, quite unstained. Logwood, the aniline dyes, or iodine may be substituted for carmine, with the general result that the same staining reactions as regards the different parts of these cells are almost invariably obtained.

[1] Analysis of myco-protein : Water, 84.81 ; albumen, 13.207; fat, 1.198; ash, 0.638 ; extractives, 0.327.

Exactly the same reactions are given when one of the above colouring reagents or stains is added to a mass of micro-organisms. The individual cells or organisms are brought prominently into view, the central jelly-like speck of protoplasm is most vividly stained in each, and reasoning from analogy, certain authors have looked upon this active part as the nucleus of the micro-organismal cell. Surrounding the central protoplasm is a dense, sometimes lamellated, thin skin or sheath which acts as a limiting or protecting membrane. It very frequently contains a substance known as cellulose, almost, if not quite, identical in composition with that forming the hard covering of the vegetable cells that are found in the higher plants. In other bacteria, in fact in the majority of them, the limiting membrane seems to consist of a firm layer composed of gelatinous material, sometimes stiff and rigid, or it may be more or less elastic or pliable. Between the central stained portion and the limiting sheath there sometimes exists a narrow unstained area which by some is said to be a space produced artificially by the chemical action on the protoplasm of staining and other reagents ; others, however, hold that it is a kind of modified protoplasm which, surrounding the more active nuclear protoplasm, divides it from the limiting membrane. Outside this limiting membrane, or more strictly speaking, continuous with it, there may usually be seen a mass of gelatinous or mucilaginous matter which does not take on any special stain, and which serves to separate the individual cells, or, to speak more accurately, to bind them together into little groups. It is due to the presence of this gelatinous material that we have those frog-spawn masses or jelly-like lumps that are met with in certain germ fermentative processes. These masses, in which the organisms are embedded, as it were, in the softened jelly-like part of their sheaths, are known as zoogloœa masses or masses of living glue. If the cells remain isolated when the membrane becomes gelatinous, capsules or highly refractile areas are seen around each cell, when examined under the microscope. These may sometimes be very delicately stained by certain methods (Gram's method, see Appendix). As examples, may be taken the capsule that surrounds the bacillus of pneumonia (Friedländer), the false Diplococcus of pneumonia and certain

other organisms such as the Leuconostoc mesenteroides, which is not pathogenic, and the Actinomyces or Ray fungus. It is rather a curious fact, as Friedländer pointed out, that this gelatinous capsule is formed only under certain conditions, and he noted that in the case of the bacillus of pneumonia the capsule could not always be demonstrated ; that it occurred in the bacilli found in the lung tissue or in the prune juice sputum so characteristic of the disease, but that it could not be made out in the organisms grown on such cultivation media as peptonized beef jelly. In the case of the Ray fungus or Actinomyces, the organism that causes wooden-tongue or Actinomycosis in cattle, this swelling of the capsule at one end of the organism and not at the other gives rise to a very peculiar club-shaped appearance of the threads, and as these are frequently arranged so that the thin ends of the rods are grouped together, whilst the swollen ends are placed at the periphery of the radius, a most peculiar and characteristic radiate arrangement of wedge or club-shaped rods is the result. In some cases bacteria first divide and multiply whilst they are embedded in this common gelatinous mass, and it is only after they have undergone a certain development that each organism becomes invested with its own capsule and is allowed to lead an independent life.

It appears that in most cases in which the organisms contain natural colouring matter, it is deposited in the capsule and not in the protoplasm itself, and that the red, magenta, blue, and yellow colouring particles that are met with in this position give rise to the naked eye colour that is seen where large masses of these organisms are growing. The beautiful brown that is seen in Crenothrix and Cladothrix, not only in the capsule, but in the surrounding cultivation medium, is due to the presence of oxide of iron which the organism is able to separate from water, or from other media in which that substance is held in suspension or combination.

The composition of the limiting or external membrane, as already pointed out, varies somewhat in different organisms ; in many cases it appears to consist merely of an altered myco-protein, but in others, as, for example, in Bacillus anthracis, it is composed of a material analogous to the casein of plants, combined with a substance which

has many points of resemblance to the mucine found in the tissues, especially the embryonic tissues of animals. This capsule of the B. anthracis, unlike myco-protein, contains no sulphur, is soluble in dilute alkalies, but insoluble in water and acetic acid.

Dallinger some time ago pointed out that at each pole of an organism which he had studied most thoroughly (the Bacterium termo, a small oval organism not now recognized as a species) there was developed an extremely delicate flagellum which, he assumed, had something to do with propelling the organism through the fluid in which it was growing, as this organism exhibited most active movements in such fluid. As a considerable number of other organisms also possess this same motion in fluid media, it was argued that as in the case of the above organism and of other cells that have an extreme degree of motility, there would also be found flagella or cilia such as are met with in the swarm cells of certain algæ, where cilia or flagella are continued directly from the protoplasm, through the cell membrane, if present, and so to the outside of the organism. Although the existence of these flagella was suspected in many bacteria, they could actually be demonstrated in the case of some of the larger organisms only. Now, however, the number of bacteria in which flagella have been seen has been gradually but surely increased, and lately Löffler has described and photographed most exquisite lash or thread-like filaments, single or in little groups, in Koch's cholera bacillus, in the bacillus associated with the causation of typhoid fever, and in a considerable number of other motile bacteria.

Whether these cilia and flagella are developed from the protoplasm of the organism, or whether they are merely secondary modifications of the external membrane, remains as yet a doubtful point. It is quite possible that in many, even of the motile forms, flagella are entirely absent ; the organism in such case relying for its motile power on contraction of the protoplasm within an elastic or not too rigid membrane. In a straight rod-like organism an undulating movement may often be observed, whilst both rotary and undulating movements are met with in spiral organisms, though these latter are very frequently supplied with well-formed flagella. It is possible that in certain species,

in which motion is very slight, if not entirely absent, the flagella serve to set up currents round the organism by which food is brought to, and excretions removed from, the bacillus. These flagella may be met with as a single pair, or we may have three or four pairs attached to the same organism. They stain like the membranes, and appear to develop only in those

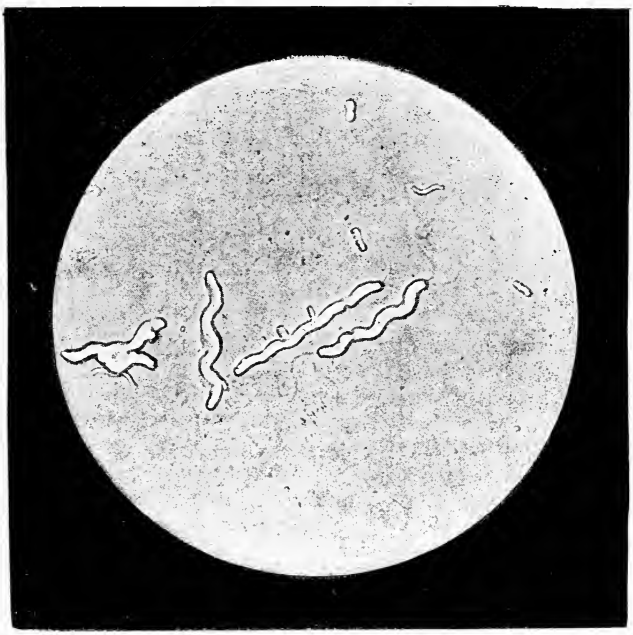

Photo-micrograph of Spirillum Undala, with pair of well-marked flagella at each end. × 1000.

organisms that have special affinity for oxygen, for as soon as the ciliated forms reach the surface of a fluid, they lose their cilia or they become much less active. This latter, however, seems to be the more probable explanation, for Löffler has shown that many organisms are provided with cilia, which at one time were supposed to possess nothing of

the kind. Up to the present, however, micrococci, the Bacillus anthracis, and many other organisms, cannot be said to be supplied with flagella or cilia, and in many organisms in which there seems to be independent movement, really nothing but the so-called Brownian movement can be distinguished when they are examined in fluid, a movement that may be observed equally well when particles of

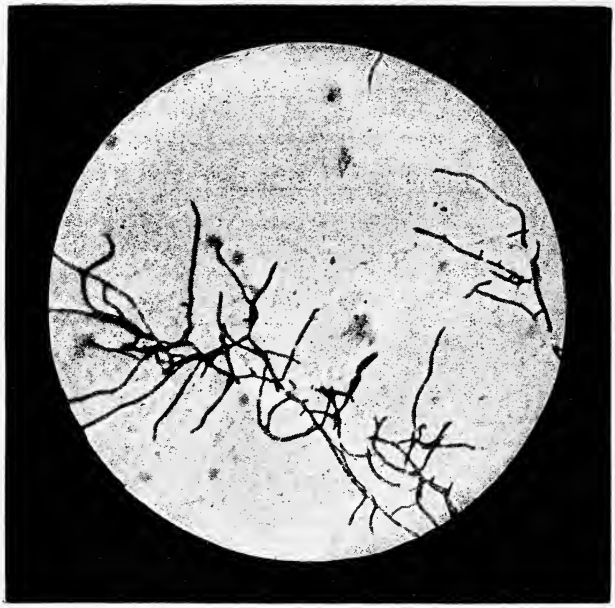

Photo-micrograph of Cladothrix Dichotoma with pseudo-branching filaments, with well-marked tranverse divisions. × 1000.

inorganic colouring matter are suspended in a fluid medium and examined under the microscope.

The simplest form of division or multiplication met with in bacteria is that known as vegetative multiplication, in which, taking as an example a short rod-shaped bacillus, there is first an increase in the length of the rod, then a zone of constriction in the middle of this lengthened rod, and then

division, complete or partial, of this lengthened rod into two shorter daughter-cells. Under suitable conditions these two again increase in length, each again is divided into two, and so regular chains or swarms of bacteria are developed. The first sign of division is a delicate transverse mark or line across the middle of the bacterium in a plane at right angles to the longitudinal axis of the cell.

By staining, as De Bary recommends, with an alcoholic solution of iodine and causing the young protoplasm to retract, it may be made out that this line is due to the ingrowth of the cell membrane from the periphery towards the centre so as to form a septum, more or less complete, between the little rods of protoplasm, which are thus gradually cut off from one another by the constricting, and growing in, cell membrane.

In the rod-shaped bacteria this division takes place in one plane only—at right angles to the longitudinal axis of the rod—and when it is imperfect or incomplete it gives rise to chain-bacteria or Strepto-bacteria.[1]

The individual bacteria of which the chain consists are held together in series by the constricted but incompletely divided membranous portion.

Similar divisions take place in the rounded bacteria or micrococci, and we have then chain-cocci or strepto-cocci formed, but instead of going on to form or remaining in long chains, they may be arranged in pairs, and are then spoken of as diplococci. There is sometimes division taking place in two dimensions of space, the one at right angles to the other, a good example of which is seen in the Bacterium Merismopœdioides described by Zopf, in which the lines of divisions are placed at right angles to one another, but in one surface plane, *i.e.*, at the surface of a fluid. In other cases there appear to be division, multiplication, and growth in three directions. To obtain an idea of what occurs, suppose that a two-inch cube is divided into single cubes, each one inch in diameter : and that these single-inch cubes then grow until each reaches the size of the original two-inch cube, when it, in turn, is again divided into one-

[1] A long spiral may divide into short curved rods, as in the case of the Cholera bacillus. In Cladothrix, where there is a false branching, the terminal cell before branching, instead of dividing transversely divides vertically, and then the division again goes on transversely.

inch cubes. This mode of division was first demonstrated by Goodsir in the Sarcina ventriculi.

We have already seen that these minute organisms are endowed with great power of movement and locomotion, a fact on which stress has been laid in connection with their rapid diffusion through fluids; but an equally important factor in the bringing about of this rapid diffusion is their extreme prolificness. If they can obtain sufficient food, and if the food is of exactly the right nature, the rate at which bacteria grow is marvellous. From actual experiment it has been found that if in a cubic centimetre of any specimen of water, we find, say, a couple of hundred organisms; on standing for twenty-four hours the number will have risen to about 5,000 per c. c.; at the end of another twenty-four hours, to 20,000; and on the fourth day they are uncountable. Cohn calculated that a single germ could produce, by simple fission, as above described, two of its kind in one hour; in the second hour these would be multiplied to four, and in three day they would, if their surroundings were ideally favourable, form a mass which can scarcely be reckoned in numbers—or if reckoned, could scarcely be imagined—4,772 billions. If we reduce this number to weight, we find that the mass arising from this single germ would, in three days, weigh no less than 7,500 tons. Fortunately for us, they can seldom get food enough to carry on this appalling rate of development, and a great number die both for want of food, and because of the presence of other conditions unfavourable to their existence. Vegetative multiplication only takes place when the conditions are extremely favourable to the growth of the organism. If nutrition is interfered with in any way, or if the removal of excretory products is obstructed, or if there be a large amount of oxygen present marked changes may at once be observed in the appearance of the protoplasm of the micro-organism. It becomes granular, then a small bright point appears in each cell; this point gradually increases in size until its diameter may be greater than that of the original organism. This large, clear, rounded, ovoid, or rod-shaped node is known as a spore, or resting-spore. It is really the seed or egg formed by the bacterium, by which the species may be continued although the parent should perish. The shape varies slightly in different species, but in every case it has a dark limiting out-

line ; it is devoid of colour, and is highly refractile. The dark outline of the spore is usually surrounded by a pale, soft, gelatinous envelope, the substance of which may, in some cases, be accumulated in rather larger quantity near the two poles of the refractile body. As soon as these glistening

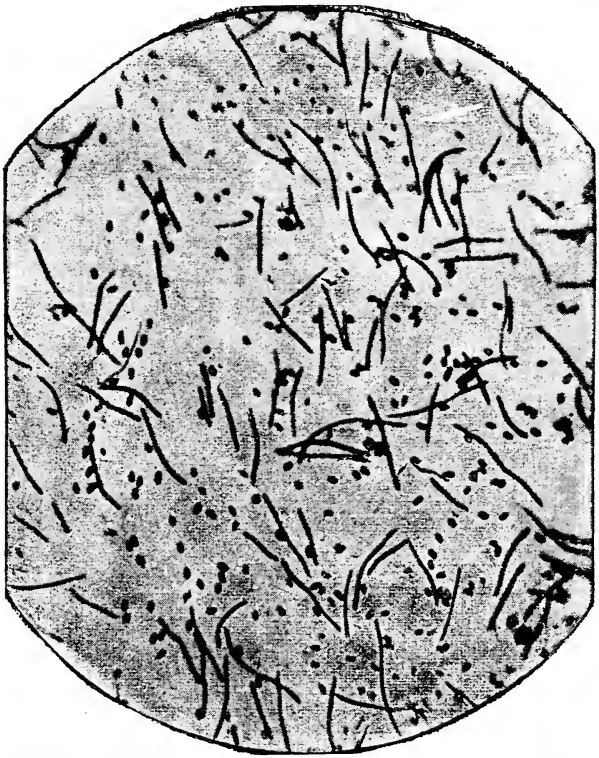

Photo-micrograph of Bacillus subtilis, with well-developed free endospores. Those in focus are seen to have clear centre and dark outline. × 1000.

bodies make their appearance, degeneration of the protoplasm of the bacteria in which they are found invariably follows, but the period at which the death of the protoplasm actually takes place varies in different cases. Where the spores are small they may lie for some time embedded in the protoplasm

of the cell, which, only as it degenerates or dies, leaves the resting-spore free to be carried about from place to place by currents of air or water, to be developed when the conditions of moisture, temperature, and food supply again become sufficiently favourable. Where the diameter of the spore exceeds that of the bacterium, it may be situated in the centre, giving rise to a spindle-shaped organism, or it may be at one end, when the organism becomes clubbed or pendulum-shaped. The spore in this case appears to escape more readily, and before the complete disintegration of the bacterium has taken place ; the side or end of the swollen organism gives way and allows of the spore being set free. This is only one kind of spore formation, and is spoken of by De Bary as endospore formation. It is met with in some varieties of vibriones, in many of the bacilli and in certain of the spirilla (Cornil and Babes) ; whilst Zopf describes similar spore formation as occurring in certain micrococci, and Escherich points out that he obtained undoubted spores in sarcina pulmonum, *i.e.*, bodies that admitted of double staining.

A second kind of spore, to which is given the name of Arthrospore, is also described by De Bary, Hueppe and others. In this there is a combination of spore formation and of fission ; the mother-cell undergoes division into a series of daughter-cells, a few of which differ from the rest in very important and essential points. There appear to be two kinds of arthrospores ; one form, met with in Leuconostoc, for example, where simple vegetative division of small round bacteria goes on regularly, so long as the conditions are favourable, and a regular chain is formed. In this chain there appear at intervals, micrococci, which differ from the remainder of the elements of the chain in the following points : As soon as the conditions of nutrition are altered they do not, like the other parts of the chain, die off, but they become "somewhat larger than the rest, acquire a more distinct outline, become thicker-walled, and their protoplasm grows darker. Eventually they become free by the deliquescence of the gelatinous envelopes, and may claim the name of spores, because, when placed in the fresh nutrient solution, they develop into new rows of beads like those of the mother plant." Here is a body which has most of the characteristics of the resting-spore or seed, but it is not formed within the protoplasm of the vegetative organism,

but by a process of fission, and as a result of the vegetative division of the organism. It is quite possible, however, that there is just as much differentiation of the protoplasm in this case, as there is where the spore is formed within the cell; the only distinction being that the separation between the spore and the vegetative element of the chain takes place at an earlier stage, and more completely than in endosporous reproduction. The reverse takes place, however, in the Bacterium Zopfii, which during the vegetative or fission stage consists of short rods, then of motionless filaments, and, if the temperature be lowered from 30° C. to 25° C. of short motile rods. As soon, however, as the conditions become unfavourable, especially when the nutrient material in which they are growing is exhausted, the rods, apparently, by a simple process of fission are divided up into short roundish cells, which retain their vitality for a considerable time, and which, when again placed under favourable conditions, act as spores, *i.e.*, they develop into the original characteristic rod-shaped bacteria.

In organisms in which spores are not found, the conditions for their existence and propagation must always remain favourable or they die out very rapidly, having no specially resistant phase to enable them to tide over their period of adversity; and had we to deal with asporous organisms only, they could, probably, by an organized attack, be rapidly and completely exterminated. The vegetative organisms, as distinguished from their spores, cannot survive the prolonged action of heat;—a comparatively low temperature, 60° C. or less, being usually sufficient to kill them, whilst the weaker chemical germicidal reagents are quite sufficient to render them altogether innocuous and inactive. The spores, on the other hand, can withstand the action of a temperature anything short of 100° C. for a considerable length of time. Cold and dessication have no effect upon them, for after being submitted to any of these, spores will, if placed under suitable conditions, develop into the more characteristic vegetative forms.

It must be borne in mind, however, that certain organisms may, by careful acclimatization, be accustomed to exist and even develop at extreme temperatures. Globig has shown that certain bacteria found in the soil can only grow at a temperature of 50° C. or more, and that they can flourish up to

70° C., whilst certain forms of "Light" bacilli can, according to Fischer, grow luxuriantly at a temperature of 0° C.

It should be remembered, however, that arthrospores are much less resistant to all germicidal reagents than are endospores; indeed, they are able to withstand a temperature only about as high as that at which the vegetative forms succumb; whilst some of the endospores are capable of withstanding dry heat of 105°, 110°, and even 130° C. They are certainly more resistant to the action of some of the weaker germicidal reagents, but they withstand for a short time only, or not at all, the action of the more powerful ones.

From the nature of the dense membrane that surrounds these spores, their staining has always been a matter of extreme difficulty. They stood out most distinctly as clear spaces in the deeply stained protoplasm of the cell, but could not be stained. By subjecting the spore-containing organisms to the action of dry heat at 110° C. for half an hour or an hour, as suggested by Buchner, or by exposing them to the action of concentrated sulphuric acid for fifteen seconds, or to a longer treatment with concentrated caustic potash, the membrane is so altered that the staining reagents are enabled to penetrate into the substance of the spore and act on its protoplasm, and impart to it a characteristic colour. If this heating be excessive the protoplasm of the bacillus may be destroyed when it in turn refuses to take on the stain, although the spore itself may, under these circumstances, be stained most beautifully, this fact also indicating that the spore is more resistant to the action of heat than is the vegetative cell. Hueppe, Babes, and Neisser have all described arthrospores as making their appearance at the end of Koch's cholera bacillus, which may become free, says Hueppe, and from which, he thinks, he has seen the bacillus being developed.[1]

[1] In order to stain endospores, the best fluid to use is probably Ehrlich's aniline water fuchsin solution. Sections are left in this for several days, they are then decolorised with 25% solution of nitric acid, washed thoroughly in water and alcohol, to which a trace of ammonia has been added; a contrast stain is obtained by treating for a few minutes with a dilute solution of methylene blue. In some cases the acid removes the fuchsin from the spores also; it is then well to wash simply with alchohol and stain with methylene blue as a contrast stain. Instead of leaving cover glass preparations for so long a period in the fuchsin they may be heated along

These so-called arthrospores of the cholera bacillus do not, however, give the ordinary reactions of spores, nor are they any more resistant to the action of heat and germicidal agents than the vegetative forms themselves: they must therefore still be looked upon as pseudo-spores. True spores, then, appear to be special protoplasmic cells, which are first developed in the mother cells and are then surrounded by a very thin, but hard and dense membrane. It is this dense covering that protects the delicate protoplasm within, against the action of the numerous destructive influences to which the spore is exposed.

If this structure be borne in mind, it becomes evident that dry heat must necessarily be less efficacious than moist, in determining the destruction of the spore. Dry heat causes no swelling of the protoplasm within, whilst moist heat causing swelling brings about rupture of the softened membrane by pressure from within, and the unprotected protoplasm, exposed under most unfavourable conditions, is at once rendered inert. It appears probable that this process of expansion from within also comes into play whenever spores are placed in conditions favourable to their development, *i.e.*, when they are placed in a warm medium in which are present both nutriment and moisture.

The first change then noticed is that the clear, strongly refractile protoplasm becomes cloudy or granular, the dark outline is not quite so prominent and the clear boundary line or limiting membrane appears to swell somewhat, and the spores gradually assume the form of an ordinary vegetative cell. In some cases, however, as soon as the spore begins to swell, the delicate outer sheath may be seen to split either longitudinally as in the Bacillus amylobacter, or across the middle as in Bacillus subtilis. In either case there is a swelling of the softened gelatinous layer which causes the removal of the firm membrane. This thin, firm, delicate membrane may come away in the form of two separate cups, or there may be simply a transverse slit through which the germinating spore escapes. Having once made its way from the membrane, vegetative division at once sets in, the segmentation always taking place at

with the fluid almost to boiling point for ten or fifteen minutes. The after procedure is as above. The spores most difficult to stain are those of the bacilli of tuberculosis, and of typhoid fever.

right angles to the long axis of the young organism, as has already been described. It was at one time thought that in the case of Bacillus subtilis division at first went on longitudinally, but it has now been demonstrated that the general rule is not departed from, the well-known appearance being due to the fact that after rupture of the membrane the two halves remaining attached by a kind of hinge are thrown outwards, the ends of the vegetative spore protoplasm remaining within these little cups, and the body growing rapidly before division takes place ; a kind of loop is thus formed which gradually becomes longer and longer, and the two limbs which were supposed to be the result of a longitudinal division are nothing more than the two ends of the same rod. After a time transverse divisions may be seen in the different parts of the loop, the ends escape from the cups formed by the halves of the opened-up spore capsule, the rods straighten out and assume the regular straight form. There are other modifications of the same process of development from the spore, but the above are the essential or most important forms.

Classification.

Our present classification of Bacteria is based upon that given by Cohn. He arranged these organisms into four groups, taking as his characterising feature the form that was most commonly assumed by each organism. His first group consisted of rounded bacteria or cocci—the Sphæro-bacteria ; the second group was made up of short rods, or cylinder-shaped bacteria—Micro-bacteria ; longer rods or thread-like organisms—the Desmo-bacteria—were placed in the third group ; and to the fourth group were assigned screw-shaped or spiral bacteria—the Spiro-bacteria. According to this author the cocci consist of rounded or ellipsoid bodies, .5 to 2μ [1] in diameter, the smaller ones being spoken of as Micrococci, the larger as Mega- or Macro-cocci. They are found singly, or may be grouped in pairs or in longer chains. The micro-bacteria or short rods are more variable in their size, measuring 1μ in diameter and a little more than that in length ; but when the length of an organism reaches more

[1] 1μ = 0.001 mm. = $\frac{1}{1000000}$th part of a metre = $\frac{1}{25000}$th part of an inch.

than about twice its diameter, it is usually spoken of as a Bacillus :—the length of a bacillus may be eight or ten times the diameter, which ranges from 1μ to 2μ. In some cases these bacteria take the shape of short stout spindles or lemons, especially during the stage of spore formation, when they are

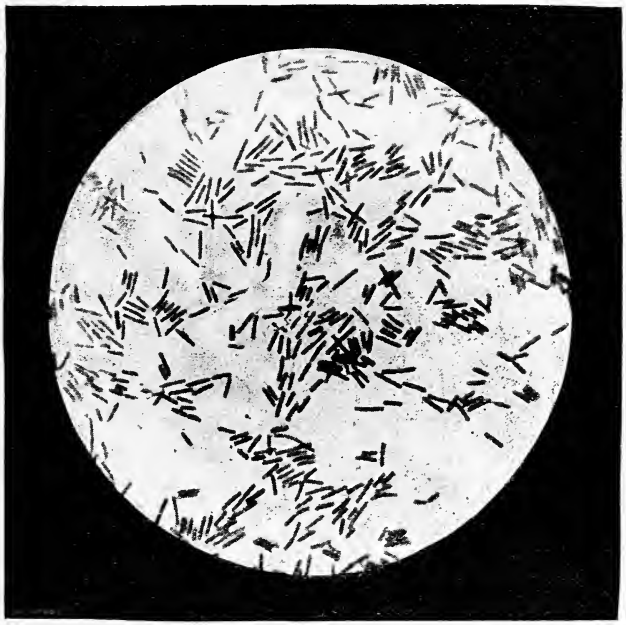

Photo-micrograph of Proteus Vulgaris Bacillus, in the form of short rods. × 1000.

spoken of as Clostridium forms, of which an exceedingly good example is the spore-bearing Bacillus butyricus.

Bacilli increase in length, and, becoming more or less jointed as vegetative division takes place, form the long delicate jointed threads which are spoken of as Leptothrix forms when there is no apparent branching, but if pseudo-ramifications, such as are found in Cladothrix dichotoma and are due to vertical division taking place in one of the terminal cells, are present, we have the Cladothrix form.

The Spiro-bacteria vary very much, not only in size but also in length and in general appearance.

In some instances other isolated features have determined a nomenclature. For example, the appearance of sulphur in one series of forms has determined a species of Ophidomonades; whilst the form and length of curves have also been used as a distinguishing feature. The organism is spoken of as a Vibrio when the curves are slightly marked ; if the curves

Photo-micrograph of Bacillus Figuraus in which Leptothrix form is well seen. × 150.

are short and slightly pronounced and the organism is thin, it is known as a Spirochæta ; a ribbon-shaped spiral is a Spiromonad, and a spindle-shaped spiral, a Spirulina.

COHN'S CLASSIFICATION.

Schizophytes, Thallophytes that develop by division or by endogenous germinating cells.

TRIBE 1.

A. Free cells arranged in pairs or fours.
Cells round—chroococcus (Naegeli).
Cells cylindrical—synechococcus (Naegeli).
B. Cells united into zooglœa masses by homogeneous gelatinous substance.
a. Cellular membrane shading off into the intercellular substance.
Cells round—micrœcoccus (Hallier).
Cells cylindrical—bacterium (Dujardin).
b. Intercellular substance arranged in concentric layers.
Cells round—glæocapsa.
Cells cylindrical—glæothece.
C. Cells forming circumscribed zooglœa masses with a definite shape.
a. Families arranged in flat layers in a single plane—merismopedia.
b. Cells rounded, arranged in a zooglœa mass forming a network—clathrocystis.
c. Cells cylindrical or wedge-shaped, the families divided by constrictions—cœlosphærium.
d. Cells forming families, dividing in several planes, colourless cubical cells with a quadrate arrangement—sarcina.
e. A large and indefinite number of colourless cells—ascococcus.

TRIBE 2.

Filamentus forms in which the cells are thread-shaped.
A. Without branching.
1. Colourless cylinders with little sign of division, very delicate, when short—bacillus, when long—leptothrix.
2. Similar filaments, but thicker and longer—beggiatoa.
3. Filaments deeply divided at intervals with colourless spore-bearing tissue and a well developed sheath—crenothrix.
4. Spiral filaments.
Short and undulating—vibrio.
Short with rigid spirals—spirillum.
Long with flexible spirals and containing phycochrome—spirochæte.
Filaments long and spirals flexible—spirulina.
5. Filaments in chains without phycochrome—streptococcus (streptobacteria).
6. Zooglœa masses or cylindrical cells.
When colourless—myconostoc.
In chains—nostoc.
Filaments thinner at one end—rivolaria.
B. Filaments with pseudo ramifications—cladothrix.
Cylindrical colourless filaments—streptothrix.

It may be of interest to some to glance over a few of the classifications suggested by different authors, and to compare the bases on which these classifications are made. Cohn, as we have seen, classifies entirely according to the elementary form of the organism, the nature of the membrane and the mode of division. Van Tieghem founded his classification

on much the same features, but he also takes into consideration some of the physiological and biological characters, the nature of the processes set up by them, the resulting products and the nature of the division.

VAN TIEGHEM'S CLASSIFICATION.

Van Tieghem places all the schizomycetes in a family which

Photo-micrograph of Bacillus Figuraus Leptothrix and rod forms. × 1000.

he calls bacteria, a family closely related to the nostocaceæ and the oscillaria of the Algæ; he divides them into micrococci; bacteria or short rods; bacilli or short threads; longer threads, without any definite sheath, being called leptothrix; with a sheath, crenothrix; with a sheath and undergoing division, cladothrix.

The genus vibrio consists of spiral filaments which break up into short fragments.

The spirillum consists of longer filaments that have a helicoid arrangement.

The spirochæte, of greater length, are spirilla with more numerous turns of the spiral.

Micrococci, arranged in zooglœa masses and held together by a thick layer of gelatinous material, he called ascococcus; when held together, but without or with less of this gelatinous material, he gave them the name of punctula.

Bacteria united by this gelatinous material are called ascobacteria ; without the gelatinous envelope, polybacteria ; bacteria in the form of spiral threads, and massed together, form the myconostoc.

These bacteria are again divided according to their properties of forming colouring matter, chromogenes ; of setting up fermentation, the zymogenes ; and of giving rise to disease in animals or plants, the pathogenes.

He gave a further classification based on the mode of division of the primary cells :

1. The bacteria in which the division takes place in one axis only. This includes micrococcus, bacterium, bacillus, leptothrix, crenothrix, cladothrix, vibrio, spirillum, spirochæte, ascococcus, punctula, ascobacteria, polybacteria, and myconostoc.

2. The meristæ, in which a membranous thallus divides in two directions but on one plane only, giving rise to the formation of characteristic tetrads.

3. The sarcinæ, in which there is division in three directions, so that the resulting masses always remain cubical in form.

Zopf's classification rests (Flügge, "Etiology of Infective Diseases," p. 180) on the doctrine of pleomorphism, which cannot be accepted as in any way proved except in the case of a few well-known non-pathogenic forms ; but his classification may be accepted as a basis from which to work in bringing proof or disproof of the theory of pleomorphism, although it is not at present, at any rate, founded on a large number of facts.

Zopf, who has studied most carefully the subject of pleomorphism, holds that several forms may occur in the cycle

of development of any species, and he has determined that both form and developmental series must be used in drawing up a classification.

ZOPF'S CLASSIFICATION.

Group 1.—COCCACEÆ.—These are as yet only known in the coccus form. To these the following genera belong:

1. *Streptococcus* (cocci arranged in threads like strings of beads).
2. *Merismopedia*, tablet cocci (division in two directions, leading to the formation of tablet-like flat layers of cells).
3. *Sarcina*, packet cocci (division in three directions, leading to the formation of bale-like colonies).
4. *Micrococcus* (the cocci become aggregated in irregular heaps).
5. And *ascococcus* (the heaps of cocci accompanied by marked formation of gelatinous material).

Group 2.—BACTERIACEÆ.—These possess chiefly coccus, rod, and thread forms; the former may be absent; in the latter there is no distinction between base and apex. Threads straight or screw-like. Genera:

1. *Bacterium*, forms cocci and rods, or only rods which are arranged in rows to form ordinary threads; spore formation absent or unknown.
2. *Spirillum*, threads screw-like, formed only of rods, or of rods and cocci; spore formation absent or unknown.
3. *Vibro*, threads screw-like, spore formation in the longer or shorter joints
4. *Leuconostoc*, forms cocci and rods, spore formation in cocci.
5. *Bacillus*, cocci and rods, or only the latter in the form of simple or twisted threads; spore formation present.
6. *Clostridium*, the bacillus form in which the spore formation occurs in peculiar enlarged rods.

Group 3.—LEPTOTHRICHEÆ.—Coccal, rod, and thread forms; the latter show a distinction between base and apex; threads straight or screw-like, spore formation not demonstrated. Genera:

1. *Crenothrix*, threads jointed and enclosed in a sheath, cells contain no sulphur granules; inhabit water.
2. *Beggiatoa*, threads thicker than Crenothrix, indistinctly articulated, cells contain sulphur granules; inhabitants of water.
3. *Phragmidiothrix*, threads without sheaths, successive divisions very numerous; cells contain no sulphur; inhabit water.
4. *Leptothrix*, threads with or without sheaths, divisions not very numerous or well marked; cells devoid of sulphur.

Group 4.—CLADOTHRICHEÆ.—Show coccus, rod, thread, and spirillar forms. The thread form is provided with a sheath, well-marked segments and pseudo-branches. Spore formation not yet demonstrated. Genus: Cladothrix.

Winter and Rabenhorst's classification, though very convenient, is far from scientific, but it serves especially well for the classification of micro-organisms that are found in disease, as these, as met with in their host, usually correspond to one stage only of Zopf's developmental cycle of any special organism.

DE BARY AND HUEPPE'S CLASSIFICATION.

Coccus Forms during the Vegetative Stage.

Single cocci arranged in chains.	Zoogloœa masses of medium size.	With endospores	Endostreptococcus.
		Without „	Arthrostreptococcus.
	Ditto, very large		Leuconostoc.
Arranged in fours or in short chains.	Without endospores (?) / Only arthrospores (?)	... ...	Merista.
Arranged in fours or eights, but not in chains.	With or without endospores		Sarcina.
Arranged in irregular masses.	Without any definite arrangement		Micrococcus.
	Like bunches of grapes		Staphylococcus.
	Arranged in rounded zoogloœa masses		Ascococcus.

Rod-shaped Forms during the Vegetative Stages.

As ribbons or single cells, shorter or longer chains or threads, in which there is no difference in appearance or structure between the two ends or between base and point. Single cells or threads, flexile or stiff.	Threads straight or wavy, no endospores, containing arthrospores ...		Bacterium.
	Threads straight, wavy, or spiral; no endospores, but with arthrospores		Spirulina (Proteus).
	Threads straight or wavy, with endospores.	No alteration of the straight rods during the process of spore formation... ...	Bacillus.
		Spindle-shaped or the process of spore-formation, accompanied by alteration of the straight rods	Clostridium.
No threads, spindle-shaped rods, undergoing longitudinal division, containing endospores			Pasteuria.
Threads with differentiation between base and point.	Threads without divisions or septa.	No deposition of sulphur granules ...	Leptothrix.
		Containing sulphur granules	Beggiatoa.
	Threads with divisions.	Unbranched	Crenothrix.
		Branched	Cladothrix.

Spiral Forms during the Vegetative Stage.

As ribbons or single cells; spiral threads; cells and threads flexible or rigid.	No endospores, but forming arthrospores		Spirochæte.
	With endospores.	No alteration in the form of the cell during spore formation	Spirillum.
		With alteration of the shape of the cell during the process of spore formation...	Vibrio.

FLÜGGE'S MODIFICATION OF COHN'S CLASSIFICATION.

- **Cells spherical or egg-shaped.**
  - Cells isolated, or united in chains or in amorphous gelatinous masses ... ... — **Micrococcus.**
  - Cells united in definitely circumscribed families.
    - Colonies solid, entirely filled with cells.
      - Cells united in large and indefinite numbers to form irregular colonies ... ... — **Ascococcus.**
      - Cells bound together in small but definite numbers to form regular groups ... ... — **Sarcina.**
    - Colonies with a simple layer of cells at periphery ... ... — **Clathrocystis (Cohnia).**

- **Cells cylindrical.**
  - Cells short cylinders, single or grouped together to form a few loosely-united or gelatinous families ... ... — **Bacterium.**
  - Cells longer, cylindrical, united to form threads.
    - Threads isolated, or matted together, or in bundles.
      - Threads unbranched.
        - Threads straight.
          - Threads short, distinctly segmented ... ... — **Bacillus.**
          - Threads long, segments not distinct.
            - Threads very thin — **Leptothrix.**
            - Threads thicker... — **Beggiatoa.**
        - Threads wavy or spiral.
          - Short, rigid ... — **Spirillum (vibrio).**
          - Long, flexile ... — **Spirochæte.**
      - Threads with false branch formation ... ... — **Streptothrix / Cladothrix.**
    - Threads enclosed in roundish gelatinous masses ... ... — **Myconostoc.**

Van Tieghem and De Bary, followed by Hueppe, laying great stress on the method of reproduction, have made two great divisions of bacteria—those which form endospores and those which form arthrospores; but as Flügge points out, this division is of little service for practical purposes, as spore formation is at present so imperfectly understood, that it is most difficult to follow the exact method of sporogenous reproduction in a Schizophyte. Winter and Flügge attempt to get rid of their objection to Cohn's classification by specially modifying it to contain only the pathogenic organisms. They take no account, as already stated, of any morphological developmental cycle or of any biological features, but they give a useful and practical classification.

Quite recently it has been suggested that bacteria should be classified according to the number and arrangement of the flagella developed, but as such classification is necessarily based on a single characteristic, and that one of the least important, and as it will be some time before a complete examination can be made in order to determine the number and arrangement of these flagella, such a classification may for the present be left out of consideration. The same holds good as regards Baumgarten's classification; he divides bacteria into two groups—those which appear to assume a single form only (the Monomorphic) and those in which pleomorphism is a well-developed characteristic. In each he has three genera. In the first: the coccus, the bacillus and the spirillum; and in the second: the spirulina, the leptothrix and the cladothrix. A similar remark applies to these, however, that applies to Zopf's classification. It may be of some service as a provisional classification, especially to those who are engaged in the study of the morphology and life history of bacteria. To the pathologist, however, these classifications are of comparatively little value except in so far as by their aid the morphologist is able to supply him with information as to the life history of bacteria outside the body.

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CHAPTER III.

The History of Bacteriology.

Earliest Workers—Kircher's *Contagium Animatum*—Bacteria in Fermentation, Putrefaction and Disease—Early Classifications—Müller—Abiogenesis — Needham — Biogenesis — Bonnet — Spallanzani — Schultz's Experiments — Schwann — Later Experiments — Pasteur — Bastian—Colour and Fermentation—Cohn and Naegeli's Classification—Henle's Researches and Postulates—Pasteur's Researches on Fermentation and Putrefaction—"Flower of Wine"—"Flower of Vinegar"—Bacteria as Scavengers—Pasteur on Silkworm Disease, and Wine Disease—Germs killed by Carbolic acid—Origin of Antiseptic Treatment.

Since Athanasius Kircher mistook blood and pus corpuscles for small worms, and built up on his mistake a new theory of disease and putrefaction, and since Christian Lange, the professor of Pathological Anatomy in Leipzig, in the preface to Kircher's book (1671) expressed his opinion that the purpura of lying-in-women, measles, and other fevers were the result of putrefaction caused by worms or animalculæ, a "Pathologia Animata" has, from time to time, been put forward to explain the causation of disease. Crude as was his theory, and imperfect as were the observations on which it was based, it is marvellous that Kircher, with the simple lenses that he had at his disposal, magnifying only some thirty-two diameters or one thousand times, was able to make out as much as he did. The observations that he made were, naturally enough, not generally credited; and the theories he formulated were received with chilling incredulity by most of his contemporaries.

Remarkable as were Kircher's observations, still more wonderful were those of Anthony van Leeuwenhoek, a native of Delft in Holland, who in his youth had learned the art of polishing lenses, and who was able, ultimately, to produce the first really good microscope that had yet been constructed. Not only did Leeuwenhoek make his microscope, but he used it to such good purpose that he was able

to place before the Royal Society of London a series of most interesting and valuable letters giving the result of his researches on minute specks of living protoplasm. The results of these observations are fully detailed in his collected works.

Towards the end of 1675 he discovered in water, in an infusion of pepper, in the intestinal canals of horses, flies, frogs, pigeons, fowls, and even in his own diarrhœa stools, small moving and living forms of such extreme minuteness that other observers with the best apparatus they had at command, even with the accurate and lucid description he gave, could not for long confirm his results. It was not, however, until 1683 that he actually described and depicted minute organisms in material taken from the teeth, that we can at present recognize from his descriptions and drawings as bacteria. Describing them, he says: "I saw with very great astonishment, especially in the material mentioned, that there were many extremely small animals which moved about in a most amusing fashion; the largest of these" (represented by him in an admirable figure) "showed the liveliest and most active motion, moving through rain-water or saliva like a fish of prey darts through the water; this form, though few in actual numbers, was met with everywhere. A second form moved round, often in a circle, or in a kind of curve; these were present in greater numbers. The form of a third kind I could not distinguish clearly; sometimes it appeared oblong, sometimes quite round. They were very tiny, in addition to which they moved forward so rapidly that they tore through one another: they presented an appearance like a swarm of midges and flies buzzing in and out between one another. I had the impression that I saw several thousands in a single drop of water or saliva which was mixed with a small part of the above-named material not larger than a grain of sand, even when nine parts of water or saliva were added to one part of the material taken from the incisor or molar teeth. Further examination of the material showed that out of a large number which were very different in length, all were of the same thickness. Some were curved, some straight, lying irregularly and interlaced." Since, he says, "I had seen minute living animalculæ of the same shape in water, I endeavoured most carefully to observe whether

these also were living or not, but I was unable to recognize even the slightest movement as a sign of life."

In the material taken from the teeth of an old man who never cleaned his teeth, Leeuwenhoek found an inconceivable number of living animalculæ which darted about more quickly than any he had ever seen before; "the largest were present in very great numbers, and waved about by the locomotion of their bodies. Besides these, other animalculæ were present in such large numbers that the whole water seemed to be alive."

This admirable account really contains the first accurate description of the rod-shaped bacteria, motile and motionless, of longer threads or bacilli, of the spiral threads or spirilla, and of rounded micro-organisms or micrococci. It was considerably improved upon in a letter to the Royal Society, dated October 1, 1692, in which he speaks of small rounded animalculæ, the diameter of which is a thousand times less than that of a fine grain of sand; of organisms having a somewhat greater diameter than the round ones, and being five or six times as long as they were broad, equally thick throughout their whole length, and which moved slowly backwards and forwards through a bending of their bodies. Along with these he describes what are evidently spirilla, with their characteristic movements, "a few organisms about the same length or slightly longer, which moved their bodies in comparatively marked curves, swam forwards or backwards, or twisted themselves in an extremely lively fashion." He also observed still longer and more sluggish organisms, sometimes straight and sometimes bent.

Although Leeuwenhoek did not attempt to theorize as to the meaning of the presence of these organisms in the mouth, we find that, in 1713, after finding similar organisms in the greenish pellicle formed on the surface of the water in an aquarium, he came to the conclusion that the organisms seen on the teeth found their way into the mouth through the medium of the drinking-water that had been stored in barrels, and that some of these found there a nidus in which they might multiply.

The world that Leeuwenhoek thus opened up so thoroughly was rapidly invaded by other observers and theorists. The thoughtful physicians of the time believed that at last they had found the *fons et origo mali*, and Nicolas Andry, reviewing Kircher's "Contagium Animatum," replaced his worms by these newly-described

animalculæ or germs, and pushing the theory to its legitimate and logical conclusion, he also evolved a germ theory of putrefaction and fermentation. He maintained that air, water, vinegar, fermenting wine, old beer, and sour milk, were all full of germs; that the blood and pustules of smallpox also contained them, and that other diseases, very rife about this period, were the result of the activity of these organisms. Such headway did he make, and such conviction did his arguments carry with them, that the mercurial treatment much in vogue at that time was actually based on the supposition that these organisms, the *causæ causantes* of disease, were killed by the action of mercury and mercurial salts.

With a kind of prophetic instinct, and certainly as the result of keen observation, Varro and Lancisi ascribed the dangerous character of marsh or swamp air to the action of invisible animalculæ; in fact the theory was so freely and forcibly propagated that even where no micro-organisms could be found their presence was inferred, with the inevitable result, as Löffler points out, that these "inconceivable" worms became the legitimate butts for the shafts of ridicule; and in 1726 there appeared in Paris a satirical work, in which these small organisms received the name of "fainter," "body-pincher," "ulcerator," "weeping fistula," "sensualist;" the whole system was thus laughingly held up to satire, and the germ theory of disease completely discredited. Linnæus, however, with his wonderful powers of observation and deduction, considered that it was possible that there might be rescued from this "chaos" small living beings which were as yet insufficiently separated and examined, but in which he firmly believed might lie not only the actual contagium of certain eruptive diseases, and of acute fevers, but also the exciting causes of both fermentation and putrefaction.

The man, however, who of all workers earliest recognized the importance of Linnæus' observations was a Viennese doctor, Marcus Antonius Plenciz, who with great shrewdness recognized the prime importance of these organisms in connection with the etiology not only of contagious diseases, but also of putrefaction. He it was who, at this time, insisted upon the specific character of the infective agent in every case of disease: for scarlet fever there was a

scarlet fever seed or germ—a seed which could never give rise to smallpox. He showed that it was possible for this organism to become disseminated through the air, and for it to multiply in the body; and he explained the incubation stage of a febrile disease as dependent on the growth of a germ within the body during the period after its introduction, when its presence had not yet been made manifest. He very rationally explained the differences in the character of the symptoms and the severity of the same disease by referring them to differences in the constitutions and surroundings of the patients. As regards putrefaction, having corroborated Linnæus' observations and found countless animalculæ in putrefying matter, he came to the conclusion that this process was the result of the development, multiplication, and carrying on of the functions of nutrition and excretion by these germs; the products of fermentation being the volatile salts set free by the organisms, which, multiplying rapidly by forming seeds or eggs, rendered the fluid in which they developed thick, turbid, and foul. This theory, admirable as it was, and accurate as it has since been proved to be, could not then be based on any very extensive or detailed observation, and we find that some of the most prominent and brilliant men of the period did not feel justified in accepting the explanation that Plenciz had offered as to the causes of disease and fermentation processes; and it was not until the years following 1831 that any real advance could be made in our knowledge of the presence of a "Contagium vivum," or living contagium element in the production of disease and fermentation. Previous to this, however, there was being gradually accumulated a large mass of facts bearing on these wonderfully interesting minute living organisms, and numerous isolated observations were constantly being made by various workers, none of whom, however, were sufficiently master of their subject to enable them to make any systematic attempt at classification of their accumulated facts, and the scientific results were consequently comparatively small, standing in no proportion to the amount of work expended and the number of observations made.

The first attempt to reduce this chaos to something like order was made by Otto Friederich Müller, of Copenhagen. He thoroughly appreciated the work he was taking in hand.

With a well-defined plan he set himself to systematize and arrange the various organisms that had been described by previous observers—commencing with Leeuwenhoek and ending with Spallanzani.

When the nature of the optical apparatus Müller had at his disposal is taken into consideration, it must be acknowledged that he succeeded in a most marvellous manner in classifying, on the Linnæan system, the minute organisms with which he had to deal. Under the head of Infusoria he divided them into two classes—those that could be seen with the naked eye, and those that were invisible except with the assistance of a microscope. The latter class he again divided into Membranacea, or those forming thin surface membranes, and Crassuiscula, or those forming thick membranes; these latter, including Monas, Proteus, Volvox, Enchelys, and Vibrio, representing, he maintained, the lowest forms of animal life. Of Monas he described no fewer than ten species, and of Vibrio he was able to distinguish, by utilizing the characters of form, motion, nidus or cultivation medium, and other biological features, thirty-one species. Relying, however, principally on the form of the organism, he described rounded and slightly oval forms, shorter and longer rods, rounded and truncated cork-screw-shaped and snake-like organisms, undulating but not spiral in their movements, and also long threads or bacilli.

Although he did not fully recognize the importance of his observation he described in certain organisms little shining points, arranged in series at regular intervals, especially in the rod-shaped forms, points which we must now conclude were spores. It is certainly not remarkable that he should never have understood the full significance of these spores, as even ninety years later, with all the additional light that had then been thrown on the subject, these bodies were still not properly understood. Later observers laid stress on the rapidity of movement of the vibriones or lineolæ, which were gradually separated from the other forms of lower organisms. The vibriones then described, including lineola, rugula, bacillus, and spirillum, correspond more or less perfectly with our bacteria of the present day.

Many advances were made after Müller's work was completed as regards the morphology of these organisms, but the question as to whence these minute forms came still remained unanswered. Whether they were the result of spontaneous generation, or were the progeny of pre-existing forms, was the question which for over a century occupied the minds of those engaged in scientific research and speculation. Some observers, prominent amongst whom

were Hartsoeker, Reaumur, and Joblot, considered, though they had no great amount of evidence to adduce in support of their theory, that bacteria were the progeny of minute organisms which were present in myriads in the air, from which they were deposited on fruits, plants, and other matter, whence they made their way into the various infusions prepared from them. In this country a prophet arose in the person of Dr. Needham, who was really the first to suggest an attempted solution of the question by a theory of abiogenesis, or spontaneous generation. Needham at first thought that these vibriones, or "plant animals," as he called them, arose from plants by special vegetative power, and that from the plant-animals, by a process of evolutionary accretions, other organisms again arose. He tried to prove, by boiling a beef infusion and keeping it and allowing it to putrefy in a well-stoppered bottle (a most scientific method), that these zoophytes could not owe their origin to germs which outside insects or organisms had brought into the infusion, as he considered that the boiling should have destroyed the germs originally in the fluid, and as no new germs could, he thought, make their way into the closely-stoppered vessel, the resulting organisms must be the result of the action of a special vegetative force. This apparently logical and fascinating theory was accepted by many whose names had great weight in the scientific world. Needham's observations were repeated time after time with the same results, and his theory met with wide acceptance.

To very critical minds it appeared, however, that these experiments of Needham's left loopholes for the insertion of other explanations than those which he gave, and Bonnet, of Geneva, suggested that the vessels used by Needham were not hermetically sealed, that an almost invisible opening would be quite sufficient to serve as a means of entrance to organisms so minute as those with which he was dealing, and that on the other hand there was a possibility that the germs were so far resistant to increase of temperature that they might live through a short period (a few minutes only) of treatment with boiling water. Abbot Spallanzani followed up, by his wonderful experiments, the theoretical criticism of Bonnet. After convincing himself that organisms did actually develop in unboiled infusions even when the outer air was rigorously excluded, he argued

that the germs of micro-organisms, or eggs, as he termed them, might exist on the walls of the vessel, on the material of which the infusion was made, or suspended in the air within the vessel. To get rid of these germs from the vessels he heated the latter on the fire, then filling them rapidly with his infusions he allowed them to cool, and sealed them hermetically, but he still found that after a few days a number of organisms made their appearance. Could the organisms have got in along with the air during the process of cooling ?

To set this question at rest he made a number of infusions in hermetically-sealed flasks, and boiled them for a whole hour, with the result that in flasks so treated no organisms made their appearance : if, however, the sealing was in any way interfered with, organisms soon made their appearance, and he concluded that living germs were necessary for the development of putrefactive organisms. This fact once established, the whole question was much simplified, and the principle on which it rested was soon utilized in Paris and elsewhere in the methods adopted for insuring the preservation of various food stuffs—methods which, with few modifications, have been handed down to the present day. It was of course objected that Spallanzani had shut out air from his vessels, or, that he had so altered the constitution of the air which still remained, that it was not possible for these minute organisms to develop in it. This objection was met in 1836 by F. Schulze, who put the question to himself, "If the access of atmosphere, light, and heat, to substances in flasks included of itself all the conditions for the primary formation of animal or vegetable organisms." To prove that this was not the case, Spallanzani's conditions of absolute freedom from germs capable of development in the infusion must be obtained, and, secondly, air must be admitted to this infusion in considerable quantities ; but the air so admitted must be perfectly free from germs.

Schulze proceeded as follows : he filled a flask half full of distilled water, to which he added various animal and vegetable substances. He gives the following description of the further methods of procedure :—"I then closed it with a good cork, through which I passed two glass tubes bent at right angles, the whole being air-tight ; it was next placed in a sand bath and heated until the water boiled, and thus all parts had reached the temperature of 212° F. While the watery vapour was escaping by the glass tubes I fastened, at each end, an apparatus which chemists employ for collecting

carbonic acid gas; that on the left was filled with concentrated sulphuric acid, and the other with solution of potash. By means of the boiling heat every living organism and all germs in the flask, or in the tubes, were destroyed, and all access was cut off by the suphuric acid on the one side and by the potash on the other. I placed this easily-moved apparatus before my window, where it was exposed to the action of light, and also, as I performed my experiments during the summer, to that of heat. At the same time I placed near it an open vessel, with the same substances that had been introduced into the flask, having also subjected them to the boiling temperature. In order now to renew constantly the air within the flask I sucked with my mouth, several times a day, the open end of the apparatus filled with a solution of potash, by which process the air entered my mouth from the flask through the caustic liquid, and the atmosphere entered the flask from without through the sulphuric acid. The air was of course not at all altered in its composition by passing through the sulphuric acid in the flask, but if sufficient time was allowed for the passage the portions of living matter, or matter capable of becoming animated, were taken up by the acid and destroyed. From May 28th till the beginning of August I continued uninterruptedly the renewal of the air in the flask without being able, without the aid of a microscope, to perceive any living animal or vegetable substance, although, during the whole of the time, I made my observations almost daily on the edge of the liquid, and when at last I separated the different parts of the apparatus I could not find in the whole liquid the slightest trace of infusoria confervæ or of moulds; but all the three presented themselves in great abundance a few days after I had left the flask standing open. The vessel which I placed near the apparatus contained on the following day vibriones and monads, to which were soon added larger polygastria, infusoria, and afterwards rotatoria."

Schulze was thus able to prove that the sterility was not dependent upon any alteration in the air within the flask, or to the small quantity of air contained in it, and that it was not due to any alteration brought about in the liquid by the heating process, as on the one hand a large quantity of air was passing through the flask, whilst on the other the fluid that had been boiled, but which was left exposed, rapidly underwent decomposition, a decomposition that was accompanied by the development of micro-organisms in very large numbers. The objection that some particles of sulphuric acid drawn in with the air might affect the growth of organisms was met by Schulze by further experiments; and Schwann, who, instead of using sulphuric acid, used heat as a means of destroying any particles that might be present in the air that was drawn into the flask, corroborated Schulze's statements. Now came further objections from the supporters of abiogenesis, who stated, most definitely and categorically, that these workers were not dealing with germs at all, but simply with particles of

albuminoid matter floating in the atmosphere, as a result of the vegetative power of which, organisms of various kinds, according to the conditions by which these particles find themselves surrounded, were caused to be developed. In 1854 Schroeder and Von Dusch made a great advance; they proved that simple filtration through a layer of cotton wool was sufficient to deprive the air of its organisms, and so to render it unfit to produce decomposition in infusions from which germs had already been eliminated by heat; and no longer than thirty years ago Hoffmann, Chevreul and Pasteur demonstrated that it was quite sufficient to draw out, and bend downwards, the neck of a bottle in which the germ-free infusion was contained, in order to ensure the continuance of a non-putrefactive condition, and the perfect freedom of the fluid contained within the flask from germs: they argued that germs obey the law of gravitation, like all other solid particles, when not blown about by currents, and must settle down upon an upper surface, so that when the tube was bent downwards the organisms could not fall into the mouth. Tyndall gave demonstrative proof of this in his exquisite experiment of removing all particles from a glass chamber, first proving their entire absence by passing through the chamber a ray of light which could only be seen so long as particles remained suspended in the atmosphere. As soon as the ray of light disappeared from view he placed vegetable infusions which had been sterilized by heat within the chamber, with the result that they remained free from any trace of organic life for several weeks together. Schwann had already pointed out that blood, taken with certain precautions and introduced into a flask in which the air was kept germ free, might be preserved for a considerable length of time, without the development of micro-organisms, and later (1857) Van der Broek showed that the juice of grapes, and urine as well as blood, might be kept free from decomposition and the presence of organisms if the apparatus into which they were received was first thoroughly sterilized by means of heat, and if the substances were not allowed to come in contact with the outside air.

Numerous observers, especially Burdon Sanderson, Roberts, Lister, Chiene and Ewart, and Watson Cheyne in this country, and Rindfleisch, Klebs, Cazeneuve and Livon, Leube, Hauser and Marchand abroad, con-

firmed these observations and made many new ones in connection with the behaviour of milk, egg albumen, vegetable substances kept under certain conditions, and pieces of organs from freshly-killed animals to which organisms from the external air were not allowed to gain access.

It seemed as though the adherents of abiogenesis had not a leg left on which to stand; but owing to the fact that certain organisms, especially when contained in such media as milk and cheese, withstand the action of very considerable heat, they still contested every inch of ground, though their foothold was being gradually but surely cut away from beneath them. Milk, which was one of the strongholds of the abiogenists, was first sterilized, with absolute certainty, by Schroeder, who attained his end by subjecting the fluid for a considerable time to a temperature of 100° C., and then by Pasteur, who heated it to 110° C., for a short time only. Cheese still remained to them, and as late as 1872 Bastian placed a small piece of this substance in an infusion of white turnip which had been filtered and carefully sterilized. This was then boiled in a flask for ten minutes, and whilst still boiling was hermetically sealed; at the end of three days countless living organisms were produced, as Bastian held, from non-living albuminoid material; but Cohn, repeating the experiment, explained that the resting spores or resistant germs were enclosed in the substance of the cheese, and that they were thus able to resist the high temperature to which the outer surface of the cheese, but not its centre, was exposed. Duclaux's later experiments with the Tyrothrix of cheese, which resists the action of a very high temperature for a considerable time, also helps to explain Bastian's results. The matter has now been set at rest, and it is an accepted belief that bacteria or microbes, as these lowly organized forms are now called, may be destroyed by heat and by certain chemical reagents, and that when once destroyed in any medium, no other organisms can rise from their ashes, the medium remaining perfectly free from putrefactive changes until fresh germs are introduced from without. Harvey's famous dictum, *omne vivum ex ovo*, has thus come to have a far wider meaning than that which he originally attached to it. The triumphs of surgery, of preventive inoculation, of hygiene in relation to specific infective diseases, of preservation of food, have had their origin in the knowledge gained during the battle which waged round

the question of Spontaneous Generation or *generatio æquivoca;* and to the disciples of that school every acknowledgment must be made and due credit assigned for the attitude of scepticism and free ingenious and honest criticism which they passed concerning half-formed and inadequately-supported theories and imperfectly-conducted experiments, for to their efforts is certainly due the fact that the experiments of their opponents became more and more perfect, and if to-day we have perfect methods of sterilization and of making pure cultivations, it is because nothing was taken for granted, and because able men on both sides of the controversy were ranged against one another to fight the matter to the death.

Whilst this battle over the origin and development of these micro-organisms was going on, intermittent attempts were made to improve on the classification that had been drawn up by Müller, but it was only as the optician supplied observers with better microscopic apparatus that any further advances could be made. Ehrenberg, however, took up the question and divided the Monad family into rounded and rod-shaped forms; these latter—Vibriones—he described as undergoing transverse division, as they increased in length. These he sub-divided into Bacteria, or short, straight, inflexible organisms; Vibriones, longer and more flexible; Spirilla, or inflexible spiral forms; and the Spirochætæ, or the flexible spiral organisms. If to this classification we add the Cocci or rounded forms, we have practically a rude model of that adopted by authors at the present time. In his vibriones he had six varieties, of which lineola, rugula, and bacillus, had already been described by Müller, but subtilis, tremulans, and prolifer, were new. In consequence, however, of the want of marks of characterization, Ehrenberg himself was very doubtful as to the propriety of his system of division or nomenclature. His spirillum comprises three forms, the old vibrio undula of Müller, the ordinary spirillum, and a new kind of spirillum (Tenue). These three differed from one another only as regards length and thickness, the Vibrio undula having only from one to one and a half spiral windings, the others being merely longer or thicker. His genus, Spirochæta, contained a single form only, the Spirochæta plicatilis, an organism of great length, but of very small diameter.

In consequence of the active snake-like and rotary movement of these organisms, Ehrenberg was fully convinced that he had to deal with animals, and this opinion was universally accepted down to the time when Davaine gave his opinion that bacteria must be classified as really belonging to the vegetable kingdom.

In 1840 colour characteristics were brought into play as a means of distinguishing certain organisms, and we find that Fuchs and Ehrenberg describe Vibrio cyanogenus and Bacil-

lus xanthogenus, as giving rise during their growth in milk to characteristic blue and orange colorations. He described the exciting cause as small chain-like organisms, and considered that they were the cause of both the colour formation and the acid fermentive changes. As early as 1819 we find the first description of bleeding bread, the organismal cause of which, Ehrenberg, in later years, was able to cultivate on

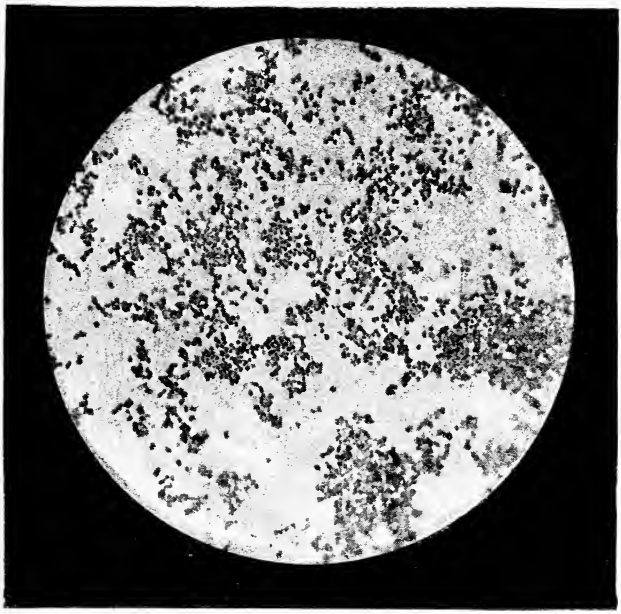

Photo-micrograph of Bacillus Prodigiosus. × 1000.—Organism forms beautiful red colour of bleeding bread, bloody sweat, &c.

various food media, boiled potatoes, Swiss cheese, and white bread. He describes the organisms of which the coloured mass was made up as being exceedingly minute, and as having a characteristic movement quite distinct from the so-called Brownian or molecular movement.

Some idea of the size of these organisms may be obtained from Ehrenberg's calculation that a cubic inch would contain from 46,656,000,000,000 to 884,736,000,000,000 plants ; this organism he called Monas prodigiosa,

but it is difficult to associate the characters that he gave with any of the red pigment forming organisms now known.

Felix Dujardin added very little to the actual classifications given by Müller and Ehrenberg, but he brought out several most important facts. In certain large vibrios he was able to make out distinct bifurcation, the two new limbs becoming segmented transversely; he was also able to recognize an outer membrane or resistant covering on these organisms, and within this a gelatinous or protoplasmic material. This led him to doubt whether, after all, he was dealing with an animal form, and several of the forms described he relegated to the plant kingdom as Algæ—Vibrio subtilis of the Oscillaria.

As Löffler points out, although Dujardin was not able to make any further advances on the classifications of Müller and Ehrenberg, his observations on the chemistry of bacteria were most important. He describes as specially suitable for the development of bacteria, fluids containing such substances as phosphate of soda, hyponitrous acid and oxalate of ammonia and carbonate of soda, and he points out that the nitrogen from the oxalate of ammonia is gradually used up in the presence of organic substances.

With the exception of Dujardin, as we have seen, all observers up to 1852 had looked upon bacteria as belonging to the animal kingdom, but in this year Perty announced that of these minute organisms, some belong to the animal and some to the vegetable kingdom, whilst a certain number appeared to him to stand on the borderland between the two. These vibriones, he says, are colourless or sometimes blue, yellow, or reddish, never green, organisms, with scarcely a trace of any differentiation of their substance; they have a spontaneous or automatic movement; they increase in number by transverse division, partial or complete; when this is incomplete, chains or threads are formed. He divides them into Spirilla or spiral threads, Bacteria or winding or straight threads, and he thinks that the bacteria have not only an active animal life, but that they also pass through a stage during which they must be looked upon as vegetable in character: a double existence which he assigned to other forms.

In 1854 Cohn insisted even more strongly on the plant nature of these micro-organisms. He, too, described zooglœa masses, and he summed up his researches as follows:—(1) All vibriones seem to belong to the vegetable kingdom, and they exhibit a very close relationship to the larger algæ. (2) In respect of their want of chlorophyll, and of their occurrence in putrefying infusions, the vibriones belong to the group of water fungi (mycophyceæ). (3) Bacterium termo is the active, moving form of a closely-related species of palmella and tetraspora zooglœa. (4) Spirochæte plicatilis belongs to the species spirulina, which we can at once indicate as a

particular species. (5) The long non-wavy vibrios (vibrio, bacillus, &c.), are related to the more delicate beggiatoa (oscillaria). (6) The shorter vibrios and spirilla, which correspond in both form and motion to the oscillariæ and spirulinæ, he was unable to localize in his system of classification.

In 1857 Naegeli collected all the forms then known, which had certain characteristic physiological features in common, into a group which he termed Schizomycetes or fission fungi, a group which is now fully recognized by botanical morphologists and physiologists. He included all those lower forms of plant-life in which chlorophyll was absent, and which contained carbon, oxygen, hydrogen, and nitrogen in definite proportions, which elements they were not able to assimilate and utilize in building up their own substance from inorganic materials. They, like the other fungi and animals, can utilize as food only such material as is presented to them in the form of living or dead organic matter held in solution, or combined with a considerable quantity of water. The processes going on within their protoplasm are so intimately connected with oxidization that they usually set free no uncombined oxygen, and this characteristic feature along with their want of chlorophyll colouring matter Naegeli looked upon as the special feature by which they might be distinguished from ordinary fungi. Amongst his fission fungi he placed the forms bacterium, vibrio, spirillum, sarcina, the mother of vinegar, the yeast fungus, the organism associated with the silkworm disease—a small colourless oval organism, somewhat resembling a yeast, which he named Nosema bombycis—most of which were characterized by the features which are now recognized as belonging to the fission fungus group. The relation of these to disease and fermentation Naegeli declined to discuss.

Although Leeuwenhoek had described certain microorganisms in the tartar of the teeth and in various secretions and excretions so accurately and minutely, it was not until 1837 that any definite attempt was made to associate them with the products of a disease; in that year, however, Donné described an Infusorian, which he likened the vibrio lineola of Müller, as occurring in pus in syphilitic diseases. This he thought at first was simply a vibrio associated with the putrefaction of the pus,

but as he was afterwards unable to find this same infusorian in the pus taken from other abscesses, and in putrefying material that had been exposed to air, he was led to inquire whether it was really characteristic of syphilitic contagion, and whether it played any part in the transmission of syphilitic infection. Although he afterwards retired from his first position he carried out a series of most careful and ingenious experiments, through which he was led to believe that very characteristic vibriones were associated with the causation and transmission of syphilis, and he thus opened up a series of most interesting questions, the answers to which, though differing somewhat from those given by Donné himself, were nevertheless destined ultimately to be very much on the same lines as those that he had laid down.

It is interesting to notice that the monad forms which at the present day are again coming prominently forward in connection with the production of certain diseased conditions, were early recognized and described by Donné, and that Rudolph Wagner also described a species of monad as occurring in cancer of the lip.

As was to be expected, however, the connection of micro-organisms with fermentation was proved long before a similar association was made out between micro-organisms and disease, and in the same year that Donné published his results, Cagniard-Latour and Schwann, who had been working independently, announced that the yeast cells (torula cerevisiæ), originally described by Leeuwenhoek, and which were found to grow in grape juice and malt wort were to be associated with fermentation—that they were indeed the cause of this process.

For long, although the intimate connection between the process of fermentation and specific infective diseases was widely recognized, the efforts of most scientific observers were directed towards the elucidation of the causes of fermentation and putrefaction. It was, in fact, suggested that cholera might be due to the action of some ferment-causing organism, which might become lodged in and multiply in the intestine.

In 1837, moreover, Bassi described a kind of yeast fungus, which he thought must be the cause of a miasmatic contagious disease in silkworms. He found extremely minute spores, on and within the bodies of the silkworms affected,

and although parasites had long been accepted as the cause of certain diseases in plants, this was really the first fully-described and well-authenticated instance of a fungus parasite giving rise to disease in any members of the animal kingdom.

As the direct outcome of these researches, Henle, in 1840, was led to believe that the cause of miasmatic, infective and contagious diseases must be looked for in living fungi or other minute living organisms, and although he was unable, experimentally, to satisfy himself of the accuracy of his position, he was fully convinced that he was working in the right direction. It would indeed have been difficult, at that period, to satisfy every condition that he required to be fulfilled; the methods now in use were then unknown and have only been perfected by workers as it has been found necessary, from time to time, to comply in the most minute detail with Henle's conditions, and as, one point being carried, it has been found necessary to advance on others. The first of these was that a specific organism should always be associated with the disease under consideration. As such presence, however, might be accidental, these organisms were to be found not only in pus but actually in the living body. As they might be, even then, merely parasitic, and not associated directly with the causation of the disease, it would be necessary to isolate the germs, the contagium organisms and the contagium fluids, and to experiment with these separately with special reference to their power of producing similar disease in other animals. We now know that it has only been by strict compliance with all these conditions, again postulated by Koch, that the most brilliant scientific observers and experimentalists in England, France, and Germany have been able to determine the causal connection between micro-organisms and disease. After Henle's work appeared a regular fungus fever set in; many skin diseases were proved to be the result of the action of fungi, and numerous internal diseases were, on very imperfect evidence, said to be due to parasitic agency; and a large number of diseases which we now consider to be caused by the action of certain specific organisms in the system, were deemed, on very imperfect data however, to be due to these fungi. Cholera and typhoid fever became subjects of great interest, the stools from patients suffering

from these diseases were carefully examined for organisms, and bacteria and monads were carefully described, but in no case could any definite proof be obtained that any of these stood in any causal relation to either of these diseases.

It was at this stage that Pasteur took up the work initiated by Cagniard-Latour and Schwann, who had first noted the connection between the growth of the yeast organism and fermentation. He applied to other processes of fermentation, such as those of lactic acid, butyric acid, and acetic acid, the same process of experiment and reasoning which they had followed, and he was able eventually to prove that the organic ferment in each case had specific characteristics, not only as regards its physiological action in setting up a certain definite form of fermentation, but also as regards the special morphology and mode of growth of the organisms that were found during and at the end of the process.

As these experiments of Pasteur's are now classical it may be well briefly to indicate the 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 his purpose ; he then, by careful microscopical study, determined what organisms developed most rapidly during the special fermentation process. After making an artificial solution of the substance to be fermented, he added a small quantity of albuminoid material, and a trace of the ash of the special yeast that he wished to grow, in order that there might be sufficient of the necessary salts for the nutrition of the organism. This fluid was carefully sterilized by being boiled in flasks, to which only filtered air afterwards had access. To the germ-free solution he added a small quantity of his special yeast, and if he then obtained a characteristic fermentation with the production of the natural special fermentation products, accompanied by rapid growth and multiplication of the organism that he had introduced, he came to the conclusion that this organism was the cause of the special fermentation.

In 1857 Pasteur described a new yeast (the cells of which were much smaller than the ordinary beer yeast), which gave rise to the formation of lactic acid from sugar, and he pointed out that the nitrogenous material which was necessary for the production of lactic acid was really needed for the

nutrition of the growing yeast, but that otherwise it did not exert any influence in transforming the sugar into lactic acid, as had hitherto been maintained by Liebig.

Some idea of the delicacy of Pasteur's experiments may be gathered from his observations on the conversion of racemic or paratartaric acid by a living ferment into right-tartaric acid (which in turn was capable of undergoing further fermentation) and into left-tartaric acid, which remained unaltered. He had already noted that the material in which fermentative changes took place, determined, in a very marked degree, the nature of the fermentation process. For instance, on adding dust, which of course contained a considerable number of different organisms, to sterilized urine, an ammoniacal or putrefactive fermentation took place; whilst on adding the same dust to sterilized milk an acid fermentation, as evidenced by the curdling of the milk, ensued, whilst in each case there seemed to be a special development of one particular organism. He had of course to contend with the difficulties involved in obtaining isolated organisms or pure cultivations, and for this reason he was for some time heavily handicapped.

All the forms of ferment-producing organisms which Pasteur had studied up to this point he spoke of as vegetable or yeast forms, and it was long before he was able to induce butyric acid fermentation. He at length found, however, a form which he distinguished as an infusiorian, in contradistinction to the vegetable or yeast form. This he describes as occurring in the form of small, straight, cylindrical rods, somewhat rounded at the ends, occurring either singly or in jointed chains of three, four, or more, about 2μ broad and from two to ten times as long as broad. The organism has a slight gliding motion, and its reproduction takes place apparently by vegetative growth and transverse division. The physiological or biological peculiarity of this organism is that it can exist apparently without a trace of organic nitrogen; whilst, like the vegetable ferments with which Pasteur had been working, it can also live without oxygen, and although in form it is like a vibrio, it differs from the vibrios in this respect and also in that it is able to bring about fermentation.

In 1863 he found a second anærobic "vibrio," which he succeeded in cultivating. He prepared a solution containing tartarate of lime, ammonia, potassium, and yeast ash, rendered it sterile or germ-free by boiling, and covered it with a thick layer of oil. To the fluid thus prepared he added a minute quantity of the organic deposit resulting from spontaneous fermentation of tartarate of lime; the

was followed by a typical fermentation in the fluid, from which air was excluded by the film of oil spread over the surface. On microscopical examination of both the artificial and the spontaneous fermentations, he found "vibriones" 1μ thick and 50μ long. It was objected that in the spontaneous fermentation a certain quantity of air must necessarily remain in the fluid in which the organism was growing, so that the presence of oxygen could not be inimical to the growth of this "anærobic" organism (a name that Pasteur gave to those organisms that are not dependent upon the oxygen of the air for their growth and development). He met this objection with proof that, grown along with other organisms, such as bacterium termo, which were dependent upon free oxygen for their existence, the latter developed and grew for a short time in the fluid and so used up what oxygen there was present in solution, at which point they were unable to develop further, so that other resulting changes must be due to the growth of the special anærobic organism. Passing from the butyric acid fermentation, and taking it as an analogy, Pasteur continued his researches on putrefactive processes in which nitrogenous substances and acrid and offensive smelling materials are formed, and he eventually came to the conclusion, which had already been expressed by Mitscherlich in 1843, that as yeasts gave rise to fermentation so "vibriones" must be the cause of putrefaction; and, going further, he assumed, what has since been proved to be erroneous, that the whole of the vibriones of putrefaction were anærobic, that is, they could give rise to their specific products only when they were removed from the influence of the action of the oxygen of the air.

In connection with the subject of anærobiosis, Pasteur pointed out that the mycoderms known as "flower of wine," "flower of vinegar," &c., were able to produce different forms of fermentation according to the presence or absence of oxygen. Mycoderma aceti, for instance, bringing about the splitting up of sugar into alcohol, *i.e.*, setting up an alcoholic fermentation when there is too little oxygen present, but in presence of abundance of oxygen giving rise to the formation of acetic acid, then setting up what is known as the acetic fermentation.

These researches eventually led Pasteur to the conclusion well stated by Duclaux, that "whenever and wherever there is decomposition of organic matter, whether it be the case

of a herb or an oak, of a worm or a whale, the work is exclusively done by infinitely small organisms. They are the important, almost the only, agents of universal hygiene ; they clear away more quickly than the dogs of Constantinople or the wild beasts of the desert, the remains of all that has had life ; they protect the living against the dead ; they do more : if there are still living beings, if, since the hundreds of centuries the world has been inhabited, life continues, it is to them we owe it." Without them the surface of the earth would be covered with dead organic matter, the remains of plant and animal bodies, which, retaining the elements necessary for the building up of new plant-life and animal bodies, would soon cut off the food supply of new plants and animals ; life would be impossible because the work of death would be incomplete, or, as Pasteur puts it, "because the return to the atmosphere and to the mineral kingdom of all that which has ceased to live would be totally suspended." From his experiments on fermentation and putrefaction Pasteur, by a very natural transition, turned his attention to the diseases of wine, and then to those of the silkworm—diseases that specially affected two important French industries. After most careful research he found that the acetic fermentation, viscosity, bitterness and turning flat of wines, were all due to the action of certain organized ferments, most of which he was able to isolate and study.

The acid fermentation, he found, was produced by the mycoderma aceti, which consists of short rod-like forms, about double as long as broad, slightly constricted in the middle ; these individual elements, being joined together in long chains, which, as they grow on the surface of the wine, interlace with one another and form a regular film or skin. The bitterness of wine he ascribed to the presence and action of branched tortuous filaments of about 1.5μ to 4μ in diameter, these chains having a peculiar knotted appearance. The turning flat of wine was due to the presence of delicate unbranched filaments about 1μ in diameter, which under certain conditions are broken up into short segments which somewhat resemble the bacilli met with in a lactic acid fermentation. The cause of the viscosity of wine was an organism made up of cocci about 1.2μ in diameter, often arranged in chains of considerable length. He found, indeed, that the relations of cause and effect were invariable ; wherever certain forms were present in addition to the yeasts, or were introduced after the yeasts, the result was a special fermentation superadded to the wine fermentation.

The genius who had shed such a flood of light on the

causation and prevention of wine disease now turned his attention to the ravages of the "spot" disease or "pebrine" amongst silkworms, a disease that at one time threatened to destroy the flourishing silk industries of France. The organisms found in this disease had already been described by Naegeli as *Nosema Bombycis*, and by Latour as *Panhistophyton*, small, glistening, oval corpuscles which appeared to be endowed with life and to lead a parasitic existence in the tissue of the silkworm caterpillars. Pasteur was able to demonstrate their presence not only in the butterflies which develop from these worms, but also in the eggs they laid, and he found that where any one of the three forms was affected, the corpuscles were passed on to the next stage. He was able to show that they increased in the body, that they were the cause of the disease, and that by careful examination and destruction of the affected eggs and the preservation of the healthy ones, the disease could gradually be eliminated. He found that another disease, the lethargy of silkworms, was also probably caused by the presence of micrococci, arranged in the form of chains, in the intestinal canal of the worms. Pasteur had thus succeeded in showing the relationship between certain micro-organisms and certain wine diseases, but he had also been able to demonstrate a causal relation between certain lowly-organized, parasitic organisms and a special disease in animals or insects. He had, in fact, demonstrated that certain specific organisms, endowed with definite morphological and physiological characters, gave rise by their presence to specific and characteristic diseases; he had by observation and experiment extricated the theory of a living contagium from a condition of chaos, and he had assigned to definite organisms, each a special rôle in the production of certain forms of fermentation, of putrefaction, and of disease; and although much was still left, and still remains to be done, in the identification and classification of those organisms, he had separated a few distinct forms, and instead of assigning to these a general and common action in the production of the above processes, he had allotted to each one its own part. This he was able to do with such clearness and to place his experiments so lucidly before the scientific world, that there could be no doubt as to his meaning; the consequence being that he soon secured a large following of enthusiastic

workers from amongst his contemporaries. On the other hand, however, he brought forward those who were by their researches led in opposite directions, or who, with less perfect methods, could not make their facts fit in with his theory, or who could not repeat or confirm his experiments.

The most important point that he wished to demonstrate was that which related to the specific character of the various ferments in fruit juices. He was attacked most vigorously on this subject by Lemaire, Bechamp, Hoffmann, and others, each of whom pointed out that in any fermentation experiments that he had made he had never seen a single organism only. (He had, in fact, never been working with pure cultivations). This was undoubtedly a very forcible objection, and one which had to be met, but one which was easily enough overcome as methods of separation and isolation became perfected. Bechamp, who had found what he termed *granules* in the cells of living plants and animals, and even in fossil remains, held that these microzymas, as he called them, remained alive ; that they set up various forms of fermentation ; that under different conditions of food, separation from the cell, and external influences generally, they ran together, became altered in shape, and underwent various changes, so giving rise to the various forms which Pasteur had described ; all these processes going on concurrently or subsequently to the various changes that occurred in fermentative and pathogenic processes. He would, however, have nothing to do with any specific organism ; he considered that all organisms were merely the result of a new grouping and alteration of these microzymas separated from the cells, and that they were specifically affected by the various altered conditions in which they found themselves when removed from the cell in which they naturally occur. Both Pasteur's positions were thus attacked. His first contention was that germs were the cause of fermentation in disease, and secondly, that each fermentation was due to the specific action of a definite organism. In his first contention his position was materially strengthened by the observations of Lemaire who, after proving that the presence of carbolic acid was inimical to the life of the higher animals and plants, carried his researches a step further, and proved that the lower organisms were similarly affected by the same material, and he found that the addition of a small quantity of carbolic acid

to fluids, in which putrefaction and fermentation would ordinarily take place, prevented the incidence of these processes. He found at the same time that the fermentations set up by chemical ferments, such as diastase and synaptase, remained entirely unaffected by the action of carbolic acid, and the result of his earlier experiments led him to believe that the process of fermentation was due to the action of living organized creatures which, like the higher plants and animals, could be killed by the carbolic acid. When they were allowed to develop freely they brought about fermentation; when their growth was stopped, or they were killed, fermentation could not go on. The same reasoning, he thought, might be applied to infection and miasma, and he concluded that disease processes were the result of fermentations or decompositions going on within the tissues, and brought about by the above or similar organisms. Pus formation was the result of the action of germs falling from the surrounding air into a wound. By the application of his germicidal reagents to wounds, vaccine vesicles, and suppurating surfaces, he attempted to destroy these organisms outside the body whilst they were actually attacking the weak points.

This was really the first step in the direction of an antiseptic treatment of wounds. Whilst treating by his method wounds in the human subject and in the dog he saw "that pus remained entirely absent, or was reduced to a minimum, putrid alterations were absent," and the wound healed rapidly. All these results were due, he maintained, to the destruction of the microzoa or infusoria by his carbolic acid lotions. The paramount importance of this theory was only afterwards fully appreciated and worked out by Lister, who saw that, owing to the difficulty of killing germs after they had once made their way into the tissues, it was absolutely necessary that such organisms should be prevented from gaining access to the wounds at all, and it is upon the attainment of this end that his well-known antiseptic treatment depends for its success.

Accepting the truth of the statement that germs were the cause of fermentation, Lister also came to the conclusion, independently, that germs entering the wounds from outside might be the cause of suppuration, and since germs were

floating in the air, were suspended in water, and were attached to the instruments and bandages that were used in the treatment of wounds, he determined that it was necessary, by using some germicidal reagent, to kill all such suspended and adherent organisms before the various materials mentioned were allowed to come in contact with the wounded tissues. With a combination of experimental resource, patience, and brilliancy almost unparalleled in the history of surgical science, he, step by step, built up a theory and practice of antiseptic surgery, a theory and practice which rapidly revolutionized the treatment of wounds and the routine of ward management. He thus introduced a system which has affected the practice not only of those who believe in its accuracy, but of those who cannot bring themselves to accept all its details, but who have nevertheless accepted its principles, sometimes even unwittingly.

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CHAPTER IV.

Bacteria as the Causes of Disease.

Anthrax—Pollender—Davaine—Rayer—Laplat and Jaillard's Observations controverted by Davaine—Pyæmia and Septicæmia—Salisbury on Bacteria as the Cause of certain Fevers—Johanna Lüders' and Hallier's observations on Pleomorphism or Polymorphism—Burdon Sanderson, Hoffman, and others, state that there is no Connection between Bacteria and the Higher Fungi—Demonstration of Infective Element in Anthrax Blood—Bacteria found in Organs in certain Diseases—Sarcina in Stomach—Specific Organisms—Specific Activities.

The bacterium which, in its relation to the cause of a specific infective disease in man and the higher animals, has been most thoroughly studied is the Bacillus anthracis, an organism which, not only on account of its size but also because of its powers of adapting itself to conditions both outside and within the body, has been recognized comparatively easily, and cultivated in artificial fluids and on other nutrient media in which it grows luxuriantly, so that it has been possible to study more or less carefully its morphological and physiological characters. This bacillus was observed by Pollender as early as 1849 in the blood from the enlarged and pulpy spleens of cows that had succumbed to anthrax or splenic fever. He recognized that the bacilli were not fragments of broken-down vessels or coagulated fibrin, as had been suggested, but that they were probably small vegetable organisms similar to those described by Dujardin as "vibrio bacillus," and he suggested that it was quite possible that these organisms were in some way or other associated with the appearance of anthrax disease.

In the following year, and before Pollender's description had been published (his full description was published in 1855), Davaine and Rayer described motionless thread-like organisms and rods in the blood taken from animals affected with splenic fever. These observations were confirmed by other observers, but it was not until 1863 that the micro-organ-

ism was supposed to have any definite relation to the fever. Davaine, at that time stimulated by Pasteur's investigations on the relation between micro-organisms and the butyric fermentation, was led to suggest that these rods were, in all probability, the actual and specific cause of the disease, which suggestion, along with his observations on certain cases of malignant pustule that were published in the following year, may be looked upon as the first real attempt to demonstrate the connection between the Bacillus anthracis and the diseases known as malignant pustule and splenic fever. Although he did not furnish rigorous proof of the connection he was so far successful that, except for the cultivation of organisms outside the body and the production of the disease by means of the inoculation of pure cultivations, work which Koch afterwards completed, Davaine left no proof wanting.

In the meantime, however, Delafond had demonstrated the constant presence of the rodlets in the blood of animals affected with splenic fever, and had also suggested their plant-like nature. Davaine found that he was able to transmit the disease to healthy animals by means of the inoculation of blood that contained these rods; whilst where the rods were absent, although the blood might be exactly the same in other respects so far as he was able to observe, he could not produce the disease. He found that a single drop of diseased blood contained from eight to ten millions of these forms, and that by diluting the blood a million times, he was still able to produce the disease by inoculation. Subsequent observers, especially Leplat and Jaillard, objecting to his conclusions, said that similar rods had been found in other diseases. They pointed out that other elements in the blood—an extremely complex fluid—might be the cause of the infective disease, and that the rodlets themselves might be only an accidental factor. Leaving this safe ground, however, their further criticisms were based on the supposition that all the rod-shaped bodies were identical in structure and life history, and that consequently they should have the same effect when introduced into the body, and they concluded that because similar organisms taken from vegetable infusions did not produce anthrax, that therefore the rodlets in the blood could not be the specific infective agent. Davaine's answer was that their contradiction of his statements was due entirely to misconcep-

tion. He pointed out the different physiological characters of organisms taken from different media, and showed that different conditions were essential to the existence of different organisms, and, in connection with a number of inoculation experiments that they had carried on (with material sent by him, which they allowed to undergo decomposition, and by means of which rabbits were killed but no rods were afterwards found in their blood), that they had produced no anthrax because the incubation period was too short, because the splenic enlargement was absent, because the animals underwent much more rapid putrefaction, and because the disease was capable of being transmitted to birds—a class which up to that time had not been found to be susceptible to this disease. During this controversy Davaine pointed out that Pyæmia and Septicæmia could not be produced by the inoculation of true anthrax virus. He described the bacteridia of anthrax as totally devoid of movement in the blood, and carefully distinguished between them and putrefactive bacteria ; he demonstrated how these latter could, by their presence and activity, diminish the activity of the anthrax bacteria, and could in turn produce a septic condition, which was in all essential respects absolutely different from splenic fever. He demonstrated that these vibriones were vegetable and not animal, and recognized the most important fact that the environment, mode of nutrition, and products of excretions of the anthrax micro-organism had a most marked influence in modifying its activity and virulence. He did not believe in the possibility of infecting the organism of the fœtus in utero, basing his belief on Brauell's and his own experiments, and advanced the theory that the immunity enjoyed by the fœtus was due to the filtering action of the placenta which, he contended, did not allow of the passage of solid particles either from the maternal to the fœtal circulation or in the opposite direction.

He concluded also that certain species of animals were much more susceptible to the disease than others. He was convinced at that time that malignant pustule, malignant œdema and anthrax were all due to the presence of the same organisms in animals or in man, and in fact he initiated the whole theory of contagion from animals to man and *vice versâ*, and opened up the immense and fertile field of the comparative pathology of infective diseases. In 1868 he

almost completed his proof of the causal relation of the organism to the disease by the ingenious method of mixing a drop of virulent blood with a large quantity of water, and using the bacteria which fell as sediment as the medium with which to inoculate susceptible animals. With this sediment he was always successful in producing anthrax, whilst inoculation with the water taken from near the surface invariably gave negative results.

It was not a perfect proof, however, and it was left for Pasteur with his filtration process, and for Koch by his pure cultivation process on solid media, to complete the proof that Davaine so ardently desired and worked to obtain.

In view of our latter-day knowledge of bacteria, it is interesting to note that as late as 1870, or only twenty years ago, these bacilli of anthrax were declared to be albuminoid crystals ; whilst within the last ten years they have been described as being built up from the *débris* of fibrinous filaments.

About this time a great impetus was given to the theory of a living contagion—an impetus which unfortunately impelled numerous workers and theorizers to see the cause of disease in every germ that they found. First, Pasteur had formulated his germ theory of fermentation and putrefaction ; Davaine, in his descriptive and controversial papers, had insisted upon the connection between his anthrax rods and splenic fever, and in the animal parasitic world the etiological relation between trichina spiralis (a small round worm) and an acute fever, met with especially in pigs, had been fully demonstrated. Salisbury, in this country, thought that he had found the organisms that were the cause of intermittent and remittent fevers, of malaria, and of certain other forms of specific disease, but he was quite unable to give proof in any single instance. In Germany, Hallier took up the subject with eagerness. He was led to investigate the subject of Polymorphism of the bacteria or fission fungi—a theory that had been advanced by Tulasne in 1851, and had been later worked out by Tulasne and De Bary. It was held, indeed, that yeast was simply a form or stage in the development of certain mould fungi, such as the Penicillia or Aspergilli.

Pasteur had already pointed out the physiological likeness between the yeasts and the bacteria in their power of pro-

ducing fermentation processes, and Hallier, who was well acquainted with these researches, concluded that if the yeast cells were part of a developmental cycle, the bacteria might also be taken to represent only short resting stages of the same or similar cycles. This was an especially seductive theory, as up to this time the origin of these minute forms had, as we have seen, been enshrouded in mystery, and had provided matter for the keenest controversy.

A lady, Johanna Lüders, was firmly convinced that the lower bacteria and yeasts developed in some way or other from the individual parts of the mycelium of certain fungi, or from their spores. Her observations were repeated, and there was a general concurrence of opinion that the bacteria were derived in some way from fungi and from other higher plant forms.

Hallier, with his isolation apparatus, which consisted really of Schwann's apparatus, to which an air-pump and a cotton wadding filter were added, and his cultivation apparatus, which corresponds practically to the potato-jar of to-day, with water to take the place of bichloride of mercury solution, came to the conclusion that the cause of almost every infective disease was to be looked for in bacteria, monads, and cocci, which in their turn were nothing but forms produced during the developmental cycle of one or other of the fungi aspergillus, penicillium, mucor, &c.

Löffler says, in summing up the results of Hallier's researches, "He put forward the hypothesis that all contagia and miasmata are the products of fungi or algæ which alone, on account of their small size, are able to pass through the fine capillary vessels, and that it was only necessary, in order to determine the nature of the original cause, first to find out the micrococcus and then to trace it back to the fungus to which it owed its existence." By such new ideas, propounded with such an air of conviction and authority, Hallier made a most profound impression on both the lay and scientific world. The whole system was so simple and clear and every part contributed so easily and naturally to form one harmonious whole ; every assertion was so definitely supported by microscopic observation and cultivation experiments that no doubt as to the correctness of the demonstrations seemed to be possible.

It was a somewhat noteworthy fact, however, that the peni-

cillium occurred so frequently in his cultivations, and, as Brefeld and others pointed out, however complete Hallier's isolation apparatus might be in itself, he did not take sufficient care to prevent the entrance of the spores of these various fungi when he introduced the micrococci and bacteria which he wished specially to study, so that, although nothing fresh might be added after he had introduced the seed material, his seed material itself might be a mixture of various kinds, and along with it he could not be sure that the spores of the larger fungi had not entered. It was a case, said Brefeld, of covering with a waterproof a man already drenched with rain. So faulty, indeed, were these experiments considered to be by Burdon Sanderson in this country, by Hoffmann, Rindfleisch, Manasseïn, and Ferdinand Cohn abroad, that these observers undertook various experiments to prove that not only was there no connection between bacteria and the higher fungi, but that there were actually cases in which the micrococci did not develop into the longer rod-shaped bacteria. Moulds could only be developed in artificially prepared food solutions when the seeds or spores of moulds were sown ; whilst bacteria seeds or germs, when obtained free from the germs of moulds, would in similar solution give rise to the development of bacteria only. There was soon a reaction against the whole of Hallier's teaching, and it was now pointed out that he had seldom or never been able to reproduce any disease by inoculating cultivations of the organisms that he grew, and the theory of living contagion fell into discredit, though the fact must not be ignored that Salisbury's and Hallier's work led to further consideration of many points associated with the relation of bacteria to disease, and that eventually it exerted a marked influence on the germ theory of disease. Hallier undoubtedly laid great stress on the fact that a micrococcus was the cause of certain diseases, and he pointed out that its extreme minuteness was in favour of its being able to enter readily and retain firmly its position in the body.

In 1868–9 Davaine and Chauveau succeeded in demonstrating that in all probability the infectious element in anthrax blood (Davaine), and in glanders pus and vaccine lymph (Chauveau), and in vaccine lymph (Burdon Sanderson) was not merely a soluble poison, but some solid material, such as a leucocyte, and probably a

micro-organism. The blood or pus was diluted many times with water, the sediment was washed again and again, each time being allowed to settle at the bottom, after which the supernatent fluid was found to have no effect in producing any disease, whilst the sediment which contained pus organisms and "fine granulations" almost invariably set up the disease process. The virus must accordingly, these observers thought, be a solid poison, and must be looked upon as a particulate body. These observations thus confirmed, to a certain extent, Hallier's suggestion that a micrococcus or a bacterium was the cause of most specific infective diseases.

From this time onwards a large number of observations were made on various infectious diseases and micro-organisms. Micrococci were found in diphtheria, in scarlet fever, in rinderpest, septicæmia, and in other specific infective conditions, though Traube, in 1864, had made what might be considered the first practical application of what had been discovered to be an important pathological condition when he demonstrated the fact that, if bacteria found their way into the bladder by means of a dirty catheter, a severe attack of inflammation of the bladder followed—an observation which was supplemented by Klebs, who demonstrated the connection between small abscesses in the kidney and the introduction of micro-organisms into the bladder. But with all these records there was very little of definite value to demonstrate the causal relationship between bacteria and disease, and even when fragments of diphtheritic membrane and of the wall of abscesses were introduced under the skin of an animal, and gave rise to both local and constitutional symptoms, there was no proof forthcoming that these were due to the micro-organism, and not to such special chemical products as had already been separated from putrid and diseased materials. Panum had demonstrated, in 1856, that it was possible to obtain from decaying flesh infusions an extremely poisonous substance, and his results were confirmed by numerous observers, some of whom succeeded in combining with acids the "basic" substance that Panum had separated; the sulphate of this base when injected into frogs proved fatal, and eventually Zülzer and Sonnenschein prepared what they described as a septic alkaloid which was stable in character, and in its reactions resembled most remarkably the vegetable alkaloids, atropin and hyoscyamin. It was natural that as these materials could be separated by chemical means from diseased and putrefying materials, they should be looked upon as the primary and real etiological factors in the transmission of disease. In 1871, however, Recklinghausen, turning his attention to bacteria, was able to show that in the organs of patients affected with various infective diseases (such as blood-poisoning, and puerperal fever, typhoid fever, acute articular rheumatism, gangrene of the lung) small accumulations of micrococci were present, and that these were probably the cause or the agent by which deposits of the abscesses or gangrenous patches occurred in different parts of the body and in different organs. These micrococci were described as having an exceedingly sharp outline, as being extremely resistant to strong acids and alkalies, and, in fact, as being in most other respects like those that had been described as

occurring in diphtheria and in abscesses of the kidney. These results, with some modifications and additions, were almost immediately confirmed by Waldeyer and Weigert; Recklinghausen and Weigert concluding that these micrococci were all in the lymphatic vessels, Waldeyer, on the other hand, holding that some, at any rate, were contained in the blood-vessels.

In 1872 E. Klebs found in pus, organisms which he describes most graphically as rod-like bodies, the so-called bacteria, motionless, frequently grouped in short chains, or in longer threads. He also found numerous micro-spores, very minute refractile organisms, whose diameter might be at most .5μ, some lying isolated and free, and having oscillating movements, others linked together in rosary-like threads. Having found them in the discharges he examined granulation tissue (raw or "proud" flesh), in lymph canals, in spaces in the septa or partitions between muscles, in inflamed marrow of bones, and in the ulcerating cartilage of diseased and injured joints, in the walls of blood-vessels and in thrombi or clots formed in the vessels and attached to their walls. These organisms were in all cases situated near the primary wound (the researches were carried on during the Franco-Prussian war), but having found the micro-organisms in this position he next traced them to the abscesses that formed in distant internal organs in cases of pyæmia. In fact, wherever there were points of secondary disease or suppuration, there he was able to demonstrate the presence of these organisms; whilst in very severe cases of blood-poisoning he was actually able to demonstrate their presence in the circulating blood. In consequence of this constant presence of the bacteria and micrococci in these various disease areas, he felt justified in concluding that the organisms he had observed and described were the cause of the pyæmic and septicæmic conditions, and that also to their action was due the formation of pus, of abscesses, and even of ulcerative inflammations of the vessel walls. There can be little doubt that the ingenious experiments devised and carried out by him and his pupils formed a groundwork on which succeeding investigators found it possible to build up the present magnificent structure. He conceived the idea of separating the micro-organism from the poison which it produced by means of baked clay cylinders, and found that fluid so treated, although it gave rise to constitutional disturbance when injected into the blood or under the skin, did not induce

suppuration, nor did it cause death : but if to this a quantity of the micro-organisms were added, and the fluid was then injected into dogs, these animals succumbed to a true pyæmia, accompanied by the formation of abscesses, especially near the points of inoculation. He showed that these organisms were not present in the normal healthy blood ; he also, by means of ingenious apparatus, which he contrived or modified, was able to observe the actual multiplication of the micrococci under the microscope. He introduced the method of fractional cultivation, but not in the complete form in which it was afterwards used by Lister and Pasteur, as he relied on the more vigorous growth of a certain organism that was placed in a special fluid and under special conditions, rather than on the dilution and isolation of individual organisms in small drops of fluid. Klebs was, however, able, by his biological method, to obtain comparatively pure cultivations of several bacteria and micrococci, and he was the first to distinguish the division going on in various planes.

Klebs' anatomical investigations were confirmed by numerous observers, Birch-Hirschfeld, Heiberg, Orth, and Hueter, the last of whom tried to build up a system of pathology on the basis of the researches that had already been carried out ; these, however, afforded very incomplete data on which to work. Pyæmia was, he considered, the result of the invasion of the tissues by micrococci, with the formation of little plugs in the vessels, around which abscesses were developed ; septicæmia was a general poisoning by absorption from a localized formation of bacteria in the body ; and a third form, which he looked upon as putrid poisoning, was the result of the absorption of poisonous matter formed by vibriones existing outside the body. The only difficulty in the matter was that the same organism appeared to produce very different conditions, such as pyæmia, septicæmia, puerperal fever, pyelo-nephritis, typhoid fever, phthisis, small-pox, diphtheria, cholera, rinderpest, whilst even in the healthy body organisms were sometimes found, especially in certain positions, a fact which at that time was not reconcilable with the various theories that had been advanced. Another objection stated was that as the bodies of patients who had died of pyæmia and septicæmia putrefied rapidly, they formed an especially good medium in which innocuous organisms might grow, and it was maintained that organisms were found in such bodies after death, because of the special suitability of the soil for their growth, and that they were therefore probably rather to be looked upon as accidental concomitants and consequences than as essential factors in the production of disease.

There now also appeared great activity in the French school : Pasteur had shown that most of the processes of putrefaction were due to the presence of motile, anærobic vibriones, whilst Davaine had observed only non-motile rod-

lets in the blood of animals that died from anthrax. The latter was further able to prove to Jaillard and Laplat that these non-motile organisms of virulent anthrax were actually rendered inert by the growth amongst them, or alongside of them, of motile putrefactive organisms, and that the action of the anthrax organism was also considerably modified by the growth of septic organisms, which, in turn, when introduced into an animal by inoculation, were capable of producing a disease which might perfectly easily be distinguished from anthrax. The knowledge that the septic condition was not due to ordinary putrefactive organisms was further augmented by Birch-Hirschfeld, who demonstrated that pus which when fresh was infective lost much or all of its specific activity when putrefactive organisms were allowed to grow in it; so that these putrefactive bacteria at any rate could not be looked upon as playing any part in the production of wound infection. Then specific forms began to be associated with specific disease conditions, or kinds of putrefaction and fermentation; the Goodsirs described sarcina in the acid contents of dilated stomachs; Trecul found his Bacillus amylobacter, with its characteristic tadpole shape, which gave rise to no wound infection, and numerous others were observed, none of which could be proved to have produced septic conditions. An organism which produced the coloration met with in blue milk was described by Fuchs, and another perfectly distinct micro-organism was described by the same observer as giving rise to a yellow colour in milk. That the milk was not absolutely necessary for the nutrition of these organisms he proved by making artificial cultivations on other media, and he gave very fully the conditions under which they could continue to exist; they were destroyed by a temperature of 50° R. to 55° R.; freezing did not interfere with their power of propagation when again thawed, and after being dried and again moistened they were able to develop in milk and to give rise to the characteristic coloration.

The way was thus being prepared for the discovery that specific organisms had, under certain conditions, specific actions and activities. If a peculiar organism was found to be associated with the production of a special kind of colouring matter, and if special fermentation and putrefaction processes were induced by individual organisms, was

it not probable, in the light of Davaine's work, that special diseases would ultimately be found to be associated, not all with the same organism, but each with a special form ?

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CHAPTER V.

Fermentation.

Fermentation: a Key to the Whole Position—Chemical Fermentations—Illustrations—Organic Fermentations—Alcoholic Fermentation—Result of Activity of Living Protoplasm—Lactic and Butyric Fermentations—Cagniard Latour—Schwann—Reess—Hansen—Liebig's Theory of Fermentation—High Beer—Low Beer—Method of obtaining Pure Yeasts—Hansen's Classification of the Saccharomyces—Spore Formation—Film Formation—Characters of Species of Saccharomyces—Metschnikoff's Monospora—Torulæ.

From what has preceded it will be evident that if any light is to be thrown on the subject of the production of organic poisons in the course of disease a careful study of the subject of fermentation is a necessary preliminary; for, taken in its widest sense, fermentation includes all those processes in which there is the formation of special ferments and of special products as the result of the life-history of certain vegetable organisms. It is quite as rational to speak of a putrefactive as of an alcoholic fermentation, and we might even go beyond this and speak of a colour fermentation or a disease fermentation, as the organisms by which one or the other is started, and the conditions under which they are carried on, have many features in common, and, in fact, do not differ more than do the causes and conditions that are already known as associated with true alcoholic fermentations.

Fermentation consists, essentially, in the breaking up of chemical compounds, the molecules of which they are composed being separated from one another for a brief period, and then allowed to combine and form simpler and more stable compounds. Owing to the setting free of such energy as has been stored up in the highly complex fermentable substance which is no longer required to maintain the high level of combination, a certain proportion of this energy is released in the form of heat, the temperature of

a fermenting fluid being found to rise without the addition of any external heat. An object lesson may be used to illustrate the processes of breaking down and re-combination that go on in fermentation. Let us suppose that we have a tall tower built up of two kinds of blocks, placed one on the top of another ; first a large block and then three small ones, then another large block and then three more small ones ; let us consider that there are pulling on these blocks elastic bands of different strengths, one very strong one between the two large blocks, another very strong one between each pair of the small blocks, and two sets of three weaker bands running from the larger blocks to the smaller ones ; these sets of blocks are so arranged that if left perfectly undisturbed they will remain piled up one on another forming a tower of considerable height. The higher the tower the more easily, as a rule, will it be upset, though this depends on the way in which the larger and smaller blocks are distributed in the structure. If, now, some disturbing element comes in, and if one of the blocks is withdrawn or is even slightly shaken, the whole structure may collapse, even the movement of a small top block may bring this about by altering the tension on one of the elastic bands, so disturbing the equilibrium of tension on the whole tower, that the two large blocks, with their strong elastic band, are set free and fall to the ground ; the pairs of smaller blocks, with their strong uniting bands, also fall to the ground, and the other thin bands of which we have before spoken are now so far stretched or broken that their influence in holding the different sets of blocks together may now be left altogether out of account. But in falling from the top of the tower to the bottom energy of position has been lost, and in falling and striking the ground a block may do a certain amount of work ; if it were in connection with a series of pulleys it might be made to lift a certain weight ; if it were to strike the ground with sufficient force it might produce a flash of light, or a certain amount of heat. We are, perhaps, not justified in stating that this is exactly analogous to what takes place in fermentation, but it is sufficiently so to explain the nature of the process, if we consider that the kinds of blocks that are built into our tower are multiplied. In the most characteristic and best form (for our purposes) of fermentation that can be taken as an example, we have

grape sugar, consisting of three kinds of blocks, six of carbon, twelve of hydrogen, and six of oxygen, all built into a high tower of twenty-four blocks (sugar, $C_6H_{12}O_6$). In the process of fermentation the equilibrium is disturbed, and we have the tall tower replaced by four smaller ones, two consisting of three blocks each ($2CO_2$, carbonic acid), and two consisting of nine blocks each ($2C_2H_6O$, alcohol), and the heat that is evolved in the process may be represented by the sum of the figures representing the distances which these various blocks have had to fall from the high tower into the lower ones, multiplied by the figures representing the mass of the volumes. Other features have to be taken into account, but the above example will serve to explain part of our meaning. But this is not all. The children's line of "soldiers" may be used to explain another feature: a child arranges its wooden "bricks" in a long line, placing them on end; if they are all of equal length the distances between them are also made equal; if they are of unequal length, they are so arranged that one block falling will just touch the one next to it. If the end block be now so disturbed that it loses its *unstable* equilibrium, and so falls as to acquire a stable equilibrium, it not only loses its own unstable equilibrium, but it upsets that of the brick next to it, which in turn acts on its neighbour, and so on till the whole line is brought to the ground. If, instead of upsetting the first brick at once, you simply give it a slight tap and only just move it, instead of falling over, it sways backwards and forwards for a little but then settles into its original position; you give it another tap, it sways a little further, but still returns to its erect position; whilst if now you give it a good strong push, or if two of you tap it at the same time, over goes the brick, bringing along with it the whole line to a condition of stable equilibrium. The towers or the rows of erect bricks are fermentable substances, the disturbing forces are those agents that bring about fermentation, and the smaller towers or the bricks laid down in rows instead of on end are the products of fermentation. The larger number of smaller towers contain exactly the same elements as did the smaller number of larger ones. They had energy of position, which is converted into kinetic energy, exactly in proportion to the distance that the elements have to fall; some of the energy appearing in the form of heat, other parts being used up in

the rearrangement that takes place when the smaller towers are formed. It may be asked, How does the tilting over of the bricks bear on all this? The first tap that you give them may be said to represent the action of some ferment; the tap that your friend gives at the same time may be held to represent one of the conditions essential for the production of a ferment, say the presence of a certain amount of light; whilst the tap that a second friend gives may be looked upon as representing a certain degree of temperature; it may be a forcible tap, representing 40° C., or it may be a slighter one, representing a temperature of 20° C. only, in which case you do not get sufficient energy out of the ferment to start the process. One or two only of your taps may upset certain towers or certain lines of bricks, but other towers that have a somewhat broader basis or a more definite arrangement require the whole three forces to be applied, and these have to be applied in certain definite proportions if the same results are to be obtained in every case. Thus, for example, by graduating the power of the disturbing force, the equilibrium of your tower may be simply upset and the elastic bands drawing in certain directions cause a rearrangement of the blocks according to the strength with which these bands pull; if, however, instead of simply just distributing the equilibrium to allow of the new arrangements taking place, your tower is struck with a sledge hammer, or overturned by a pistol shot, or by the explosion of a charge of dynamite near it, the conditions are so altered that you cannot rely upon any plan of rearrangement being adhered to. As a result you simply obtain a series of disconnected blocks or of much smaller and irregular towers. It will be said that this regular breaking up is exactly what takes place in the breaking up of chemical combinations. This is perfectly true, for that is exactly what fermentation is. It is the upsetting of the equilibrium of unstable compounds by most delicately adjusted forces which are so accurate, so constant, and so delicate, that the stronger affinities of certain elements for one another are allowed to act to their full extent, and regular stable combinations are formed always in a definite manner. Let us take first such a substance as nitrogen trichloride or nitrogen teriodide. If a sharp blow be given to a small quantity of either of these materials, or if either be heated to a certain temperature, it will immediately break down into nitrogen

and chlorine, or into nitrogen and iodine. Thus a tower made of $2NCl_3$, or of two large and six small blocks, is transformed into two towers, one composed of two large blocks and one of six small ones, or rather into four towers, one composed of the two large blocks and three each composed of two small ones ($2NCl_3 = N_2 + 3\ Cl_2$), and along with the falling of these molecules into their lower positions there is a setting free of energy which manifests itself in the form of heat and light. Consider now that in your tower or in your row of bricks you have continual oscillation going on ; the elastic bands are being continually pulled upon by certain forces which we may say are light and heat ; there is continual motion in every part of the tower, or the bricks standing on end are continually oscillating backwards and forwards, but never sufficiently far to disturb the equilibrium completely, when suddenly a third element of disturbance sets in, a small organism comes near and wishes to take out one of the blocks from the tower for its own use ; it seizes the time when the oscillation is greatest, and giving a little extra pull it removes the block, seizes on it firmly and immediately, and the rest of the tower collapses ; or in the case of the swaying bricks, although it has no power alone to upset the first brick in the row, by striking it just when its oscillation is at one extreme phase it assists light or heat, pushes this first swaying brick a little further and causes the collapse of the whole line. Bunge gives a series of examples of the breaking down of such chemical substances into simpler materials, and shows how certain ordinary chemical substances along with heat, or even heat alone, may act as fermentation exciters. Thus he points out how a blow can initiate the breaking up of nitro-glycerine into carbonic acid, water, nitrogen, and oxygen.

Nitro-glycerine is highly unstable, not so much from the elements which it contains as from the method of arrangement of the atoms of the elements. Some oxygen has been induced to unite with nitrogen, a substance for which under ordinary circumstances it has little affinity, it having at the same time a much stronger affinity for both carbon and hydrogen than these have for one another ; rapid and extensive oscillations are constantly going on, the slightest increase of which must be followed by a new arrangement of molecules. A sharp tap so increases these oscillations that the equilibrium of the tower is disturbed, the weak bands between

the oxygen and the nitrogen are severed, and the free oxygen is immediately pounced upon by the carbon and the hydrogen, which are set free from one another, each of these elements taking up a certain quantity of the freed oxygen ; the atoms of nitrogen having, of course, a strong affinity for one another, combine, and a small portion of oxygen is set free. The amount of energy released here is very great indeed, and it is the more readily observed, and even measured, from the fact that the process goes on rapidly and violently, as it usually does where the resolution is that of a very complex body, into extremely simple substances. Other examples given by Bunge are the resolution of nitrogen trichloride into its constituent elements by "contact with various substances, such as phosphorus, phosphorus compounds free from oxygen, selenium, arsenic, some resins (other kinds being inert), non-volatile oils, &c. ;" and he instances chlorate of potash, which splits up into chloride of potash and oxygen at a certain temperature, and, in the presence of binoxide of manganese, ferric oxide or oxide of copper at a much lower temperature ; he says : "The presence of this substance probably so modifies the heat-wave, that the atoms of the chlorate of potash are more easily thrown into responsive vibrations, and thus decomposed. In the same way peroxide of hydrogen decomposes on being brought into contact with platinum, gold, silver, binoxide of manganese, &c. In these cases it is called an effect of contact, or a catalytic effect. We can form the following hypothesis of the process which goes on here, as in the cases above cited : The substance which acts 'catalytically' exercises an attraction on one of the atoms in the unstable molecule. It does not necessarily always unite with the atom, but the unstable arrangement of the atoms in the molecule is invariably altered to a stable one."

Now let us see what actually takes place when grape sugar, of which we have already spoken, is being split up into alcohol and carbonic acid. We have seen that there is a rise of temperature quite distinct from any heat that is applied to bring about the fermentation. It has been proved that in some way or other the presence of the yeast-plant has a very definite effect in starting a process of fermentation, and there are theories as to the *rôle* that this yeast-plant plays in starting the fermentation. In the first place,

it can only bring about the detachment of the molecules or bricks of a substance when the motion of the molecules of that substance is started or extended by the action of a certain degree of heat; thus fermentation will not take place unless the material to be fermented is kept at a temperature of from 10° to 40°. This increased temperature acts, probably, in two ways: first, by increasing the motion of the molecules as above stated, and secondly, by enabling the protoplasm to act more energetically, by increasing the rate and extent of molecular motion within the organism itself. The determining motion, according to Bunge, "might proceed from the vital functions of the cell. But it is likewise conceivable that certain substances occur in the cell, and that these substances act in a similar manner to the catalytic bodies in the examples adduced above." Heat and moisture are both necessary factors in all processes of fermentation, but neither of these alone can give rise to it.

Pasteur, who was really the first to understand this subject so far as to be able to throw light upon it for others, looked upon "fermentation, properly so called, as a chemical phenomenon, co-relative with physiological actions of a peculiar nature," the elements in which the peculiar physiological actions were manifested being spoken of as ferments which were not dead albuminoid matter, as held by Liebig and his school, but actually living organisms "of a peculiar nature in this sense, that they have the property of exercising all the functions of their life, not excepting 'vegetative multiplication,' without necessarily employing the oxygen of the atmospheric air;" and he thus generalizes his results: "Guided by all these facts, I have been gradually led to look upon fermentation as a necessary consequence or manifestation of life when that life takes place without the direct combustion due to free oxygen." This opened up exceedingly wide and important questions: Was it possible that all living plant-cells might have the power of inducing fermentation in a more or less marked degree? and did yeasts differ from other living cells only in the fact that they had more marked powers of acting on certain carbohydrates, and of exciting alcoholic fermentation? Experiments on fruit, on barley, on leaves, all went to prove that the elementary cells of plants possessed within themselves this power of inducing fermentation of sugar that was already

present or of sugar that was artificially introduced. By a natural transition from the observation of vegetable cells to a study of animal cells, we are led to consider how important may be the part played by these latter in the digestive tract and in the tissues of the body, especially when they are called upon to act in conjunction with vegetable ferments, either in normal or in abnormal positions. Fermentation, then, may be looked upon as an ordinary chemical transformation of certain substances taking place as the result of the action of "living" cells, the nature of the fermentation and of the substances ultimately resulting being due, firstly, to the nature of the fermented body; secondly, to the nature of the organism which induces the fermentation; and thirdly, to the physical conditions under which the fermentation takes place. Thus the results may be extremely complicated if a mixture of ferments, say an alcoholic, a lactic, and a butyric, be sowed in a single nutrient material; but if we sow only one, say the alcoholic, the sugar will split into alcohol and carbon dioxide; if we sow the butyric fermentation, butyric acid will be formed as a result of the splitting up of the sugar; and so on. As will later be seen, the special name of any fermentation serves to indicate merely that some special product predominates. In the alcoholic fermentation, alcohol is the chief product, but there are also formed as bye products, glycerine, succinic acid and a number of other substances, the amount and nature of these bye products varying, first, with the yeast, and second, with the conditions under which it is allowed to grow. The character and aroma of beer and wine indeed depend essentially on the formation of such bye products—compound æthers. It is of course possible, nay, even probable, that what bacteriologically may be termed impurities, may effect the same result, and that special aroma and flavour may depend upon the presence of small quantities of other organisms than the special yeast used. Further, the activity of the process is dependent in a very marked degree upon the nature of the fermenting substance, and a medium which may afford ample material for the carrying on of one kind of fermentation is absolutely valueless as a medium for other fermentations. The only real difference that exists between a pure alcoholic or butyric fermentation and the complicated fermentations which take place in the animal body or in putrefactive processes is,

that in the one we have a single ferment only, playing its part, acting on comparatively simple and non-complicated media, whilst in the other we have a complex substratum for the growth of the organisms and a considerable variety of organized ferment cells.

Fermentation may be considered from two points of view—first, as merely a chemical process which is started by the *products* of micro-organisms or yeasts, in which it resembles many other chemical reactions which are initiated by light, heat, a blow, or some other molecular activity unassociated directly with organic life ; whilst, under the second heading, it may be looked upon as due to the action of living protoplasm or cells, special fermentations being induced by special organic forms. The soluble products of these organisms, however, appear to play a secondary part in the process of fermentation, some accelerating, others interfering with it. The process appears to be associated with the necessity which there is for the organized ferments to obtain certain elements for their growth and development—elements which can only be obtained under special conditions, and which, if obtained otherwise, do not lead to the breaking down of the substance which should be fermented in the usual fashion.

As we have already seen, the early history of bacteriology was almost entirely associated with the work that was done in connection with fermentation, and it was not till Cagniard-Latour demonstrated that his yeast was made up of small cells which appeared to be capable of reproducing themselves by budding, that the inevitable conclusion was drawn that these globules of yeast were really composed of vegetable protoplasm, and that it was in consequence of their growth and proliferation that sugar solutions underwent the process of fermentation with the evolution of carbonic acid gas and the production of alcohol. Schwann and Kützing, independently, arrived at the came conclusions after obtaining the same results, and other observers[1] soon corroborated the observations made by these pioneers, although the whole facts were not discovered at once, and the knowledge of the structure and life-history of the yeast-cell that we now

[1] Kieser, 1814 (*Schweigger's Journal*, No. 12, p. 229) described spherical corpuscles, all of nearly the same size, which were transparent and motionless, and Desmazières depicted yeast globules in 1826 ("Ann. des Sciences Naturelles," p. 4).

possess has only gradually been accumulated. These cells are composed of a granular protoplasm surrounded by a definite envelope. When these vesicles or cells are watched during their development, growth, and multiplication, there may be seen, at or near one or other extremity of each, small protoplasmic bodies, which are projected beyond the general outline of the cell, and which gradually but surely increase in size. Ultimately there is a constriction, more or less marked, between the original cell and the bud, and the bud grows to the size of the parent cell; the same process is repeated time after time, until there is formed a chain or series of ellipsoidal or rounded yeast-cells. At one time it was supposed that there was no development either of spores or of mycelial chains, but, thanks to the researches of Reess, by whom the presence of spores within the cells of certain forms of yeast was demonstrated, and to those of Hansen, who was able to confirm their observations as regards spore formation, and also to demonstrate the presence of typical chain mycelia as well as of the budding form of mycelium, these organisms have been put into a separate family by botanists, who have given them the name of Saccharomyces, sugar fungi, or yeasts. These saccharomyces are indeed to be looked upon as fungi, for although they are closely related to the algæ in many respects they contain no chlorophyll.

Many of the later observers made very definite statements, founded apparently on very accurate data, that the process of alcoholic fermentation is closely bound up in the question of organized ferments; nevertheless Liebig continued to defend his doctrine of unorganized[1] ferments with great ingenuity and vigour. His theory was that fermentation was the result of "internal molecular motion which a body in the course of decomposition communicates to other matter in which the elements are connected by a very feeble affinity" (Schützenberger, on Fermentation, p. 40). "Yeasts, and in general all animal and vegetable matter in a state of putrefaction, will communicate to other bodies the condition of decomposition in which they are themselves placed. The motion which is given to their own elements by the disturbance of equilibrium is also communicated to the elements of

[1] The term unorganized is not here used in its modern signification, which will be mentioned in the next chapter.

bodies which come into contact with them." This was nothing more than an extension of Willis' and Stahl's view of fermentation ; they held that a ferment is a body which has a peculiar internal motion which is capable of being transmitted from the ferment to a fermentable matter. So fascinating and plausible a theory, of course, received wide recognition, and until Pasteur's admirable demonstrations of his theory of fermentation were made, had received very general acceptance, especially amongst German chemists and biologists. The mechanical theory and the theory of catalytic forces as used in the old sense have now been laid aside, and the vitalist theory—expressed in the following words by Turpin : " Fermentation as effect, and vegetation as cause, are two things inseparable in an act of decomposition of sugar "—has taken the field against all opponents. This theory is that living organisms build up structures and develop energy from the materials in which they live, and break up by their vital activity, either directly or through a soluble ferment, the sugar in which they grow. In this theory albuminoid material is considered to be necessary for the process of fermentation or decomposition only in so far as it is required for the nutrition of the micro-organism, it being denied that nitrogeneous elements play any such part, as that ascribed to them by Liebig, of producing the molecular motion, which brings about the splitting up of the sugar, by undergoing a spontaneous decomposition. Albuminoid material, in fact, is merely an accompaniment of the process of fermentation—a necessary one, no doubt, but one not in any way playing the part of causal factor.

What takes place in brewing, a process which, though until recently incompletely understood, has long been carried on on an enormous scale in most northern countries ? Malt is barley in which a certain proportion of the starch of the grain has been converted into sugar by the process known as "malting." This consists essentially in moistening the grain several times, keeping it at a temperature high enough to promote its sprouting, during which a substance called diastase is developed as the result of the vital activity of the cells in the germinating grain which acting on the starch converts it into sugar. As soon as this takes place the sprouting is stopped by raising the temperature and then by

drying the grain to kill the young plant and so prevent further sprouting. To obtain a fermentable liquid, a solution of the sugar and of the other soluble constituents of the malt is made in hot water; this is allowed to cool to a temperature of about 16° C. A certain quantity of "high" yeast is then added to the solution, and during the process of fermentation the temperature may run up to 18° or 20° C. After a time little bubbles of carbonic acid gas are seen to rise, the yeast increases in quantity and gradually rises to the surface, whence it is skimmed off, and may be again used to set up fermentation, if still pure. The fluid becomes bright, clear, and sparkling (from the presence of carbonic acid), and contains a certain proportion of alcohol; whilst the sugar, if the fermentation has been properly carried on, has almost entirely disappeared. This is what is known as "high yeast" fermentation. It goes on most readily at a comparatively high temperature, and the yeast rises to the surface as it is formed, bringing up with it a certain proportion of the impurities contained in the liquid, the heavier particles falling to the bottom. The process goes on rapidly, but unless great care is taken it is said that there is a danger that impurities may get in, and that secondary fermentations may be set up, though this is a position now scarcely tenable in these days of India Pale Ales.

The "low" fermentation is brought about by a ferment which acts more slowly, at a much lower temperature, and through the agency of yeast-cells that sink to the bottom as they are formed. This fermentation of beer must be allowed to go on at a temperature of from 4° to 5° C., and the fluid is not completely ripened until the end of about fourteen days. This low temperature is maintained in the small breweries by inverted cones of metal, containing ice, which are allowed to float in the fermenting liquid; they are kept constantly supplied with ice, and the number used is regulated according to the temperature of the external air. In the larger breweries the same results are obtained by passing currents of purified cool air over the surface of the fermenting tanks, which, as a rule, are underground, so as to allow of the temperature being maintained at an extremely equable level. Formerly all beer was made by the high fermentation process, a system that still prevails in this country, but

in Germany, Austria, and Scandanavia, and also in France, the low fermentation has now almost entirely ousted the high form. There can be little doubt that this is due, in part at any rate, to the impetus that Pasteur's studies gave to the subject of the careful examination of yeast; he pointed out that at the higher temperature there was greater danger of contamination by organisms which produce other fermentations than the alcoholic, and that these micro-organisms unable to flourish at the lower temperature, would at the higher temperature grow most luxuriantly. This of course is undoubtedly true, but it should be remembered that where the brewing is in the hands of men in a small way of business the conditions are very different from those where all the apparatus and skill that capital can command are at the disposal of the brewer. If it were once known which was the best kind of yeast for high beer fermentation it would be possible to obtain pure cultivations, and with it so to conduct the beer fermentation by attending most carefully to the cleanliness of the vessels in which beer is made and stored as to obtain a very pure beer, having excluded all organisms that by their fermentation might render it unsound. The low beer may be brewed in smaller quantities and under less favourable conditions as regards the possibility of keeping the yeast pure than at higher temperatures. The yeast then used causes the fermentation to go on at such a low temperature, that a large number of the organisms which usually give rise to impurity in the beer cannot multiply. Such beer, however, can only be stored when the temperature is kept very low, for as soon as this begins to rise the dormant spores of other organisms begin to develop, set up various acid fermentations and spoil the beer. The different methods of brewing are also determined in part at any rate by the climates of the different countries the cool, light beer apparently being more palatable in warm continental countries; the heavier, with its own peculiar flavour, being usually more sought after in this country.

Given pure yeasts, thorough cleanliness, and means of keeping out other organisms, both beers may be kept sound even though the temperature be comparatively high. To obtain such pure yeast it is necessary first to take a single cell, and from this to grow a series of buds and chains in a sterilized wort, to break this up into separate portions of seed

material, to produce other crops from this, and so on until a sufficient quantity of pure yeast is obtained to bring about the special fermentation in the large mass of wort. This method has another very great advantage—it enables an investigator to take a single yeast-cell and to follow it in its life-history ; in doing this Hansen completed the work that Pasteur had commenced.

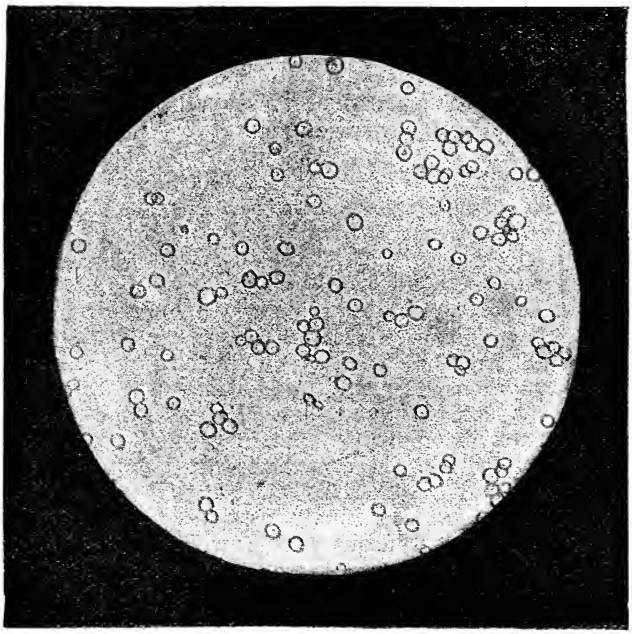
As the process of obtaining pure cultures is of great interest not only to scientific investigators but also to practical men, we may here give it in brief.[1] In a Pasteur flask containing wort, a cultivation of the yeast to be experimented on is started and carried on as vigorously as possible. A quantity of water, that has previously been sterilized by boiling, is added to the growth ; the yeast-cells in a drop of given size are then counted under the microscope. Let us suppose that ten cells are found ; a drop of similar size is then transferred to a flask containing a known volume, say 20 c.c. of sterilized water, so that we have one yeast-cell for each 2 c.c. of water. The flask containing the 20 c.c. of water with the added 10 yeast-cells, is now thoroughly shaken and then divided equally, 1 c.c. being placed in each of twenty flasks containing sterilized wort.[2] If the separation has been complete ten out of twenty flasks should contain one organism each ; but this of course cannot be absolutely depended upon. After carrying the process to this stage Hansen shakes the flasks very vigorously, by which he separates the cells as far as possible from one another, puts them in an incubator, and allows them to remain perfectly still, so that the cells may sink to the bottom or become attached to the walls of the flask. If there are more cells than one in any flask, they should have been completely separated by the final shaking, and each will probably take up a separate position on the wall or bottom of the flask, and at the end of several days "the flasks are carefully lifted and examined, and it is noted whether one or more white specks have been formed on the walls of the glass ; if only one such speck is found we have then a pure culture." This method is especially useful in the case where the yeast-plants are at all weakly, as all yeasts grow much more luxuriantly and strongly in a fluid medium than they do on gelatine plates, even when wort is added to the gelatine in order to render it more suitable for the nutrition of the yeast. Where the species are mixed, but where the individuals that we desire to separate are vigorous, the gelatine method may be used with advantage, especially as the individual colonies can then be examined from time to time, or continuously observed under the microscope as they pass through their various phases of development.

The characteristic appearances that were said at one time to belong to the yeast-plants have been proved by Hansen to be really non-existent, except in a very limited sense. He maintains that the shape and relative size of the cells and the appearance of the protoplasm of a yeast-plant are

[1] For the method of carrying on the process on a large scale see Jörgensen's work.

[2] 1 c.c. or one cubic centimetre = about 16 minims.

not really specific characteristics. He finds that within certain limits the same species, under different external conditions, may exhibit very different appearances, but he also holds, and brings forward very strong proof in support of his position, that there are limits to the influence which can be exerted on the cells of a species, and that different species exhibit very different characteristics when placed under similar conditions ; thus, for example, Saccharomyces



Photomicrograph of Saccharomyces Cerevisiæ. × 500. Mother cells with small buds coming off. Yeast from an Edinburgh Brewery.

cerevisiæ, when developed in the ordinary manner and then grown vigorously for twenty-four hours at a temperature of 27° C., exhibits the ordinary appearance of rounded or ovoid cells with a formation of septa in some cases, and with more or less well-developed cells in others ; whilst this same organism, when cultivated at 7.5° C., occurs in the form of dense colonies with beautiful mycelium-like branchings.

As Hansen's work is the most recent and most complete that has yet been carried out, we may give a short account of the species that he describes in his classification as quoted by Zopf. He divides the yeasts into three groups: Saccharomyces cerevisiæ, 11–5μ most frequently 8–6μ in diameter, which contains a single species only; Sacch. Pastorianus, 17–2.5μ average diameter 8–7μ, in which he describes three species, all of them oblong or sausage-shaped; whilst the third group, Sacch. Ellipsoideus, 13–2.5μ in diameter, the most usual size being about 8–7μ, in which most of the cells are oval or rounded. These forms so overlap one another that by mere microscopic examination it is impossible to determine whether we are dealing with absolutely pure cultures or not.

Hansen's researches were carried on practically, and on a most extensive scale, in connection with a large brewing industry at Old Carlsberg in Copenhagen, and he very naturally turned his attention to the classification of beer yeasts for the purpose of obtaining pure and healthy yeasts for the production of beer.

Taking up the subject where Pasteur nad left off he, however, again went over some of the old ground, and studied the various yeasts with the greatest care; cultivated them in all kinds of media, noted their behaviour under different conditions as regards temperature, moisture, presence or absence of oxygen and the like; and as the result of prolonged and ingenious experimental work he was able to dispel much of the inaccuracy that had grown up around the subject, and to give detailed accounts of the life histories of the saccharomyces. He found that the older observers had not understood the conditions under which spore formation occurs; that it was not possible, as Reess had stated, to classify the saccharomyce by a mere microscopic examination, in which the characters relied upon were necessarily merely the form and size of the cells and spores. He also pointed out that even Reess' observations on the conditions under which spores were formed were not to be relied upon. That they were not the result of the yeast being attacked by mould fungi or bacteria, as Reess and Van Tieghem had maintained, both of whom believed that spores were evidence of disease in the yeast-cells, he was soon convinced. Nor could he believe that Wiesner and Brefeld, who made very careful study of yeasts, without, however, enjoying the advantages of

methods for obtaining pure cultivations, were right. These observers had ascribed to certain forms of yeasts the power of forming spores; whilst in others the same power was denied, Wiesner holding that spores could not develop from pressed yeast, although they could from beer yeast, and Brefeld maintaining that cultivated yeast had lost its power of forming spores, whilst the wild yeast still retained this faculty. To evolve some kind of order from this confusion was the task that Hansen set himself; he wished to determine the conditions under which ascospores could be formed.

For this purpose he used the method of complete aeration that is obtained by the use of Engel's gypsum blocks. To well-baked plaster of Paris add distilled water until the plaster is nearly liquid; pour this on to a sterilized glass plate, on which rests a small mould of thin metal or paper, made rather less than the vessel in which the experiment is to be carried on. These blocks are first thoroughly sterilized by means of heat, after which a small particle of yeast is placed on the upper surface of one of them, an air chamber is sterilized, and in this a small vessel containing water, in which the block rests, is kept. The whole vessel may be placed in an incubator if any special temperature is required, or it may be left at the ordinary temperature of the room.

He found that the following conditions were necessary for the perfect formation of spores: a plentiful supply of air (oxygen) and moisture; a certain temperature—the most suitable for the six forms that he examined being about 25° C.; a young condition of the protoplasm of the yeast-cells, the older cells with their thickened walls appearing seldom, if ever, to give rise to spores. As regards temperature, he found that the extremes, at which the individuals of the different species grow, vary somewhat, the lowest temperature at which they are developed being from .5° to 3° C.; whilst at the other extreme he found that they could still grow at a temperature of 37.5° C. He found that spores became visible as irregular bodies formed from the cell contents in a period of about thirty hours from the commencement of the sowing of the yeast-cells when the temperature was kept near the higher extreme, or as low down as 25° C.; but working with lower temperatures, differences occur in the different species, though in none of them does the development of spores take place so rapidly as at the higher temperatures; for example, he found that at 11.5° C. the Saccharomyces cerevisiæ does not form spores until a period of ten days has elapsed; whilst, on the other

hand, Saccharomyces Pastorianus II., kept under exactly the same conditions, gives evidence of the commencement of spore formation after seventy-seven hours ; *i.e.*, supposing that the previous conditions have been the same for the species experimented upon.

This single observation proved to be of enormous importance to brewers, for Hansen's pupils, Holm and Poulsen, determined that it was possible by Hansen's method to show that pure cultivated yeasts produced spores at a much later date—under similar conditions—than did those saccharomyces that were capable of producing certain diseases in beer when present in proportions of $\frac{1}{40}$th of the yeasts introduced ; and as it was found possible to determine by this method the presence of wild yeasts when they occurred in the proportion of $\frac{1}{200}$th of the whole of the yeast used, it was an easy matter to determine within a very short time whether a yeast was fit to be used for brewing purposes or not ; in the case in point, forty hours at the temperature of 25° C. was sufficient for the purpose. It was found, indeed, that all the bottom yeasts might be separated from one another by making cultivations at a temperature of 15° C. and at 25° C.

Having obtained these pure cultivations, and being so successful with the study of the spores, Hansen turned his attention to the other characters, by the use of which he was able to separate in a still more definite manner the various species of saccharomyces one from another. He subjected to a most careful examination the films which appeared on the surface of liquids that were undergoing fermentation. It had been supposed that the Sacch. mycoderma was the result of a yeast fermentation ; but Hansen found that the saccharomyces film was something quite different from the film formed by the Sacch. mycoderma, and he came to the conclusion that film formation must be looked upon as a phenomenon that may be met with under certain conditions wherever micro-organisms are or have been growing. Here again the presence of a plentiful supply of air is an absolute necessity ; another essential condition is a state of perfect rest of the surface of the fluid in which the process of yeast growth and fermentation is going on. It is interesting to note that when carbonic acid gas is passed through the fluid and is allowed to accumulate on the surface these films are not formed,

so that in fermenting tanks in which, of course, large quantities of carbonic acid gas accumulate the films are not usually met with. From this one may gather how laboratory experiments may go wrong, or give untranslatable results, when the exact conditions met with in nature, or on a large scale, are not adhered to; it also explains the different results that have been obtained by various workers, the conditions in their experiments not always being the same. For instance, in Chamberland or ordinary flasks covered with filter paper the film forms will develop rapidly as soon as the primary fermentation is completed; whilst in the closed Pasteur flasks, in which the air cannot be changed rapidly, the films grow comparatively slowly.

To produce these films Hansen proceeded as follows: Having obtained his pure cultivations, drop cultures were made into carefully sterilized four-ounce flasks, half filled with sterilized wort and protected from falling particles by being covered with sterilized filter paper. As soon as the films became apparent to the naked eye they were examined. They appear, according to Hansen, as small opaque points, which gradually increase in size and then run together, forming irregular patches floating on the upper surface, with a convexity on the side resting on the fluid. The film at length overspreads the whole surface and becomes adherent to the wall of the flask at the margins; on shaking the flasks much of the film can be made to sink, but a new one forms to fill up the gaps that are left.

These young films he divided into two groups, the first consisting of Sacch. cerevisiæ, Sacch. Pastorianus II., Sacch. ellipsoideus II., in none of which could he find mycelium-like colonies (at one time it seemed that Hansen's researches had demonstrated this fact); whilst in the second group, which included Sacch. Pastorianus I. and III. and Sacch. ellipsoideus I., such mycelial colonies were early developed. Later researches have tended to show that this division is very much a matter of time, and that it cannot now be looked upon as having any real scientific value.

As regards the temperature at which these films are developed Sacch. cerevisiæ and Sacch. ellipsoideus I. are developed between 38° and 6° C., the three Pastorianus varieties between 34° and 3° C.; Sacch. ellipsoideus II., 38° to 40° C., and 3° C. The film of this latter appears to be the

most vigorous at all temperatures, at 13° C. it develops so quickly and vigorously that it out-distances all the others, giving at 23° C. a film covering the whole of the surface in from six to twelve days ; whilst the others at the same temperature only gave a much more delicate film in from three to five weeks. The next to this as regards rapidity of the formation of the film is Sacch. Pastorianus III., which at the temperature of a warm room forms a film much more rapidly than any except Sacch. ellipsoideus II.

It may be noted, in connection with the limits of temperature at which this film formation takes place in the three Pastorian varieties, that they cease to develop and form spores at a temperature of 36° to 38° C., if this be continued for ten or eleven days ; whilst the other three species all continue to grow for a much longer period.

In addition to the forms studied by Hansen others may be mentioned. Below is appended a short classification of the more important forms of yeasts, with some of their more characteristic features.

The Saccharomycetes or yeast fungi are a family of ascomycetes, divided into two very unequal groups—the Saccharomyces of Reess and the Monaspores of Metschnikoff; the mode of spore formation is somewhat similar in the two, the only difference being that in the saccharomyces the spores are rounded and are sometimes multiple in the same sporangium, whilst in monaspora there is in each cell a single needle-shaped spore developed. In the first genus are included the following forms :

1. *Saccharomyces cerevisiæ*, may be looked upon as a typical English high yeast, some of the characteristics of which have already been described. It grows as rounded or slightly ellipsoidal cells, which give off small cells by budding ; in the earlier stages of film formation there are formed delicate mycelial-like threads, but as the film becomes older longer and more regular threads are formed. In the yeast-cells nuclei are frequently to be made out, especially on being stained with osmic acid or hæmatoxyline. These nuclei are very distinctly seen in old cultivations.

The development of ascospores takes place most rapidly (after twenty hours) at 30°C., most slowly (after ten days) at from 11° to 12° C., and stops altogether below this. The spores may be very distinctly seen, as they are highly refractile, and their walls are well defined. They are usually from 2.5 to 6μ

in diameter. Film formation takes place most rapidly (seven to ten days) at a temperature of from 20° to 22°, most slowly (two to three months) at 6° to 7° C., and ceases altogether at 38° C., and at 5° C., at the other extreme. Between 20° and 30° C., the cells are frequently sausage-like and irregular; from 6° to 15° C. the cells are usually like the parent cells in younger cultivations, but in older cultivations the forms are like those already described.

This species, like all those investigated by Hansen, secretes a peculiar substance, which, acting on saccharose or crude cane sugar, inverts it to "invert" sugar; the yeast then brings about the fermentation of this latter substance, and also of dextrose and maltose, giving rise to the formation of alcohol and carbonic acid gas, with an evolution of heat and great multiplication of the yeast-cells. It does not seem to exert any action on lactose or milk sugar. In this respect these ferments resemble mucor racemosus, which first brings about the inversion of cane-sugar; it also secretes invertase, which causes inversion of saccharose, the products of which it ferments; it also sets up a weak fermentation in beer wort of the maltose and dextrose present in that liquor.

Reess' genus of *Saccharomyces ellipsoideus* Hansen divides into two—I. and II.

2. *Saccharomyces ellipsoideus* I. is really a "wild" species of wine ferment; in beer wort it grows as a low yeast. It is usually rounded or ellipsoidal in shape, though it sometimes assumes the sausage form. The spores, of which two to four are usually found in a single ascus, are from 2 to 4μ in diameter.

These spores develop most rapidly (in twenty-one hours) at 25° C., most slowly (eleven days) at 7.5° C., and are not formed at all at 32.5° C. at the one extreme, and at 4° C. at the other. Grown on the surface of beer wort gelatine, its colonies form a peculiar net-work along the line of the inoculation streak. The surface membrane is formed rapidly (eight to twelve days) at a temperature of from 33° to 34° C., most slowly (sixty to ninety days) at 6° to 7° C.; it is always a delicate membrane, and cannot be grown at 5° C. on the one hand and at 38° C. on the other.

The most characteristic growth takes place at from 13° to 15° C., when it occurs as a complicated branching mass, with elongated cells, or threads, arranged in rows, with several lateral processes coming off at the points of junction. Secondary branches are formed at the constrictions of the primary branches.

It appears to exert as powerful and rapid a fermenta-

tion process as does the Saccharomyces cerevisiæ on the various carbo-hydrates on which that ferment acts.

3. *Saccharomyces ellipsoideus* II. is also a "wild" or wine fermentation yeast which gives rise to the muddiness of beer.

It is essentially a low yeast, the film that forms is exceedingly delicate; it makes its appearance in from three to four days at a temperature of 33° to 34° C., but not for five or six months at 3° to 5° C. At 2° and at 40° C. no film forms. Young cultivations at 15° C. are usually somewhat rounded or egg-shaped, whilst the older cultures show longer mycelial rods, with forked transverse shoots given off at the joints. Asci containing from two to four spores may be egg-shaped, slightly irregular or elongated. The spores measure from 2 to 5μ in diameter; they are developed most rapidly at 29° C., most slowly at 8° C., and are not formed at above 35° or below 4° C.

Hansen does not look upon *Saccharomyces Pastorianus* as a pure species; he divides it into three.

4. The first of these, *Sacch. Pastorianus* I. (Hansen), is a wild yeast, spores of which frequently occur in the atmosphere of breweries. It gives an unpleasant bitter taste to beer; it, also, is a bottom ferment, occurring as elongated ellipsoidal or pear-shaped cells, from which small apical or lateral branches may be given off.

The asci are usually elongated or rounded; they may contain two spores or multiples of two, up to eight or even more. The spores vary very much in size from 1.5 up to 5μ; are developed most readily (twenty-four hours) at a temperature of 27.5° C.; most slowly (fourteen days) at a temperature of 3° to 4° C. The spore formation ceases at .5° C., and at 31.5° C. The films are usually very delicate, are developed most readily (seven to ten days) at from 26° to 28° C., most slowly (five to six months) at from 3° to 5° C., development ceases altogether at 34° and 2° C. At from 3° C. to 15° C. mycelial-like threads are developed pretty freely in this film and most irregular forms make their appearance; many irregular club and skittle-shaped and other forms are formed in the older films, but fewer in the younger ones; in these films the cells are usually smaller.

5. *Saccharomyces Pastorianus* II. (Hansen) was also separated from the air of the brewery. In gelatine made with yeast water it grows along the line of the inoculation streak (at 15° C. at the end of sixteen days) in the form of colonies with smooth edges. It is a feeble top fermentation yeast when grown in beer wort.

The sedimentary cells of this yeast are mostly elongated, but they may be slightly rounded, varying considerably in size. The cells found in the film are rounded, egg-shaped, or somewhat elongated. The spores are from 2 to 5μ in diameter; the asci are usually elongated and the spores occur in multiples of two. They are formed most rapidly (twenty-seven hours)

at a temperature of 23° C., most slowly (seventeen days) at 3° to 4° C., and cease to be formed at 29° C. and at .5° C.

This yeast gives rise to neither cloudiness nor to any unpleasant bitter taste. It secretes an invertase and causes fermentation of all the carbo-hydrates that are fermented by the other yeasts of this group. In old cultures of the films the cells are small, very irregular in shape, and thread-like, like the preceding.

6. *Saccharomyces Pastorianus* III. (Hansen) is, according to Hansen, one of the causes of turbidity in beer. Grown on yeast water gelatine at a temperature of 15° C., at the end of sixteen days the colonies present peculiarly fringed edges; grown in wort it gives rise to a top fermentation, and causes considerable turbidity with a production of alcohol and carbonic acid gas.

The spore formation is very much like that in the preceding species: it takes place most rapidly (twenty-eight hours) at 25° C., most slowly (nine days) at 8.5° C., and ceases at 29° and at 4° C. The film appears in the form of small flakes most rapidly (seven to ten days) at 26° to 28° C., most slowly (five to six months) at 3° to 5°, and ceases altogether at 34° and 2°. Here again the elongated or sausage form predominates, but large and small rounded and oval cells are also present in the sedimentary forms in the films at from 20° to 28° C. The cells are of much the same shape as are those of the sedimentary yeast, but at a temperature of from 15° down to 3° C. there are elongated mycelial-like threads which in old cultures become still more characteristic. These mycelial-like threads are developed at the above temperature, which is much lower than in the case of the threads in Saccharomyces Pastorianus I., where they are most characteristic at a temperature of 13° to 15° C. At the same temperature, 15° to 3° C., the cells in Saccharomyces Pastorianus II. are oval and rounded.

Hansen describes in less detail a number of other ferments which produce alcohol from sugar.

7. *Saccharomyces Ludwigii*, though found in the sap of oaks, grows freely in yeast water, when it appears as a peculiar caseous mass or as fungus-like specks which float in the fluid. One great peculiarity of this form is that it may be so modified by cultivating it in beer wort through several generations at a temperature of 25° C. that it does not form spores, or that it forms them but slowly. The spores, when formed, are usually from one to four in number, but there may be more; the cells of the film are usually considerably elongated. The film formation goes on most rapidly at about 25° C.; at the ordinary temperature of

the room it goes on very slowly, taking a whole month to form a comparatively delicate membrane. In very old cultures well-marked mycelium formation may be met with in which the cells are ellipsoidal, elongated, or sausage-shaped, or somewhat club-shaped. It is capable of acting on grape sugar ; it inverts cane sugar and ferments it ; but has no action on maltose, lactose, or dextrine in yeast water, nor does it attack starch.

8. *Saccharomyces Marxianus*, named after its describer, was first found in wine. Hansen studied it most closely. He found that in beer it develops as small ellipsoidal and egg-shaped cells, with here and there sausage-shaped cells, which are often combined into colonies. There are developed on a quiescent fluid small viscid masses, some of which remain on the surface whilst others sink to the bottom. The film develops exceedingly slowly, but in it are found cells which resemble very closely those of the films of the first six species of Saccharomyces described. The true film contains oval and short sausage-shaped cells. The spores are not freely developed. When it is grown on solid nutrient media spores are more frequently formed, in which case they are usually oval or kidney-shaped. In beer wort this yeast is not very active, nor is it able to ferment maltose, but it acts vigorously on saccharose, inverting it and then fermenting it with great activity ; it also acts upon dextrose.

9. *Saccharomyces exiguus*, found by Hansen in German yeast, differs from the preceding saccharomyces in the fact that it forms no mycelial threads on beer wort, or on solid nutrient media. It forms spores, but sparsely, and the film is exceedingly delicate, the cells of which this is made up being short rod-shaped or ovoid. It acts on the sugars exactly as does the Saccharomyces Marxianus.

10. A somewhat peculiar saccharomyces belonging to this group is the *Saccharomyces membranæfaciens*, which forms on beer wort a bright yellow tough scum, composed of long and sausage-like cells, which may be closely packed together or may occur singly. It forms spores rapidly, liquefies nutrient gelatine, and is peculiar from the fact that it does not cause fermentation of any of the ordinary carbo-hydrates, nor has it any effect in inverting cane sugar.

11. *Saccharomyces minor* (Engel), who describes it as spherical cells 6μ in diameter, arranged in chains of 6–9

elements. The spore-forming cells are larger 7–8.5μ, and contain from 2–4 spores 3.5μ in diameter. He ascribes to this ferment the action of fermentation in bread, a notion that has since been scouted and again accepted.

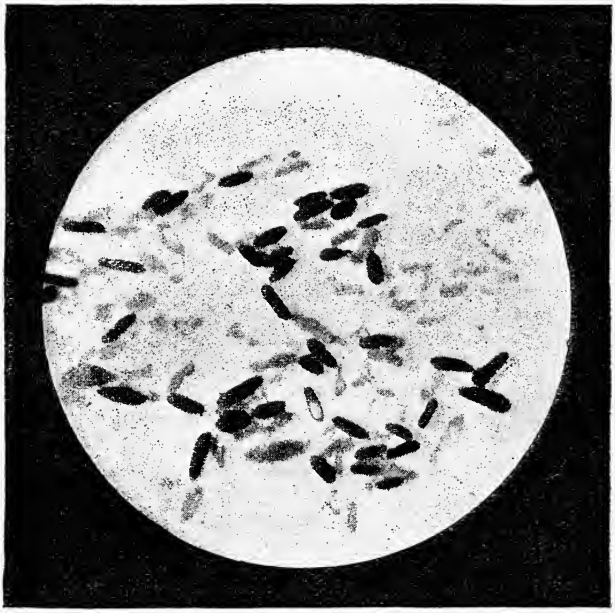
12. *Saccharomyces conglomeratus* (Reess), Hansen thinks is simply a form that may be met with in old films of all the six species that he specially investigated, and in recent works this species has been dropped.

13. *Saccharomyces apiculatus*, described by Reess, can scarcely be said to be a true saccharomyces, although it is included amongst them by Zopf as a doubtful member of the group ; it has not yet been ascertained to have any spore formation, and is therefore retained by Hansen in this group only provisionally. It occurs in fermented wine and spontaneously fermented beer, and, in the hot seasons, on sweet succulent fruits, such as cherries, gooseberries, plums, or grapes ; whilst in the winter it is found in the soil beneath the trees that bear these summer fruits. It occurs in cultivation fluids as lemon-shaped cells—hence the name—though under certain conditions it assumes elongated, crescent-shaped, and rod-shaped forms. It gives off buds of two kinds : one oval, the other lemon-shaped. It is a bottom yeast giving rise to a feeble alcoholic fermentation ; it does not invert cane sugar, but acts on dextrose in yeast water, but does not ferment it completely. Mixed with Saccharomyces cerivisiæ it retards the action of the latter.

An organism that was long classified with the true yeasts is the Rosahefe or Pink yeast of the Germans. According to Hansen, however, no spores are formed during any phase of its development and for the present he excludes it from the Saccharomyces or true yeasts. It belongs rather to the Torulæ.

Genus II. (1) Monospora (Metschnikoff). The single member of this group, *Monospora cuspidata*, is of interest principally because of the elaborate researches that have been made by Metschnikoff on its relation to a peculiar disease of the Daphnia, a small fresh-water crustacean. It occurs as a budding mycelial thread made up of elongated cells which before spore formation become elongated ; there then appears a long, thin needle-like body situated in the centre of the cell in its long axis. This spore is taken into the alimentary canal of the Daphnia, whence it is driven through the walls by the

peristaltic action of the muscles of the intestine. It thus passes into the body cavity or into other tissues, where it is immediately attacked by the white blood corpuscles, or by the connective tissue corpuscles ; or it may first become developed into the rod-shaped vegetative form. When it has once commenced to divide it is no longer attacked by the above cells (which Metschnikoff speaks of as the phagocytes) of the insect. If the Daphnia is in moderately good health the



Photomicrograph of Rosahefe. × 1000. Rose-coloured yeast (?) No spores have been found, and Hansen does not classify it with the yeasts.

monospora is gradually overcome, but if the insect is feeble, or if the monospora is ingested in very large quantities, it multiplies so rapidly that the animal may eventually succumb to its attacks. This form is specially interesting from the fact that it afforded some of the strongest proofs of Metschnikoff's phagocyte theory that he was able to obtain.

We have already seen that the yeast-cell is usually observed in a fermenting liquid as a rounded or ovoid body,

that it gives rise to buds by sending out small processes from its wall, and that these latter then become detached from the mother cell. This cell consists of a distinct membrane and of protoplasm, the former of which may be thicker or thinner according to the age of the cell, whilst the latter may vary very considerably. Whilst the cell is merely growing actively, the plasma or cell contents are homogeneous and highly refractile, but when it is placed in beer wort or other highly nutritive media, it multiplies rapidly and gives rise to the fermentation of the sugar and maltose, and the protoplasm becomes differentiated and undergoes certain changes. Large clear spaces, which are supposed to contain the more fluid part of the protoplasm (vacuoles), are formed ; cloudy granular change occurs in the other protoplasm ; and larger fat globules also make their appearance. As the cell gets older, and consequently less active, the finely granular protoplasm accumulates as a thin layer inside the cell wall, whilst the centre is occupied by clear fluid, in which are floating a number of fatty granules and globules.

It would appear that this last is a somewhat degenerated condition, and that it is due to imperfect nutrition, for if a few of these cells are transferred to a fresh rich nutrient medium, the protoplasm becomes again modified, the cloudy granules disappear, small shoots of the clear plasma pass into the large central cavity, small rounded vacuoles are formed in place of this large central cavity ; these in turn become subdivided, and eventually the whole cell is again occupied by clear protoplasm. By appropriate staining, especially of an older cell, a nucleus may be distinguished, whilst under certain conditions, to be afterwards mentioned, spores or ascospores are formed.

Other organisms which in certain respects resemble the yeast fungi are the Torulæ, which Pasteur described as being somewhat of the nature of yeasts, but different in the fact that they were unable to give rise to such marked alcoholic fermentation. Hansen, however, was able to show that this was not a sufficiently distinct characteristic, as some of the saccharomyces give rise to the formation of little or no alcohol, whilst, on the other hand, some of the Torulæ set up very marked alcoholic fermentation. The great point of distinction is, that none of the Torulæ, so far as has yet been observed, are capable of producing endospores ; all

of them multiplying by budding, some of them also giving rise to the formation of mycelia. It is of course quite possible that such a classification may not hold good, as it has been suggested that some of the torulæ could not be definitely brought within it, as far as non-sporulation is concerned. Hansen describes seven species varying in size from 1.5 to 8μ; some invert cane sugar; some give rise to scarcely a trace of alcohol, whilst others produce as much as 6.2 per cent. of alcohol. They occur as spherical or elongated cells, and cannot be distinguished by the microscope alone from the round cells of the different species of saccharomyces. As a group they have not much action on maltose, and only some of them affect dextrose.

CHAPTER VI.

Fermentation (*continued*).

Soluble Constituents of Yeast—Action of these upon Sugar—Conversion of Glycogen in the Liver and other tissues—Growth of Yeast-Cells in Organic and Inorganic Fluids—Fermentation of Fruit Juice—Ærobic and Anærobic Fermentations—Effect of Free Oxygen on Yeast-Cells—Fermentation not necessarily equal to Growth of Yeast—Enzyme or Unorganized Ferment a Secondary Function—Function depends partly on Organism, partly on Medium in which it is Growing—Peptonizing Function usually requires presence of Oxygen—Various kinds of Fermentation: Lactic, Urea, Butyric, Ammoniacal, Acetic—Formation of Fatty Acids—Mycoderma Aceti—General Processes of Fermentation—Hoppe-Seyler's Classification.

On approaching the subject of fermentation by yeast we find at the very outset that Pasteur and others had noticed a very remarkable peculiarity of the water in which yeast had been mixed, and from which it had again been separated.

It was observed that a certain substance, perfectly soluble in water but precipitable by alcohol, had the peculiar property of inverting saccharose into equal quantities of dextrose and lævulose, and it was at once assumed that this invertin, invertase, or some similar substance, produced through the vital activity of the yeast-cells, was necessary to bring about conversion of starch into sugar (diastase), or of saccharose into invert sugar (invertin), before the cells could bring about a true fermentation, or splitting up and hydration into alcohol and carbonic acid gas, with certain other products to be mentioned immediately. It was noticed by Bertholot and Hoppe-Seyler, moreover, that, even if the living organisms were first killed by the addition of ether, the invertase or invertin still continued to act, and was able to invert a quantity of saccharose altogether out of proportion to the amount of inverting material present. In consequence of the latter part of this observation, many physiologists and chemists maintain that the action of the invertin is essentially due to the setting up of a certain rate and length of

molecular vibration, such wave rate and length being transmitted through the whole body of material to be inverted. In fact, that the addition of the invertin is simply the lighting of the spark that fires the whole train. There is something very fascinating in this theory, and it certainly explains many, otherwise, obscure chemico-physical questions connected with these two subjects—inversion and fermentation. We see at any rate that part of the process takes place entirely outside the yeast-cells, and may go on even when the organisms that produce the inverting material have been killed or completely removed. There are other similar examples of conversion by purely chemical means, as, for instance, where, on the addition of a dilute acid, such as sulphuric acid at a certain temperature, starch is converted into glucose, the heat and the acid setting up such molecular vibration amongst the molecules composing starch, that in the dilute acid there is a rearrangement of molecules, water is taken up, and by hydration of the starch glucose is produced. In this case the conversion takes place much more rapidly and completely at a temperature of 130° C. than at 100° C.

In a similar manner glycogen may be converted into a sugar that will reduce Fehling's solution. This process goes on in the liver and other organs and tissues of man and animals, in which, probably, the secretions of the cells take the place of the sulphuric acid and the high temperature, or of the protoplasm of yeast and other vegetable cells. Let us now, however, see what relation the yeast-cells themselves (as apart from their products) are supposed to bear to the real process of fermentation. If it were possible to obtain an absolutely pure solution of sugar, *i.e.*, a solution containing no nitrogenous elements of any kind, and if we were to place in this a minute quantity of yeast, we should find that a very slight fermentation might take place—*i.e.*, there would be an almost inappreciable diminution in the quantity of sugar present ; a small quantity of alcohol and carbonic acid gas would be developed, but very shortly the process would stop, and there would be no marked increase in the number of yeast-cells found in the whole solution. If now we were to add a small quantity of nitrogen in the form of an albuminoid substance and a certain quantity of extractives and salts, such as are found in the ashes of burnt yeast, there would very quickly be observed a very different

state of matters ; first there would be very marked turbidity of the fluid. If we were using a high yeast this turbidity would rapidly become more and more marked, and there would rise to the surface a yellow scum ; bubbles of carbonic acid gas would be seen rising in the liquid, and a spirituous or alcoholic taste would soon become pronounced. If from the first fluid, *i.e.*, the fluid in which there was nothing but pure sugar, we were to examine the very minute quantity of yeast, we should find that, although there was apparently an attempt at budding in a few of the cells, in most cases there are a series of clear globules within the yeast-cells, that there is a very large proportion of thick walled granular cells (which have certain other peculiarities), that, in fact, throughout the whole we have evidence of very little proliferative activity, and that young, vigorous, healthy, yeast-cells, budding and giving rise to other cells by vegetative activity, are conspicuous by their absence. A microscopic examination, in the case of the yeast growing in the sugar solution containing a small quantity of albuminoid material and extractives, reveals a very different state of matters ; here the yeast-cells are in a state of extraordinary activity, buds are being thrown off from the extremities, terminally or laterally, the protoplasm is evidently exceedingly active, vacuolation is conspicuous by its absence, except in certain cells, and we are at once struck by the extraordinarily large proportion of young vigorous cells—the more active the process the greater the number of the new cells, and the larger the quantity of yeast formed. In one case the yeast-cells die of starvation, although large quantities of sugar are present ; and as the yeast-cells have not been able to grow and reproduce by the exertion of their vegetative activity, they have not been able to resolve the sugar into alcohol and carbonic acid ; badly nourished or dead yeast-cells, therefore, exert little or no influence in bringing about an alcoholic fermentation. In the other case the cells have supplied to them all the elements necessary for the nutrition of their protoplasm. The carbon, the hydrogen, and the water, may all be obtained from the sugar solution, albuminoid material of course supplies the requisite nitrogen, whilst the ash of yeast, which has been added, contains all the other elements necessary for the nutrition of the yeast-cells.

Under these conditions, the cells, as we have seen, may undergo rapid development, growth, and multiplication, and we have an increase in the amount of yeast, and at the same time in the amount of alcohol and carbonic acid gas generated. These two experiments afford most exact evidence that Liebig's theory was essentially incorrect, and that Pasteur's theory that true fermentation was the result of the action of the living protoplasm, gives us the key to the whole situation.

It was, naturally enough, objected that as the fermentation process could not go on except in the presence of an albuminoid material, this might really be the cause of the whole process, and many most elaborate experiments were brought forward to prove that it was this organized but dead material that was the real and primary factor in the process.

Pasteur, however, equal to the occasion, was able to demonstrate in a most convincing manner, that the nitrogen might be supplied to the organism in the form of inorganic salts, instead of being presented as albuminoid material. He utilized for his purposes a mixture containing 150 c.c. of a 10 per cent. solution of sugar candy, .5 grammes of the ash of yeast, .2 grammes of bitartaric of ammonia, and .2 grammes of sulphate of ammonia. He found, on introducing Saccharomyces Pastorianus into this solution, that a somewhat slow but very complete transformation of the sugar took place, and that the nitrogen from the ammonia was used up by the growing yeast-cells, which at the same time increased enormously in number. It was thus evident that these mineral salts could take the place, in fermentable liquids, of "media of natural composition." He found, however, that the process went on more slowly, that somewhat peculiar forms of yeast showed themselves, and that an essential factor for the success of the experiment was that no other organisms should be allowed to make their way into the fermenting solutions—*i.e.*, the absolute purity of the various materials of which the nutrient solution was composed, and of the ferment itself, must be guaranteed, and any relaxation of the strict conditions of extreme purity was invariably followed by an interference with the vital manifestations and physiological actions of the yeast organisms.

It would appear, indeed, that although the fermentation

is, under such conditions, very complete if sufficient time is allowed for the process, the yeast-cells experience a certain difficulty in wresting the nitrogenous elements from the inorganic ammonia salts, and that all other conditions must be extremely favourable, in order to allow of their taking up, and utilizing for their own use, inorganic nitrogenous material. The presence of other organisms, for example, that have a stronger affinity for nitrogen in this form, *i.e.*, organisms which are better adapted to exist under such conditions, and which can, by their vital activity, interfere with the growth of the yeast-cells, remove from the sphere of action of the yeast-cells material that is absolutely necessary for their rapid and perfect morphological and biological development, as a result of which fermentation and hydration of the sugar do not take place in the ordinary way ; other substances or bye products are formed, and the decomposition of the sugar is incomplete, or is irregularly carried out.

What, then, are the conditions necessary for the growth of the fermenting organism in a fermentable fluid ? In the process of wine-making it is a well-known fact that the fermentation is set up by some organism, which, though present either as young cells or as spores on the outside of the grape, cannot attack the juice so long as the skin remains unbroken—a fact brought out by Davaine at a very early stage of his researches. When, however, the grapes are plucked, the skins are bruised and the juice is set free, the fermenting organism,—the wine yeast or Saccharomyces ellipsoideus, or some similar variety—utilizing the grape juice, which contains not only sugar but also all the elementary constituents necessary for its nutrition,—grows vigorously and sets up the vinous fermentation. It is a remarkable fact, but one well known to wine pressers and fermenters, that for the commencement of this vinous fermentation there must be an access to free oxygen, or oxygen mixed merely mechanically with nitrogen of the air to the fluid that has to be fermented, as without this the yeast spores and old yeast-cells are utterly unable to develop or to give rise to active and vigorous yeast-cells. Pasteur insists that we have evidence of the necessity for the presence of such free oxygen in the fact that the fermentation of grapes takes place much more rapidly and completely when the grapes are left attached to the stalks of the bunches, by

which means there is a freer circulation of air allowed to take place through the fermenting mass than when they are plucked from the stalks and pressed and fermented. Of course, it might be objected that the air is useful merely in carrying the yeast cells into contact with the fermentable fluid, but numerous experiments have been carried on to prove that the presence of oxygen is absolutely necessary for the resuscitation of old and spore-bearing yeast-cells. This is in itself a most remarkable circumstance, and a very significant one when it is borne in mind how very different the conditions are under which the later stages of fermentation are best carried on. It must be remembered, however, that the conditions most favourable for the multiplication and production of a sufficient number of active cells are not by any means the conditions most favourable for the decomposition by the cells of the largest amounts of sugar

In our large breweries (as is sometimes brought home to us only too closely by the reports of the death from suffocation by carbonic acid gas, of men who go down into vats to clean them out) there is always, as the fermentation process goes on, a very great accumulation of carbonic acid gas on the surface of the fermenting liquid; so dense and so deep is this layer that it is at once seen how impossible it is for much free oxygen from the atmosphere to obtain access to the yeast-cells. Any oxygen they utilize for the building up of their protoplasm must be derived either from air held in solution in the fermenting liquid (which can only be a very small amount, as the boiling of the wort must have driven out a very large proportion of such air from the fluid), from the small amount of oxygen that can pass through the layer by diffusion, or it must be derived from the breaking down of those substances rich in oxygen that are contained in the malt solution.

In the same way in the later stages of wine-making the fermentation is allowed to go on in large casks; carbonic acid gas here also rises to the surface, fills the cask up to the bung-hole and gradually flows over, and, as the carbonic acid gas comes in minute bubbles to the surface from all parts of the fluid, it can scarcely be imagined that any large amount of free oxygen can be left in suspension in the fermenting grape juice; and certainly very little can find its way from without, as the carbonic acid gas is pouring out from the bung hole in such considerable quantities. The fermentation at this stage, then,

is going on very rapidly; there is a growth and multiplication of the yeast-cells, a transformation of the sugar into alcohol and carbonic acid gas—all without the presence of free oxygen. Pasteur set himself to reconcile these two apparently contradictory facts, and as a result of his observations he made one of the most important of his discoveries, important in its relation to the life history of both fermentative and pathogenic organisms; and specially important because of the parts that the ærobic and anærobic conditions under which organisms exist play in determining the growth of these organisms and the spread of certain specific infective diseases. He found that old cells with thick membranes, even containing spores, were not able to multiply in media otherwise suitable unless oxygen was present; and that the vigour of the rejuvenating process depended to a very great extent on the amount of free oxygen that was contained within the fermenting fluid. He found, in fact, that with these cells, as in the case of fungi and of other animal and vegetable cells, a sudden and complete cutting off of the supply of free oxygen immediately proved fatal to them, so that the cells which had been exposed to the air for some time—that is, which had acquired an ærobic habit—were rendered inactive by the cutting off of the supply of oxygen. Along with this he found that the yeast organisms could multiply in liquids containing nutrient materials for a considerable length of time before there was any appearance of alcohol in the fluid.

After a time, however, the free oxygen being used up, it would naturally be expected that the growth of the yeast-cells would also come to an end, but such was not found to be the case. The yeast-cells continued to multiply, the oxygen in the fluid was used up and replaced by carbonic acid gas, and carbonic-acid gas was found on the surface, cutting off any further oxygen supply from without. How was it that these cells went on multiplying? Pasteur answered the question as follows: During the gradual elimination of oxygen the yeast-cells, having once become vigorous from the presence of a good supply of oxygen, and of other nutrient requisites, had acquired an activity that they did not before possess; they had at the same time become gradually acclimatized, and their protoplasm had become so far altered that, as the free oxygen was partially cut off, it was able to wrest from the sugar what oxygen it required for the building up of its own

substance, and as the free supply was more and more cut off it became gradually more able to take what it required from the sugar solution—that is, it was gradually acclimatized. In reading this if instead of oxygen the word energy be used it would appear that we should have a more accurate physiological statement. It has already been stated that, in the presence of a free supply of oxygen, relatively less alcohol is formed than under conditions of anærobiosis; it appears as though the oxidation is too complete; there is enough oxygen to supply not only the wants of the living organism but also those open bonds of combination in the various molecules that are unsatisfied when the yeast takes for its own use certain constituent atoms from these molecules, and as a consequence very perfect oxidation is brought about and alcohol is transformed into carbonic acid gas and water. In the anærobic condition that is brought about when the oxygen in the fluid is used up, and as the carbonic acid gas accumulates on the surface in the flask or in the vat, the vigorous yeast-cells are able to separate sufficient oxygen for their own use from the sugar, or sugar and water, disturbing the compositions of the fluid, and necessitating further rearrangement of the remaining molecules; additional oxygen cannot be brought from without to satisfy the vacant bonds, and other very definite products are formed. These products are comparatively stable even when afterwards exposed to free oxygen but not to oxygen in a nascent condition, and we have as a result the alcoholic fermentation. Here, in fact, are two essentially different metabolic processes at work in the protoplasm of the yeast-cells. When oxygen is freely supplied the anabolic or building-up processes predominate, a fact evidenced by the rapid division of the yeast-cells under these conditions; whilst, when oxygen is excluded, the catabolic or breaking-down processes are in the ascendant, as shown by the larger amount of the medium decomposed, accompanied, however, by a slower multiplication of the yeast-cells. Summing up, Pasteur says :—

"This being so, it is evident, we repeat, that to multiply in a fermentable medium, quite out of contact with oxygen, the cells of yeasts must be extremely young, full of life and health, and still under the influence of the vital activity which they owe to the free oxygen which has served to form them and which they have perhaps stored up for a time. When older they reproduce themselves with much difficulty when deprived of air, and

gradually become more languid, and if they do multiply it is in strange and monstrous forms. A little older still, they remain absolutely inert in a medium deprived of free oxygen. This is not because they are dead; for in general they may be revived in a marvellous manner in the same liquid if it has first been aerated before they are sown. . . . At this point we must observe—for it is a matter of great importance—that in the operations of the brewer there is always a time when the yeasts are in this state of vigorous youth of which we have been speaking, acquired under the influence of free oxygen, since all the worts and all the yeasts of commerce are necessarily manipulated in contact with air, and so impregnated more or less with oxygen. The yeast immediately seizes upon this gas and acquires a state of freshness and activity which permits it to live afterwards out of contact with air, and to act as a ferment. Thus, in ordinary brewery practice, we find the yeast already formed in abundance even before the earliest signs of external fermentation have made their appearance. In this first phase of its existence yeast lives chiefly like an ordinary fungus."

But as soon as the process of fermentation ends, and sometimes even before the whole of the sugar has been converted, the yeast if originally not sufficiently rejuvenated gradually loses its power of living by deriving its oxygen from its nutrient medium, and the cells revert to their original condition of senescence. They become dormant, and until again supplied with oxygen can bring about no further fermentation. It is for this reason that to obtain the best fermenting yeasts, cultivations must always be made in the presence of free oxygen, and although this was done before Pasteur explained the reasons for its necessity, it is now carried on in a much more systematic manner. Brewers knew perfectly well that they had to clear out their vats from time to time, not only to get rid of foreign organisms, but also that they might obtain oxidation or more perfect aeration in the early stages of the process of fermentation.

It is very interesting to note that Pasteur, although laying such stress on the connection between vital processes in the cell and the process of fermentation, at the same time holds a very definite opinion that the vegetative activities of the yeast-cells are independent of their characters as ferments; and he maintains that the presence of oxygen, although increasing the activity of the cells as regards their subsequent power of setting up a rapid fermentation, may, nevertheless during its presence, give rise to weakening of their fermenting action. He says:—

"Free oxygen imparts to the yeast an increasing vital activity, but at the same time *quâ* oxygen, impairs rapidly its power as yeast inasmuch as under

this condition yeast approaches the state in which it can carry on its vital process after the manner of an ordinary fungus; the mode of life, *i.e.*, in which the ratio between the weight of sugar decomposed, and the weight of the new cells produced, will be the same as holds generally among organisms which are not ferments. In short, varying the form of expression a little, it may be concluded, from the sum total of observed facts, that the yeast which lives in the presence of oxygen, and can assimilate as much of that gas as is necessary for its perfect nutrition, ceases to be a ferment at all. Nevertheless, yeast formed under these conditions, and subsequently brought into the presence of sugar, *away from the influence of air*, would decompose more *in a given time* than in any other series of conditions under which it could be placed. The reason is, that yeast which has formed in contact with air, having the maximum of free oxygen that it can assimilate, is fresher and possessed of greater vitality than that which has been formed without air or with an insufficiency of air."

In other words, when the ordinary respiratory power which it has in common with the fungi is reduced to its lowest point, after the protoplasm of the cell has been thoroughly rejuvenated, its fermentive power in the absence of oxygen reaches its maximum.

It is thus evident that the processes which go on in fermentation are similar in kind to those chemical metamorphoses that are constantly being brought about in the animal and vegetable organisms; the only difference being, first, in the nature of the material that is broken up; second, in the nature of the cells that bring about the metamorphoses; and third, in the exact nature of the ultimate products of the various processes; the difference in all cases being not so much in kind as in degree. As to the exact nature of the process, there is much difference of opinion, some authorities holding that it is necessary for the whole of the sugar which is altered by the living cell of beer-yeast to penetrate or pass, by a process of endosmosis, through the membranous envelope of the cell and become an integral part of the cell protoplasm, and that, unless this takes place, the resolution of sugar into alcohol, glycerine, carbonic acid gas, succinic acid, &c., cannot take place. It is necessary, in fact, that the whole of the sugar should be, as it were, digested by the yeast-cells, and combined in great part into protoplasm before it can be converted into the various substances above mentioned, which are, on this assumption, merely the excretory products of the vegetable cells feeding on a definite kind of nutrient material. We have seen, however, that the cells of yeast secrete a definite

substance, invertin, which has the power of materially altering the carbo-hydrates to which it is added, and from our knowledge of the functions of other cells we should be led to expect that these cells may exert some influence on the fermenting fluid without the whole of the sugar actually becoming part of the cell ; or perhaps that, on the other hand, the protoplasm may set up such molecular motion in its immediate neighbourhood, especially at certain temperatures, that a certain area of the sugar present is so acted upon that some of its molecules are set free for the use of the yeast-cell, and that the others can only rearrange themselves in a definite manner ; and that, as a result, we have hydration and the formation of alcohol, carbonic acid gas, and water, the most stable elements that can be formed under the existing conditions, and out of the molecules of oxygen, hydrogen, and carbon that are available. There may be slight modifications giving rise to the formation of succinic acid or other bye products, as a greater or less number of accidental or superfluous molecules are set free to become converted into the superfluous or additional substances.

It is a well-known fact, that when yeast is placed under conditions of moisture and warmth suitable for its development, if there be sufficient nutriment present, but sugar be withheld, or even if nitrogenous elements be kept from from it, it becomes soft, and certain marked changes go on in the substance of the cell.

Bechamp found under such conditions leucine and tyrosine, both of them products of protoplasmic metamorphoses, a soluble albuminous substance coagulable by heat, an enzyme, a peculiar gummy substance, phosphates and acetic acid, along with which there was of course the production of a certain amount of alcohol, some carbonic acid gas, and pure nitrogen.

Schützenberger, repeating the experiments, found other products, such as xanthine, hypoxanthine, carnine, and guaine, that pointed most distinctly to a process of metamorphoses of the protoplasm, and by a series of most ingenious experiments he found that yeast in distilled water lost in five days about 9 per cent. of its protoplasm. That is, in yeast there are certain soluble elements that can be removed by washing with distilled water, leaving the insoluble

protoplasm, which can be filtered, calcined, and weighed; after the process of self-digestion, which goes on when the yeasts are deprived of saccharine fluids, a further quantity of 9 per cent. is transformed from insoluble protoplasm into the soluble elements above mentioned. The protoplasm has, in fact, been living on itself, and, showing how indissolubly these metabolic changes are associated with the process of fermentation, both alcohol and carbonic acid gas are formed in the process. Yeast, then, has the power of disassimilating its substance by a series of steps into simpler bodies, but under favourable conditions it may be said to exert its metabolic power only in breaking down in a very superficial way large quantities of the medium on which it is grown—sugar. It is specifically adapted to break down this sugar as far as the stage of alcohol formation, but most yeasts cannot carry disintegration further. Other organisms, however, have the power of carrying on the process as far as the formation of acetic acid for example, at which point another organism may again intervene and take up the work. We have thus the yeast forming alcohol and then dying out as it were, then the Mycoderma aceti comes in and does its work, and later various putrefactive organisms may continue the breaking-down process. On the other hand it must be remembered that whilst yeast sets up the alcoholic fermentation, Hueppe's lactic acid bacillus gives rise to the production from sugar of lactic acid, whilst the Bacillus amylobacter or the bacillus of the butyric fermentation causes the sugar to be split up directly into butyric acid. We have here an example of three specific actions or fermentations of the same substance by the intervention of three different sets of organisms, and it is quite possible that other organisms effect an even further transformation into H_2O and CO_2 at one step, the organism being specially adapted at each stage for the work that it has to carry on, or perhaps it would be better to say that the conditions are adapted to the organism.

As might be expected, such self-digesting yeast is materially weakened; it is no longer in a position to absorb much oxygen, and, if the process be long enough prolonged, both the power of absorbing oxygen and the power of inducing fermentation are lost. But if the water that has been used to wash yeast, *i.e.*, water containing the soluble products necessary for the perfect nutrition of the yeast cells, be

added to the exhausted yeast, the conditions of nutrition are rendered so favourable that the yeast cells again acquire in a most marked degree their original and characteristic power of absorbing oxygen, of vegetative proliferation, and of setting free alcohol and carbonic acid gas.

We may briefly give in his own words the position that Schützenberger holds, so that it may be compared with Pasteur's position, previously stated. "The cell ferment is not developed in the absence of free oxygen, even in its most favourable medium, the must of grapes. The ferment, in process of development, continues to increase in suitable media, even in the absence of all trace of oxygen, as M. Pasteur had already shown. The contrary assertions of Brefeld are erroneous. M. Pasteur's theory, according to which yeast, in the absence of air, takes from the sugar the oxygen necessary for its development, is not well founded; in fact, this development stops long before the greater part of the sugar is decomposed. Is it from the albuminoid matter that the ferment takes its oxygen in the absence of air? Yeast sets up alcoholic fermentation in a solution of pure sugar in the absence of all trace of oxygen, but without developing. This is contrary to the affirmation of M. Pasteur that fermentation is bound up with the organization of the yeast, or is a phenomenon correlative to the vital activity of the cells."

We find that in nature there is, in protoplasm, not only an extreme adaptability to surrounding circumstances, but an attempt to utilize, as far as possible, the whole of the energy set free from cells. We find that in this matter the protoplasm of yeast cells differs in no essential respect from other kinds of protoplasm, and we have already seen that in the process of fermentation of saccharose there is a preliminary change brought about by the soluble products of the yeast-cells (invertin), by which we obtain dextrose and levulose, both of which materials may, under certain conditions, be split up into alcohol and carbonic acid gas.

It may now, further, be noticed that if the fermentation be stopped at a comparatively early stage, there is found in the fermenting solution a larger quantity of levulose than of dextrose; *i.e.*, the dextrose is more readily converted into the characteristic products of fermentation than is the levulose. It has also been proved that if the yeast-cells be heated to a temperature of about 60° C., they are destroyed, as is evidenced by the fact that they are no longer able to multiply, and they never give further evidence of vitality. But there is left in the fluid a substance known as enzyme (ἐν ζύμη, leaven—yeast), which, added to the mixture of dextrose and levulose, does not affect the levulose in the slightest.

(This may also be done by using the Pasteur-Chamberland filter, which keeps back the yeast-cells, but allows the enzyme to pass.) It is only on the addition of living yeast, or that yeast in which there is active protoplasm capable of actually digesting and transforming food materials, that the levulose becomes transformed into dextrose.

We have here three very significant statements, all of them attested by eminent authorities, which seem to explain many of the anomalies and disagreements in individual statements. The process of inversion is reduced to a purely chemical one, in which the chemical reagents bringing it about are manufactured by the living protoplasm; apart from which, however, it is able to exist and act so that saccharose is converted into dextrose and levulose merely by the action upon it of the chemical products or enzymes contained in yeast water; whilst the conversion, and certainly the fermentation of levulose, can only take place when this material comes into actual contact with the living protoplasm, from which it seems, necessarily, to be more closely associated with the actual process of digestion.

This is a decided step in advance in assisting to explain the process of fermentation, of digestion in the higher animals, and of those changes that take place in putrefactive and pathogenic processes. It also appears to throw light on some of the points in dispute on fermentation.

For example, we can easily understand how different the results would be in cases where experimenters use yeast as the fermenting agent, and crude saccharose as the fermentable substance in one case, and yeast with dextrose in another.

The process of inversion of the saccharose by the invertin into dextrose and levulose is carried out easily enough, whether oxygen be present or not, and this dextrose is easily broken up by the yeast. When we come to the levulose, however, it is a different matter, as this substance can apparently only be converted into dextrose when it is in direct contact with the protoplasm of the cell, and so long as food materials and oxygen can be obtained from other sources there is not the same tendency for the protoplasm to take up this levulose that there is when other sources of food supply are cut off. The active and rejuvenated cells are still able to utilize the levulose, whereas older cells, which, as we have seen, after self-digestion lose even their power of transforming

dextrose, lose their power of transforming levulose at a still earlier period.

This enzyme function, although so intimately associated with the vital activity of protoplasm and so usually possessed by cells, is considered by Hueppe to be a secondarily derived function of the protoplasm, and to be really a modification or development of the primary power of digestion possessed by all protoplasm independent of the action of enzymes, only to be met with when the conditions for the growth of the organism and the development of its functions are most perfectly combined. It is, as Cartwright Wood says, a function developed in such a high degree, that it may actually be temporarily separated from the protoplasm which develops it. It must be looked upon as something separate, and as something that can act, under favourable circumstances, apart from protoplasm, economizing the energy of the protoplasm, and utilizing the most suitable products for the nutrition of the organism and for the manifestation of its special functions. Under less favourable conditions, where the protoplasm has to struggle for existence, as it were, this enzyme function is withdrawn ; and although the protoplasm can still exert its characteristic powers of digestion and of formation of special products, these processes take place within the cell, they are of a less specialized nature, and are, in fact, the primary and inherent functions of the cell protoplasm, which are capable of doing their work within the protoplasm, but are not so highly differentiated that they can act externally to it. The primary function is, then, not separable from the protoplasm. This formation of enzymes is of special interest in relation to the processes of disease; for it is found that, by modifying the conditions under which an organism grows, or by varying its food, special ferments come into operation. For instance, Lauder Brunton and M'Fadyean, experimenting with a certain organism, found that if it were grown on peptonized beef jelly, it would give rise to an enzyme which was capable of liquefying the gelatine. If this organism were now introduced into a solution of starch, it would continue to grow, but it could give rise to no diastatic ferment—*i.e.*, it was incapable of transforming, except within its own protoplasm, the starch material into sugar, or into material that it could utilize for its own nutrition. If, however, successive generations of this same organism were cul-

tivated on starch, there came a period at which it generated a diastatic ferment, which could be separated from the solution, and which alone, and without any direct intervention of the protoplasm, could bring about a transformation of starch into sugar—*i.e.*, a secondary separable diastatic function had been developed, in addition to the primary digestive function of the organism. When taken back to the gelatine from the starch, the organism had lost its power of liquefying gelatine ; but this again it regained, after being again cultivated, through a series of generations, on gelatine.

It is quite possible that this may be a somewhat too strong statement of Lauder Brunton's and M'Fadyean's position. It may be that they maintain only that the organism when grown on starch produces the diastatic ferment, whilst when on albumen it forms a peptonizing substance. It may be merely a temporary modification just as under analagous conditions, the cholera bacillus when grown on sugar produces butyric acid, whilst when grown on albumen it forms ptomaines.

It is thus evident that special forms of fermentation require that, in addition to a special kind of protoplasm, there shall be a special nutrient material—a fact that explains some of the different results that have, from time to time, been recorded. The yeasts, as we have seen, are capable of fermenting dextrose and of producing a substance, invertin, by which the saccharose, or cane sugar, is converted into dextrose and levulose—both fermentable substances. Wood points out that only three yeasts are known that are able to ferment milk sugar directly, although many of the bacteria are able to invert and bring about its fermentation. He says that—

" Of still greater interest is the varying manner in which the same organism conducts itself towards different albuminoids. . . . As a general rule, those organisms which liquefy gelatine are able to coagulate milk, and then peptonize casein which has been separated; but some organisms which peptonized gelatine are without action on the milk, and some that are inoperative on gelatine peptonize milk, though this is exceptional " ; and he further says that even the manner in which " milk is peptonized is subject to considerable variations, and that, although the vast majority first coagulate the casein and then dissolve it, certain microbes seem to peptonize the casein directly. . . . Very striking is the way in which the same organism conducts itself to the different albuminoids, gelatine, fibrin, blood-serum, and egg albumen. One organism is unable to liquefy gelatine, but peptonizes fibrin ; another liquefies the gelatine, but cannot peptonize the

egg albumen. . . . They must, accordingly, be regarded as specific in their nature, depending on the specific nature of the protoplasm of which they are merely further differentiations."

Micro-organisms, when grown under such conditions that oxygen cannot gain access to them—under conditions of anærobiosis—usually appear incapable of developing their separable peptonizing enzyme function, as they are no longer able to liquefy the gelatine in which they are growing. The fact that this separable enzyme function could be kept in temporary abeyance was utilized by Chantemesse and Widal, to prevent the liquefaction that takes place in plate cultivations where a certain specific organism requires a considerable time for its complete development. By adding dilute carbolic acid to their nutrient medium they found that the peptonizing power of the organisms was interfered with ; whilst if the carbolic acid solution was sufficiently weak, even the more delicate organisms still retained sufficient vitality to enable them to grow on the surface of the carbolized gelatine.

Having corroborated the above observations, Hueppe and Wood sought to obtain the same results while avoiding the dangers resulting from the use of such a substance as carbolic acid, by offering for the nutrition of the organism a certain preparation of a material which would enable it to develop the diastatic, instead of the peptonizing ferment ; and, by adding glycerine to the gelatine, they found, as they expected, that Lauder Brunton's and M'Fadyean's experiments were practically repeated. The organisms feeding on the glycerine did not in most cases attack the gelatine, and the peptonizing became exchanged for a diastatic ferment ; plate cultivations were not so rapidly liquefied, and bacilli of very slow growth could thus be separated from impure cultivations, in which organisms that ordinarily exert a liquefying or peptonizing function were present in considerable numbers.

Our knowledge of most other fermentations, although being gradually increased, is still in a somewhat nebulous condition, for the reason that no one has as yet (with the exception of those who have worked at the matter from the industrial point of view) taken up the matter thoroughly since it became possible to obtain pure cultivations of any single organism, the whole energy of bacteriologists having been diverted to the consideration of the very important questions that have come up to be answered in

connection with the production of specific infective diseases in man and animals by micro-organisms.

There has, indeed, been so much work to be done in separating out the causal bacterial agents in these diseases, that there has been little time or energy left to be devoted to anything more than a most cursory study of the chemical and biological processes of the non-pathogenic forms. We do know, however, something about the mannitic fermentation of sugar, the lactic, the butyric, the ammoniacal, and the acetic fermentation; and, speaking generally, these processes are essentially the same as those which take place in ordinary alcoholic fermentation, the differences being that the protoplasm cells of the ferment differ from those of yeast both in morphological and biological characteristics, and that the composition of the end-products is also different. The food material acted upon by different protoplasm, and giving rise to different results, may be the same; the fermentation is still brought about by a process of hydration or some other specific process, and is intimately associated with the vital activity of the special ferment. In the mannitic fermentation the sugar is converted by hydration into dextrose and levulose, after which comes the conversion of some of the sugar into gum, and of some into mannite, during which second part of the process there is also an evolution of carbon dioxide and the formation of water. Mannite is a sugar to which a molecule of hydrogen has been added; gum, a cane sugar from which one molecule of water has been subtracted. The viscous change that takes place as the fermentation proceeds is due to the formation of this gummy material in the fluid. Pasteur described the special mannitic ferment as consisting of chains of small cocci, each coccus having a diameter of 1.2 to 1.5μ of an inch. These, like the yeasts, require nitrogenous material, in addition to salts, extractives, and sugar, in order that they may develop. This form of fermentation is specially interesting, from the fact that it is to it that the ropiness of the white wines is due—a ropiness which, as Pasteur pointed out, might be prevented by heating the wine for a few minutes at a temperature of 60° C., or, as suggested by François, by the addition of a certain proportion of tannin, a substance which appears to interfere most markedly with the development of the viscous fermentation organism.

In the lactic acid fermentation it does not appear to be necessary that any process of hydration should take place, as there is here merely an exact division of one molecule of sugar into two molecules of lactic acid, there being neither addition nor loss of either oxygen or hydrogen. It must, however, be borne in mind that the lactic acid fermentation is frequently accompanied by the formation of CO_2, in which case, of course, the process is not nearly so simple.

By fermenting lager beer at from 30° to 34° C., Chr. Hansen found that there was developed a form of bacterium made up of long chains, of dumb-bell or hour-glass shaped organisms, of bacteria as long curved or straight threads; whilst at irregular intervals were formed spindle-shaped or club-shaped bacteria, even when the nutrient conditions still continue good at a late stage of the growth. That these organisms, however, were not all alike, Hansen proved by the fact that some of them are stained yellow by iodine, whilst with others a blue reaction was obtained. The conditions necessary for the development of this organism, and the production of acetic acid, are a high temperature—from 30° to 34° C.—and a plentiful supply of oxygen. So necessary is this latter that a film forming on the surface is all that there is to denote the presence of an organism, the fluid beneath, from which free oxygen is cut off, remaining perfectly clear; and Hansen even gives this as a diagnostic feature to enable an observer to determine whether he is dealing with pure cultivations of these mycodermi or not, as, wherever other bacteria are present, roughly speaking, turbidity is set up.

It is a curious fact that the pure lactic fermentation cannot go on when the medium is too acid, and it is only by removing the lactic acid as it is formed that a complete transformation even of milk sugar into lactic acid can be obtained. This fact was observed long before the exact nature of the process was understood; and in all the earlier methods devised for the preparation of this substance the daily neutralization of the fluid with chalk or carbonate of soda played a most important part. That the process is essentially the same as the others, in so far as it is the result of the activity of micro-organisms, was proved by Pasteur, who found that by sowing the lactic acid ferment, which he described as composed of small globules or short joints, either isolated or in mass, in a fluid which he had found specially suitable for alcoholic fermentation, active lactic fermentation was coincidentally set up, and lactic acid was found present along with the resulting alcohol; whilst, as we already know, if no lactic acid ferment be introduced, either accidentally or intentionally, no

lactic acid fermentation ever takes place. Although this is true in a certain degree, it is found, as has already been stated, that there are actual differences in degree as to the readiness with which the various sugars undergo the alcoholic and lactic fermentations. For instance, although the glucoses are readily converted into lactic acid, the saccharoses, which are specially fermentable by the saccharomyces, are not very easily transformed into lactic acid; whilst, on the other hand, sugar of milk, which does not readily yield alcohol, may be comparatively easily converted into lactic acid. Muscle sugar and mannite, neither of which can be converted into alcohol, may both be broken up into lactic acid, as may all those substances that are specially affected by the butyric fermentation.

It was for some time thought that the fermentation of urea, through which carbonate of ammonia is frequently met with in the urine, was the result of a single micrococcus, the micrococcus ureæ, a small organism about 1μ in diameter, which may occur in pairs, in tetrads, or in longer chains, and which, in the presence of suitable nutrient materials, and of urea, either in artificial solution or in urine, determines a regular hydration of these substances and their conversion into carbonate of ammonium.

Since the time of Pasteur's researches, however, Leube has found an organism about double the size of the above and arranged in chains, which rapidly decomposes solutions of urea into carbonate of ammonia. It differs, too, from Pasteur's organism in that it peptonizes or digests gelatine, causing it to liquefy, and it appears that, unlike some other bacilli and sarcinæ that can set up a feeble urea fermentation, it is quite as powerful an ammoniacal ferment as the small micrococcus ureæ described by Pasteur, as in both of them the fermentation can go on most vigorously, even in the medium that has become distinctly alkaline, *i.e.*, the carbonate of ammonia accumulates until about 13 per cent. of this substance has been formed; these two microbes may be said to bear the same relation to the other urea-forming bacteria that the Saccharomyces cerivisiæ and the other more powerful alcohol-forming yeasts have to the weak saccharomyces and other sugar fermenting organisms. That this power is not confined to a single organism is not remarkable, since it is quite possible by boiling the urea in

water, or, better still, in slightly alkaline solution, to bring about direct hydration of the urea into ammonium carbonate.

When it is asked what value the study of fermentation has been to the cause of medicine, it is not necessary to go further for an example than this urea fermentation. In the operations of the older surgeons it was very frequently recorded that the introduction of instruments into the bladder (in which, of course, was a solution of urea) was followed by a regular fermentation, which too frequently led to the death of the patient; and it having been determined that micro-organisms were the cause of this fermentation, it became an easy matter to prevent their introduction by careful sterilization of the instruments; and, as a result, such ammoniacal fermentation within the bladder is now a matter of very infrequent occurrence, and is, in those cases in which it does occur, the result not so much of accident as of carelessness on the part either of the patient or of the surgeon. So marked, indeed, has this been, that even those who sneer at bacteriology and antiseptics have been compelled to accept new methods of procedure in regard to the cleanliness of their instruments, a cleansing which means simply the removal of these minute vegetable protoplasmic organisms, which, on being introduced into the suitable fermenting medium in the bladder (or elsewhere), give rise to the alkaline fermentive, putrefactive and other processes.

In an interesting chapter on the butyric fermentation of putrefaction, Schützenberger points out that in the process of putrefaction or putrid fermentation, as it may be called, there is a formation of butyric acid, one only of a series of fatty acids formed under similar conditions; accompanying this there occurs a transformation of glucose, starch, lactic acid, albuminoid substances, and the various fruit acids, into these fatty acids, into carbon dioxide, and into certain hydrogen compounds. From a very large number of these substances lactic acid with or without carbon dioxide is formed, whilst the lactic acid may be further split up into butyric acid, carbon dioxide, and hydrogen.

From this chapter we may borrow some of the formulæ to show how easy and how rational is the transformation from these substances into the fatty acids. Other substances may also be formed, these being further broken down or separated as the case may be. Glucose, as we have already seen, is, under the action of the lactic acid ferment, converted into lactic acid, and this under the action of the butyric ferment, a small rod-shaped organism from 1.8 to 18μ in length and 1.8μ in breadth is converted into butyric acid, carbon dioxide, and hydrogen. These micro-organisms

are straight, somewhat rounded at the extremities, single, or arranged in short chains of three or four elements; if placed in a nutrient medium similar to that in which yeast grows, they are reproduced by a process of vegetative division, especially if this medium be neutral, or slightly alkaline, and if it be kept at a temperature of 40° C.

The formula of the process of splitting up is given as first

$$\underset{\text{1 Molecule Glucose}}{C_6H_{12}O_6} = \underset{\text{2 Molecules of Lactic Acid}}{2C_3H_6O_3}$$

The two Molecules of Lactic acid are then transformed into

$$\underset{\text{1 Molecule of Butyric Acid}}{C_4H_8O_2} + \underset{\text{2 Molecules Carbon Dioxide}}{2CO_2} + \underset{\text{2 Molecules Hydrogen}}{2H_2}$$

In the same way two molecules of malic acid are broken up into two of lactic acid and two of carbon dioxide, and the lactic acid is again transformed as above, that is, $2C_4H_6O_5 = 2C_3H_6O_3 + 2CO_2 = C_4H_8O_2 + 4CO_2 + 2H_2$. This butyric acid formation is only one of a group, and we find that where lactic fermentation is going on in an impure condition propionic acid, $C_3H_6O_2$, acetic acid, $C_2H_4O_2$, and valerianic acid, $C_5H_{10}O_2$, are produced, and glycerine fermented for some time with beer yeast, is split up into propionic acid mixed with formic and acetic acids, giving a kind of compound of putrefaction products.

A very ingenious formula is quoted by Schützenberger to show how the fatty acids are formed from sugar :— $\frac{n-1}{3}(C_6H_{12}O_6) = \underset{\text{Fatty Acid}}{[C_nH_{2n}O_2]} + \underset{\text{Formic Acid}}{(n-2)CH_2O_2}$ Formic acid, the lowest of the fatty acid group, being immediately converted into carbon dioxide and hydrogen. The presence of lime or organic matter appears to modify this process somewhat, and to give rise to the formation of secondary vegetable acids. For instance, two molecules of malic acid, $2C_4H_6O_5$, break up into succinic acid, $C_4H_6O_4$, and tartaric acid, $2C_4H_6O_6$, and from these are formed, from the tartaric acid the fatty acetic acid, $C_2H_4O_2 + CO_2 + H_2$, and from the succinic acid the fatty valerianic acid, carbon dioxide and hydrogen, $C_5H_{10}O_2 + 3CO_2 + H_2$.

These transformations have this in common, that they are all brought about by the presence of minute organisms, that the conditions of the development of these organisms are similar to those met with in other fermentation processes, and that theirs are the principal products formed during the process of putrefactive fermentation, which also in all cases requires the presence of these minute organisms, the spores of which, as we have seen, seem to be everywhere

present. Here, as in the case of the yeasts, we find both ærobic and anærobic organisms. The ærobic forms give rise to oxidation of certain of the products of decomposition, one of these, nascent hydrogen, especially taking up oxygen, as a result of which water is formed; the anærobic organisms, which may be said to complete the process of putrefactive fermentation, only commence to grow when the ærobic organisms have used up all the readily available oxygen such as that dissolved in a liquid, on the surface of which there has formed a kind of film, by the intervention of which aeration from the external air is greatly interfered with.

We have already noted this film growing on yeasts, when it appears to assist the carbon dioxide developed during the process of fermentation in keeping off the oxygen of the atmosphere, and to allow of these anærobic organisms carrying on their growth without the further intervention of a free oxidizing agent. The products of these anærobic organisms, of course, differ from the products of the ærobic fermentations in so far that they contain less oxygen, the sulphuretted hydrogen, hydrogen, and nitrogen being released in a more or less free condition and uncombined with oxygen, the oxygen of the original fluid being used up in the formation of such substances as leucine, $C_5H_{10}(NH_2)CO\ OH$; tyrosine $C_6H_4\begin{cases} OH \\ C_2H_3 \end{cases}(NH_2)CO_2\ OH$); the volatile fatty acids; some compound ammonias, and, of course, carbon dioxide; the two latter of which, as already seen, may be formed from urea ($CO(HN^2)_2$) by simple hydration, the more highly organized substances, such as leucine and tyrosine, being, during the process of fermentation, further converted into ammonia and the fatty acids.

From the variety of the products of these fermentations it is evident that we have to do, not with a simple conversion by means of any single organism into the simplest and ultimate elements of decomposition, but that we have a series of breakings down or stages of decomposition by which the highest (and most unstable) organic materials are gradually transformed into the lowest and most stable. It will be found, however, that the process is not quite so straightforward and simple as above stated, as some of the energy released during the formation of a number of lower

and more stable compounds is utilized in the presence of animal and vegetable protoplasm in building up higher and more stable materials ; though at the later stages of decomposition these become fewer in number, and eventually become oxidized into the more stable forms—a free access of air to the products of anærobic decomposition always leading to their oxidation. Hydration almost invariably occurs during each of these stages.

Schützenberger says, "Nothing resembles putrid fermentation, with reference to the derived products, more nearly than the change which takes place in the constituent parts of yeast, when left to itself without nourishment, deprived of sugar and oxygen.

We see, in fact, the appearance of leucine, tyrosine, sarcine, &c. This is the first step; the action stops there, and goes no further; the yeast, or the special soluble ferment which it secretes, is unfit to attack these bodies again; but if we wait for the development of vibrios, we shall find the production of ammonia, carbon dioxide, and volatile fatty acids at the same time that the leucine partly disappears."

Fermentation by oxidation can only be set up by organisms in the presence of a free supply of oxygen, and the process in such cases appears to be due to special organisms which, attacking the substance to be fermented, remove some of its constituents, set free others, especially carbon and hydrogen, which, in their nascent condition, are seized upon by the oxygen of the air, and so water, or water and carbonic gas, are formed.

In the commonest of these fermentations by oxidation, the acetic fermentation of alcohol, the free access of air is as absolutely essential for the growth of the organism as it is for the special stage of the fermentation of the alcohol into acetic acid.

If the mycoderma aceti be sowed in wine, freely exposed to the air at a temperature from 24° to 27° C., chemists hold that there is hydration of alcohol by the combination of an atom of free oxygen with two atoms of its hydrogen water, OH_2 and aldehyde C_2H_4O, being formed, the latter of which, by further oxidation, in turn becoming converted into, $C_2H_4O_2$, acetic acid. At a certain stage this process of acidification stops, owing to the quantity of acid developed, but if this be removed and fresh alcohol be added, the acidification again commences, and so on, as long as the mycoderma remains active, can obtain sufficient oxygen, and sufficient alcohol

on which to act. If, however, the supply of either oxygen or alcohol be cut off, the ordinary mode of life of the mycoderma is at once interfered with. It uses up part of its own protoplasm by internal metabolism, continues to act on the acetic acid, and converts it into simple substances, water and carbon dioxide, at the same time retaining molecules of carbon, hydrogen, and oxygen for its own use. The mycoderma is, then, no longer so much an enzyme-former or an organism with a thick celled wall, that acts principally on the suitable nutrient medium by means of its chemical products, and gives rise to a definite chemical process; but is now rather a mass of protoplasm endowed only with its primary digestive powers, by means of which it can convert organic matter into its very simplest forms or elements. There is very complete oxidation, and instead of alcohol and acetic acid being formed as intermediate products that can be easily separated, the substances are at once converted into water and carbon dioxide, the ultimate products of perfect oxidation.

It is sometimes said that the mycoderma in the true vinegar fermentation is in a weakly condition; it is certainly not in its most active condition, but the state appears to be one rather of quiescence or advanced development, in which the formation of a thick membrane and the production of a separable enzyme are characteristic features, rather than one that can be spoken of as a condition "of incomplete or tardy development."

To show that the process was not merely a physical one, as was suggested even by Pasteur, Mayer showed that by simply heating the acidifying fluid to a temperature at which the mycoderma is killed all oxidation is arrested, and he also showed that the temperature at which oxidation of *concentrated* alcohol goes on in spongy platinum is at a temperature above 35° C., at which point it is completely stopped in the presence of the mycoderma aceti, in addition to which "physiological acidification" can only take place in a weak solution of alcohol.

Pasteur's observations and results led to the adoption of what is known as the Orleans process of making vinegar, which, as given by Schützenberger, is as follows:— "The mycoderma aceti is made up of small, slightly elongated cells, with a transverse diameter of from 1.5

to 3μ, united in short chains, or curved rods. Constriction and division take place between the segments of the chain as the process of vegetation goes on. A small quantity of this mycoderma, which occurs as a wrinkled membrane on the surface of liquids that are undergoing acetic fermentation, is first sowed on the surface of an aqueous liquid containing two per cent. of alcohol, one per cent. of vinegar, and traces of alkaline and alkaline-earthy phosphates. When the surface is covered with the membrane the alcohol begins to acidify. This action being fully set up, some alcohol, wine, or beer mixed with alcohol, is *each day* added to the liquid, in small quantities; this is continued till the oxidation becomes slower; the acetification is then allowed to terminate, and the vinegar is drawn off. The membrane is collected, washed, and employed for a new operation. It is better always to give the plant sufficient alcohol, so that its activity may not be exerted on the acetic acid. Nor ought it to remain too long out of the liquid, or it would lose its active force; finally, it is better to moderate its development, to prevent burning oxidation." These last few lines are of special interest, as they show how markedly favourable conditions of nutrition, and the supply of special materials on which the organisms may act, modify the process set up by the acidifying organism; just as certain organisms will select glycerine or sugar from a peptone gelatine medium before they begin to act on the gelatine itself, so the mycoderma aceti, under certain conditions, confines its attention entirely to the alcohol, converting it into acetic acid and then leaving it; whilst, as soon as the glycerine in the one case and the alcohol in the other, are completely used up, or as soon as the activity of the protoplasm becomes so great that it cannot derive sufficient material from the one, it immediately attacks the other, and continues the oxidizing process. Not only so, but the various organisms appear to be specialized at the different stages of the resolution of organic matter; each, during the stage at which it can perform its share of the work, appearing not only to multiply much more rapidly than the other organisms with which it is found but actually holding them in check. In this respect the vegetable protoplasm of the lower organisms is only like the protoplasm of the cells of the higher animal organisms. In relation to pathogenic processes this is a

point of very considerable interest, for we find that under certain conditions there comes to be a contest between the animal cells and the lower vegetable cells. As Wood well puts it, "the reaction of the cells upon each other may turn on the sum of the conditions of existence to which they are exposed happening to favour one more than the other, thus when two organisms are sown together in a culture fluid, which will overgrow the other may depend upon the relative quantity of the two primarily introduced, the nature and reaction of the media, and the temperature at which they are held." Fermentation, then, is due to the action of highly specialized cells. That this is so is indicated by the usual presence of enzymes and also by the fact that they exert this specific action in their highest degree only under a certain set of specific conditions which vary in the case of each organism. Each organism has become adapted to a certain medium, and is so specialized as to have the power of splitting it up under certain conditions in a specific way. This is also indicated by the fact that as the conditions become unfavourable, less and less of the specific product (relatively to the bye products) is produced. Although we shall have more to say in connection with the subject of the specific infectivity of micro-organisms in disease, it may here be pointed out that fermentation is due, in great part, to the action of cells which have the power of developing a special enzyme function ; that these cells are usually more highly specialized during the stages when they bring about their specific action ; that this is associated to a certain extent with diminished activity of the protoplasm directly on the substances to be fermented ; that most processes where there is the formation of an enzyme, or a special poison, take place most actively when there is a complete or partial cutting off of the supply of oxygen ; that the cells in this condition usually develop a more or less perfect cell membrane which characterizes the formation of zoogloœa masses ; that this incomplete oxidation also characterizes the formation of toxines ; that the same rules hold good in the formation of most of the products of the lower vegetable organisms and of the individual cells of animal tissues with complete oxidation and the formation of carbonic acid gas and water ; that when incomplete oxidation takes place various specific products make their appearance ; but that even in

the presence of a full supply of oxygen, protoplasm under certain conditions cannot bring about complete oxidation, and as a result some of the intermediary products of fermentation and decomposition may be formed.

It is evident, then, that all ferments may be classed under two great heads : firstly, the organized ferments or the active unstable protoplasmic cells of yeasts, bacteria, of plants and animals. Secondly, the unorganized ferments or enzymes which we have spoken of as the separable functions of more highly developed cells, both of them being, however, associated with the nutritive and other metabolic changes of protoplasm, be this protoplasm that of simple vegetable cells or of highly organized gland cells. Hoppe-Seyler classifies the whole of the fermentations as follows :—I. ferments which bring about hydration or cause hydrolysis, these being divided into (*a*) those that act like boiling mineral acids, all of which belong to the enzyme group ; and (*b*) ferments acting like caustic alkalies in which we have the process of decomposition (1) of fats into glycerine and fatty acids as in saponification, which results from the action both of the organisms directly and of the unorganized ferments, (2) decomposition of amido —or ammonia-nitrogen compounds which is also usually associated with hydrolysis. II. In the second group, Hoppe-Seyler places fermentations in which there is transference of oxygen from the hydrogen to the carbon atoms. As examples of this we have (*a*) lactic acid fermentation in which there is decomposition of certain carbohydrates into lactic acid ; this, as we have seen, being always associated with the presence and activity of a certain group of micro-organisms, (*b*) the alcoholic fermentation brought about in a similar manner by yeasts, (*c*) putrefactive decomposition changes, (1) of simple inorganic compounds such as combinations of the fatty acids with lime, or (2) of organic compounds such as fibrin and other proteids. Then we have also the acetic fermentation already referred to, nitrification or mineralization, chromogenic fermentation and that series of changes in which we have the production of ptomaines.

Such a classification is specially important when we come to consider the relation of the ordinary fermentation processes to those that are set up during the course of disease in animals and in plants.

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CHAPTER VII.

Parasites and Saprophytes.

Putrefactive Bacteria—Pigment-forming Bacteria—Enzyme-forming Bacteria—Non-pathogenic, Saprophytic, Parasitic, Pathogenic Bacteria—Sarcina Ventriculi—Leptothrix Buccalis—Facultative Saprophytes—Facultative Pathogenic Parasites—Anthrax Bacillus—Pathogenic, Parasitic, Saprophytic, merely Relative Terms.

In order that much of what occurs in the following chapters may be understood, it is necessary that some idea should be given of the way in which the terms saprophytic, parasitic, and pathogenic are used. The first of these is not so important; the term saprophytic as applied to bacteria is being gradually supplanted by the term non-pathogenic, especially in works dealing with the relation of micro-organisms to disease. Speaking generally, saprophytic bacteria are those bacteria that have the power of obtaining the nutriment they require for their building up, and for the carrying on of their vital functions, from dead vegetable or animal substances only. They are not able to invade the tissues of living animals and plants, but they play an important part, as we have already seen, in bringing about oxidation of various organic materials and of giving rise to certain definite compounds. To this class belong the putrefactive bacteria, and many of the pigment-forming bacteria; the yeasts or fermentation fungi are also to be looked upon as purely saprophytic in character; and such organisms as the Bacillus amylobacter, the lactic acid bacillus, and the like, are all probably to be classified in this group.

The subdivision of the group has been attempted by many workers, and the saprophytic organisms have been arranged according to their power of action on the various carbo-hydrates and on proteid substances. Others have taken their products as a basis on which to classify them, and enzyme-forming, pigment-forming, or alcohol-

forming organisms have all been described ; but as their powers of decomposing different substances and of giving rise to pigment or to certain enzymes necessarily overlap one another, or are met with in the same individual under different conditions, it remains an exceedingly difficult matter to give any classification founded on the above mixed characteristics.

Some of the organisms which, at present, are supposed to be saprophytic in character, may eventually be found to be parasitic under certain conditions, and it is for this reason that the term *non-pathogenic* is gradually ousting the older botanical term of *saprophytic*.

Parasitic bacteria are those which are able to flourish on or within the substance of plants or animals. In animals they live in the various cavities of the body, or in certain cases in one or other of the nutrient fluids of the body, and they derive their food from these fluids, or from the nutrient materials that are taken in by the host. The ordinary terms of obligate parasites, facultative saprophytes, and facultative parasites, can scarcely be used in connection with these minute organisms, as, with very few exceptions, all the parasitic bacteria have now been cultivated outside the body and under such conditions that it is evident that some of those which were at one time looked upon as purely parasitic have a saprophytic stage of existence ; so that nearly all, if not all, the parasitic bacteria must be looked upon as facultative parasites, or parasites that can develop almost as well saprophytically as they can parasitically, or facultative saprophytes, or saprophytes that can develop almost as well parasitically as they can saprophytically.

We know of course that all parasitic bacteria are not pathogenic, and that we have along the alimentary tract, and even in the respiratory tract, a large number of parasitic organisms. The Sarcina ventriculi, for example, first described by Goodsir, may be taken as a purely parasitic organism. It is found especially in dilated stomachs. A drop of fluid taken from the contents of such a stomach frequently contains a number of organisms which present a curious appearance under the microscope. They are very like the typical bale of wool or goods tied with a strong cord in three directions. Other sarcinæ are saprophytic as well as parasitic, but the Sarcina ventriculi has not yet been

satisfactorily cultivated outside the body, or if it has, it has always become somewhat altered in appearance. Sarcinæ have also been found in the bladder, in the air passages, in the intestines, and even in the blood, but most of these can be cultivated on nutrient gelatine. None of these organisms are pathogenic so far as at present can be made out; they appear to be simply accidentally associated with certain conditions, as in the case of dilated stomachs, in which they are not always present, but in which they frequently occur, apparently because the acid fermentation that goes on in the accumulated contents renders them specially favourable media for the development of these sarcinæ. The non-pathogenic parasitic bacteria of the mouth were amongst the first organisms observed by Leeuwenhoek; they are found on the gums, on the sordes covering the teeth, and on the mucous membrane of the mouth. The forms of these we shall mention later. Although most of these are parasitic and non-pathogenic, one form, the Leptothrix buccalis (an old term embracing probably several species, and one not now very generally used), is also pathogenic in the sense that it invades the teeth, and gives rise to what is known as caries, or rotting of the teeth, but even this has to obtain help from some of the other non-pathogenic species found (some of the micrococci), which give rise to the formation of lactic acid from the sugar of old food, an acid that, combining with the lime salts of the teeth, softens them, and so allows of the penetration of the leptothrix form.

Pasteur has suggested that other facultative saprophytes are those met with in the alimentary tract, where he thought they appeared to assist in carrying on the decomposition of the nutrient materials, assisting, under certain circumstances, the animal tissues of the body to carry on the process of digestion without interfering in any marked degree with the nutrition of the tissues of the host by absorbing any of the material that could be utilized by these tissues for their own nutrition. This, however, is now considered to be questionable. The organism of cholera finding its way into the alimentary canal, acts as a pathogenic parasite, but here only from the fact that by its growth and metabolic activity it gives rise to irritant toxic materials, which exert most injurious effects both locally and constitutionally.

As another example of a facultative pathogenic parasite, the anthrax bacillus may be cited. It may develop actually in the tissues, as in woolsorters' disease, where it occurs in the bronchial mucous membrane; in the lymphatic tissue spaces, and in the pleural cavities; or in the blood, as in cases of accidental inoculation. It may also occur in the blood in woolsorters' disease, the bacillus readily making its way from the air vesicles and tubes into the pulmonary circulation. In this connection it may be mentioned that anthrax bacillus cannot undergo its whole developmental cycle within the body; the vegetative stages only having been found in parasitic anthrax; whilst growing on dead tissues at a favourable temperature spore formation is invariably noticed at some period or other during the life history of the anthrax rods, so that the bacillus must be looked upon as a facultative saprophyte. Another point to which attention may be drawn is that bacteria must be looked upon as essentially and primarily saprophytic organisms. By a process of long and gradual acclimatization or adaptation, certain species have become so altered, either temporarily or permanently, that they are able to exist as parasites. It will always be found, however, that the tendency to revert to the saprophytic or non-pathogenic condition is more marked than their tendency to become transformed into the parasitic and pathogenic condition. It has been found, for example, that anthrax bacillus passed through a series of cultivations in gelatine, or hog-cholera bacillus similarly treated, loses a great deal of its pathogenic power. This loss of pathogenic power may take place in two directions, either by the organism losing its power of development in living tissues, or by losing its virulent specific poison. The cholera organism cultivated outside the body usually loses much of its pathogenic power. By appropriate treatment, such as cultivating at the body temperature in specially prepared broth through a number of generations, this pathogenic activity may, however, be restored. We must therefore look upon pathogenicity, parasiticism, and saprophyticism as mere relative terms; the conditions necessary for the development of the saprophytic mode of life being more widely met with than are those in which the parasitic life may become developed, most organisms become more or less permanently adapted to

them, so that it is only under special circumstances that parasitic and pathogenic organisms are developed. The importance of this fact will be evident when we consider the epidemiological importance of the presence and mode of life of micro-organisms both outside and within the body.

CHAPTER VIII.

Cholera.

Cholera a Parasitic Disease—Early Observations on the Comma Bacillus —Characters—Methods of Staining—Methods of Isolation—Use of Plate Method in Practical Public Health Work—Tube Cultures—Motility of cholera bacilli—Potato, Blood-serum, and Milk Growths —Behaviour in Water and Sewage—Infection of Man through Agency of Bacillus — Early Inoculation Experiments — Koch — Nicati and Rietsch—Macleod—Difficulties—Cholera in Guinea-pigs—Position of Bacilli in Tissues—Presence of spores doubtful.

On account of its general interest there are few examples that can more appropriately be taken to illustrate the relations between a special bacillus and a special disease than that afforded by Koch's discovery of the comma bacillus in cases of Asiatic cholera, and its relations to that disease. The study of the disease itself, since its appearance in this country in the great epidemic of 1832, has had a peculiar fascination for epidemiologists and skilled hygienists. Its development, behaviour, and whole general history appeared to be shrouded in mystery, and phenomena, the explanation of which seemed to be utterly beyond the powers of experts of all kinds to give, were at one period constantly being observed, recorded, and discussed. Now, however, through the laborious but brilliant researches initiated by Pettenkofer at the head of one school, and by Koch in a very different one, much of this air of mystery has been dispelled, and many of the doubts and difficulties that surrounded the subject have been gradually cleared away as workers under one or the other of these great leaders have gained fresh knowledge and elucidated new facts. Take, for example, the question of the spread of cholera from its home in Lower Bengal, in the delta of the Ganges, to surrounding districts and distant countries. At first such spread was thought to be most erratic and inexplicable. Now, however, although it appears from time to time to have

made almost unaccountable leaps and divergencies, it has been found to follow a very definite line of advance in the course of the various epidemics, and although there may have occurred sporadic cases which could be traced to no definite source of contagion or infection, which have proved a stumbling-block to many conscientious workers and observers, it is generally acknowledged that the evidence accumulated through the researches of Koch and his followers, both in Germany and in Great Britain, and of several enthusiastic workers in France, can leave little doubt in the minds of most of those who study the subject carefully, that cholera is a parasitic disease, that it travels along the ordinary lines of commerce by railways, caravans and ships, from the regions in which it is endemic to those centres of trade and religion which, by their imperfect sanitary arrangements, by the want of cleanliness of their inhabitants, by meteorological conditions, and on account of bad water supply, are ready for its reception and propagation. In the European epidemics, of which up to 1885 there had been six exceedingly severe ones during the present century, the disease has, in every case, followed the lines of trade.

Before the three last epidemics (1865, 1873, 1884) cholera usually came to Europe by what may be called the Continental routes—the caravan routes through Persia, Asia Minor, and Russia; but in the three last it came by the Mediterranean or maritime route, first by land through Egypt, brought there by Mecca pilgrims, and thence to the seaports of France, Italy, and Spain, whence it gradually made its way northward and inland, spreading over the whole of Europe.

At the mouth of the Yang-Tsze, as instanced by Macleod, of Shanghai, cholera breaks out regularly at certain seasons of the year, but he adduces evidence of great value to show that although it may be imported directly from India, between which and Shanghai there is at least weekly communication, just as there is between India and the Nile delta region, it is probably, in the strictest sense, an endemic disease of that region.

It can be readily understood, after the fearful ravages which it made in places in which it was not actually endemic, and after it had decimated the population in certain parts of India, in Egypt, in the low-lying portions of Persia, and

Asia Minor and in Europe, that many observers should be anxious to find out the ultimate cause of the disease, and as early as 1848 Virchow, and in 1849 Pouchet, Brittan, and Swaine found numbers of vibriones in the discharges of choloraic patients, without, however, being able to assign to them or prove for them any specific *rôle* in the causation of the disease.

Following up these researches Philippe Pacini, setting himself to look for a causal agent, frequently found in cholera stools small micro-organisms which were characterized by active and peculiar movements. These observations, however, as well as those of Klob, who looked upon the cause of cholera as an accumulation of fungi in the intestines, and those of Boehm and Hallier (all published in 1867), who believed that they had found the cause in a peculiar fungus which was found to grow in certain forms of grain imported from India, not only did not receive adequate proof, but were, like all preceding observations, entirely unreliable. Then followed the experiments by Hayem and Raynaud, carried on during the epidemic of 1873, which, however, again were equally futile and without definite results, and it was not until Koch, going out with the German Cholera Commission to Egypt and India, was able to demonstrate a peculiar species of bacterium as the causal agent that any definite proof of the micro-organismal nature of the contagium of the disease could be obtained.

Since that time, however, the amount of work published on the subject has been so great that cholera has now a special "literature" of its own. The report of the German Commission was speedily followed by that of the French Government, who sent out MM. Straus, Roux, Nocard, and Thuillier, the last of whom fell a victim to his assiduity and zeal in carrying on the work of investigation in Egypt. In this country Klein and Heneage Gibbes, Cunningham and Lewis, Roy, Graham Brown and Sherrington, Watson Cheyne, and Macleod have, with very different voices, some supporting Koch's verdict, others opposing it, reported on the subject; with the general result that until further and more convincing opposing evidence is forthcoming, Koch's comma bacillus, of which the following is a brief description, must be looked upon as the *causa causans* of the disease.

The 'comma' bacillus, which is now regarded as belonging to the spirilla, usually occurs as a slightly curved rod, measuring from 1 to 2μ in length, with an average length of about 1.5μ ; it is .5 to $.6\mu$ in thickness, the average thickness being about one-third to one-fourth of the length. It is therefore from one-half to one-third the length of the tubercle bacillus, but somewhat thicker. In place of occurring as single rods these organisms may be grouped

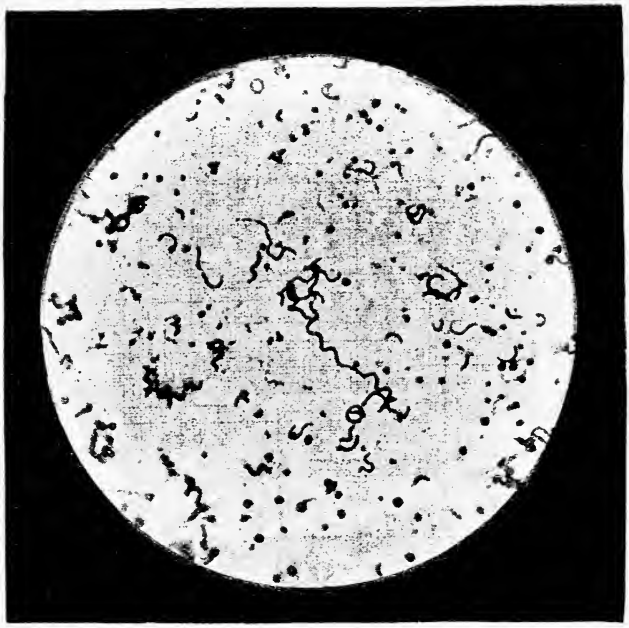

Photo-micrograph cholera bacilli. × 1000. Long spirilla, comma, S and O shaped organisms. Some involution forms.

in pairs, or in larger numbers, in which case the curve may be continuous or reversed, so giving rise to the formation either of half circles or of S-shaped curves. In cultivations in meat broth the bacilli may be so grouped that they form long wavy or spiral threads, each of which may be made up of 10, 20, or even 30 short turns.

Such are the characters of the organisms which Koch found,

almost invariably, in the stools of patients during the earlier stages of the disease, in the contents of the lower bowel and in the mucous membrane of the lower part of the small intestine. In fatal cases Drs. Straus and Roux thought that they could also see certain organisms in the blood of cholera patients, but they were unable to repeat their observations. Emmerich also described a bacillus which he said he found constantly in the blood, organs, and intestinal contents in cases of cholera. This organism was, however, according to Flügge, probably a common inhabitant of the intestine, the Bacterium Coli Communis, and it is now a generally accepted fact that nowhere, except in the alimentary canal, have the comma bacilli been found in ordinary cases of cholera ; as the blood, the liver, the spleen, and other organs have all been carefully examined, and in no case, in which no fallacy crept in, could positive results be obtained.

The best method yet described of demonstrating the cholera bacillus in the discharges is that recommended by Cornil and Babes, who spread out one of the small white mucous fragments on a microscope slide, and then allow it to dry partially ; a small quantity of an exceedingly weak solution of methyl violet in distilled water is then flowed over it, and it is flattened out by pressing down on it a cover glass, over which is placed a fragment of filter paper, which absorbs any excess of fluid at the margin of the cover glass. Comma bacilli so prepared and examined with an oil-immersion lens (× 700 or × 800) may then be seen ; their characters are the more readily made out because of the slight stain they take up, and because they still retain their power of vigorous movement, which would be entirely lost if the specimen were dried, stained, and mounted in the ordinary fashion.

The bacilli retain their curved shape and "rounded" extremities (which, however, may be either slightly pointed or thickened), but they seem to be somewhat larger when thus prepared than when completely dried. During the very early stages of the disease, and when the period of reaction is setting in, it is sometimes an exceedingly difficult matter to demonstrate the presence of the cholera organism in the dejecta, and several methods have been tried with greater or less success to obtain such a demonstration. Of these the plan described by Schottelius is found to be one of the most useful, especially in the later stages of the disease, where although the number of cholera bacilli may be comparatively small, other bacteria are found, often in considerable numbers, in the intestinal contents.

A small quantity of the material from the intestine is mixed with double its quantity of faintly alkaline meat broth, which is then allowed to stand at a temperature of 35° C. for about twelve hours; at the end of that time the comma bacilli have multiplied to such an extent, especially where they are in contact with the air, that if a small portion of the pellicle from the surface is stained and examined, as above, it is found to consist of an almost pure cultivation of comma bacilli which are, however, somewhat shorter than those usually met with. If the cultivation be left undisturbed for a longer period the bacilli generally grow larger, and eventually spirilla may be formed, but in the course of a few days the other bacteria grow so luxuriantly that the comma bacilli no longer predominate, and in many cases they are almost entirely "overgrown." From the earlier "maass" cultivation so prepared, in which the relative proportion of cholera bacilli is enormously increased, "plate" cultivations may now readily be made. The plate method as employed by Koch is the best, and the one least open to fallacy for obtaining pure cultivations. It is carried out as follows: On a sterilized platinum needle a drop of the above fluid, of the cholera discharge or of the contents of the small intestine is taken. This is introduced into a test tube one-third full of nutrient gelatine, liquefied at as low a temperature as possible by immersing the lower portion of the tube in warm water. The seeded gelatine is carefully shaken in order that the organisms may be disposed widely and equally throughout its substance; three small drops of this are then taken on a looped platinum wire and added to a second tube, which is similarly shaken, and from this second tube five drops are transferred to a third tube. After the contents of each tube have been thoroughly mixed and the seed material taken for the next tube, the remainder is poured on to a glass plate previously sterilized; each of the three plates is carefully labelled and placed under a bell jar in which the air has been thoroughly sterilized (see Appendix). The plate from tube No. 1 is found to contain a large number of organisms, that from tube No. 2 contains a much smaller number, and that from tube No. 3 a much smaller number still, so that as the organisms are developed (one colony from each organism or group of organisms) there is sure to be sufficient space on one or other of the plates between the different groups to allow of a careful study of the growths as they extend, and at the same time there is a sufficient number of points to ensure the appearance of several growths of each kind of organism that was present in the original seed material.

If plates made according to Koch's method be kept in a chamber in which the temperature is maintained at a little over 20° C. small greyish or white points are first seen; examined with a low power lens, each of these has a slight yellow tinge and a somewhat wavy margin; as the "colony" increases in size its colour becomes slightly deeper, the margins become more and more crenated, and the surface somewhat granular. Slow liquefaction is now found to be taking place, and small funnel-shaped depressions, any one of which seldom measures more than 1μ in diameter, are seen in the gelatine; at the

bottom of these depressions yellow pin-like masses are usually seen. In consequence of this liquefaction the colony has the appearance of a piece of ground glass with a finely notched margin surrounded by a clear zone. On the second or third day small air bubbles are seen, and on the fifth or sixth day the liquefaction is well marked ; at this stage the colony has sometimes a delicate pink tinge, a most characteristic feature when present. From these points or colonies growing, as we have seen, from individual organisms or small groups of the bacillus, pure cultivations may be made in test tubes containing gelatine, agar-agar, potatoes, or broth in or on which the growth and special characters of the bacillus may be watched and noted. It is sometimes made a matter for reproach to bacteriologists that the methods employed in their work, useful enough though they may be in conducting scientific experiments, are found altogether wanting when they are required for actual practical work. The following story, which went the round of the medical papers, may help to remove the idea that bacteriologists are mere visionaries, and to prove that the science, some of the problems of which they are attempting to solve, is by no means so useless as many would have us believe. An Italian emigrant steamer, touching at New York, had on board a child suffering from a suspicious form of diarrhœa, though it could not be said that all the symptoms of Asiatic cholera were present. In order to determine the true nature of the disease—whether it was cholera or not—gelatine plate cultivations of the dejecta were made by a doctor in port, and the vessel was detained for four days ; during that period bacilli identical in appearance with, and having all the characteristics of, Koch's comma bacillus were developed, and it was at once concluded that this was a case of true Asiatic or Indian cholera. The diagnosis was subsequently confirmed, as a series of other cases occurred, in all of which unmistakable symptoms of Asiatic cholera were developed. An equally interesting and instructive example of the same thing was recorded by Pfeiffer and Gaffky when they were working in Koch's laboratory. At a time when there was no general cholera epidemic, Gonsonheim and Finthen in Germany were suddenly ravaged by a most deadly form of diarrhœa which in many features resembled true Asiatic cholera. The

recorders, in order to determine the exact nature of the disease, made plate cultivations from the dejecta of some of the patients, and found Koch's comma bacillus; they were thus able to put the matter beyond all doubt. How the organism came to the district, and why the outbreak remained localized, are questions that still remain unanswered. During 1890 there was a similar, but less localized, outbreak of fatal diarrhœa in Spain. Koch's bacillus was here again separated, and all doubt as to the nature of the disease at once removed.

In view of these facts the immense importance of bacteriological methods, as permitting of rapid and definite recognition of the disease, with the possibility of taking precautionary measures as early as possible, and so preventing a wide dissemination of the disease germs, can scarcely be insisted upon too strongly.

In a puncture culture in gelatine the growth takes place along the whole track of the needle, first as a delicate white cloud, then, as it gradually becomes more and more marked, it forms a delicate streak, around which there is usually a clear space, due to the liquefaction of the gelatine along the tract of the needle. Near the surface the liquefaction goes on more rapidly than deeper down, and at the end of forty-eight hours there is a distinct funnel-shaped area, in which a clear liquid portion usually sinks somewhat below the level of the gelatine, and it appears as though the top of the funnel was closed by a small clear glass globe or air bubble. This appearance is apparently due to the slowness of the liquefaction, time being given for the water to evaporate from the liquefied gelatine. This is proved by the fact that if 5 per cent. gelatine instead of 8 or 10 per cent. be used, the gelatine liquefying more rapidly does not allow time for evaporation to take place, and the bubble is never seen. About the fourth day of the growth this "funnel" is still more marked, but the upper part has become quite clear, the central thread having fallen to the lower and narrower part of the funnel, where it may be seen as a comparatively short spiral, the thread as it sinks being arranged in regular "bights" like those of a cable. After a time the whole of the upper part, say two-thirds, of the gelatine is liquefied and perfectly clear, then comes a layer of "white" cholera bacilli, which rests on the gelatine that has remained solid (this sediment has usually a yellowish tinge); and on the surface

of the liquefied gelatine a greyish scum is found, in which "involution forms" may be frequently observed.

Cultivations made with a platinum needle on the surface of agar-agar, grow as elevated pale translucent streaks, the margins of which are well defined, and in the agar immediately below the streak there occurs a slight opalescence which is very characteristic of the bacillus. The agar, unlike the gelatine, is not liquefied. As the growth becomes older it spreads, though not very rapidly, the band increases in thickness and gradually assumes a brown colour especially in the opalescent substratum. When the organism grows in meat infusion it forms a delicate greyish pellicle on the surface, and in such a medium the movements of the bacillus are most characteristic. If a single drop taken from the surface of such a cultivation, or even a very small fraction of a drop be added to a drop of meat broth faintly tinged with a 1 in 2,000 solution of methyl violet and examined in the moist chamber (see Appendix), the bacilli are seen to have most rapid movements, especially if the temperature be slightly raised; Koch says, "when they accumulate in numbers at the border of the droplet, and are swimming about actively, it seems quite as if they were a swarm of midges, between which, diving here and there, are long spiral-shaped threads, which are also moderately active." The shorter rods become more and more curved, and then stretch themselves out, constantly moving, oscillating and twisting.

From numerous experiments carried on by several observers it appears that this motility is dependent partly on temperature, but chiefly on the desire for oxygen which these organisms exhibit. In the "hanging drop" (see Appendix), for instance, it will be seen that the cholera bacillus separates itself from most other micro-organisms by its movements in search of a supply of oxygen, which always bring it to the margin of the drop; once there, however, it loses its motility and remains in its acquired position, where its wants are supplied without any effort on its part. The cholera bacillus also grows on potatoes, but here it should be noted the growth does not go on at the ordinary temperature of the room, but only when the temperature is raised to 30° or 35° C. A fragment of blue litmus paper placed on the surface of a slightly moistened boiled potato indicates, by turning red, a slight acid reaction, which is due

(according to Koch) to the presence of an organic (pyromalic) acid. Here the acid reaction of the potato is the factor that interferes with the growth of the bacillus, and it is only when the temperature conditions become more favourable than usual that any growth can take place.

The growth when started appears as a thin, moist "light greyish brown" mass, very similar in character, as Koch points out, to the glanders bacillus as grown on potato, except that it has not the deep or chocolate brown colour so characteristic of that organism.

A most interesting fact has been observed in this connection. Potato cultivations of bacilli from the various cholera epidemics vary somewhat in colour; there are also other slight modifications in the appearance of the different masses, by means of which a skilled observer, who has cultivated the various organisms from the different localities and epidemics, is enabled to distinguish the bacilli of each epidemic; and it is stated that in some of the German laboratories the directors can take up half a dozen potato cultivations and say, This is from the Naples epidemic, that from Egypt, and so on through the whole series. There are undoubtedly differences, and to the most inexperienced eye the colour of the Shanghai cholera growth may be seen to differ slightly from that of the Egyptian and that of the Naples growths.

On blood serum and in milk these organisms grow most luxuriantly. They may cause slight liquefaction of blood serum, but in milk, which forms for them an exceedingly good nutrient medium, they give rise to no noticeable alterations; it may therefore be readily understood how deadly the cholera organism may become if it once finds a resting place in milk. Its presence cannot be recognized by any peculiar or characteristic appearance, by taste, or by smell, as it only gives rise to a faintly aromatic and sweetish smell, which can scarcely be distinguished, except by the most practised nose, from the slightly aromatic smell of the milk itself. Dr. Simpson in *The Indian Medical Gazette* records a naturally prepared experiment, which shows at a glance what an important part these milk cultivations may play in the spread of cholera. On board the ship *Ardenclutha*, in port in Calcutta, ten men partook of the milk supplied by a native milk-seller who came to the ship daily; of these ten men, four died of cholera, and five suffered from exceedingly severe diarrhœa, the tenth, who escaped, had taken only a small quantity of the milk. After careful investigation it was found that the milk had been watered with 25 per cent.

of water, from a district in which it was known that several people were suffering from cholera. A case had been imported into this district on the second day of a certain month, the dejecta from this patient drained into a tank, near which the milkman's house stood ; on the seventh day of the same month the first new case occurred among the milkman's neighbours, and on the same day the first case of diarrhœa occurred on board the *Ardenclutha*, and two days later a case of undoubted cholera occurred in the ship. These facts are in themselves interesting and instructive, but they are rendered doubly so by the fact that of the other members of the crew, fourteen in number, who had taken none of the watered milk, not a single one was attacked by diarrhœa or cholera.

Cholera bacilli are so little fastidious in their diet, that, within certain limits, they are satisfied with anything, but there are certain things at which even they draw the line—soup *maigre*, for instance, they abhor, as also sour things ; acids are to them a deadly poison ; on the other hand, however, they are somewhat exclusive in their habits, and the presence of other and more vulgar bacteria they cannot brook for long. For example, if intestinal contents or dejecta from a case of cholera are sprinkled on moist soil or damp linen kept at blood heat the comma bacilli increase at an enormous rate for the first twenty-four or thirty-six hours, and under these conditions, as already seen, there may be obtained almost pure cultivations of the specific organism, but on the third or fourth day it begins to die out, and other bacteria are found to be asserting themselves most strongly.

Babes has found that at a temperature of 30° C. the bacillus will grow upon various kinds of meat, on eggs, on various vegetables, and on moistened bread. It also multiplies in the dejecta from healthy patients, on cheese, in coffee, chocolate, *eau sucrée*, and other kinds of fluid sugars ; but it cannot exist for twenty-four hours on acid fluids or vegetables, on mustard or onions, or in wine, beer, or distilled water. Distilled water and soup *maigre* are never sufficient for its nutriment, and if the strength of any of the usual cultivation media be reduced to about a fortieth of the original strength, there is gradual diminution in the number of organisms; even in water which contains an ordinary amount of organic and inorganic material in solution, multiplication of the comma bacillus does not take place. Where, however, there is a very large accumulation of organic matters, as at the margin of stagnant water, where there are large quantities of nutrient materials " from the presence of a variety of solid

particles in suspension and of pieces of mud," development occurs, "especially on the floating solid fragments." Koch's experiments, however, have shown that cholera bacilli mixed with spring water were still alive after the lapse of a period of thirty days. In Berlin sewer water they lived only six to seven days, mixed with excrement only twenty-seven hours, and in cesspool water they were not alive at the end of twenty-four hours. Babes' experiments, as already stated, did not bear out these statistics in their entirety.

Infection through the agency of milk has already been mentioned, and water has been referred to as a vehicle by which the bacillus may be carried. Macnamara gives an experience of infection through water, for the absolute accuracy of which, in the case of such a skilled observer, Koch contends there can be little doubt. In a region in which no cholera prevailed, the dejecta from a sporadic case of cholera became accidentally mixed with some water, which, after remaining exposed to the heat of the sun for a whole day, was partaken of by nineteen persons, of whom five were attacked with unmistakable cholera within thirty-six hours; the evidence here given was so conclusive that little doubt can be entertained as to the relation of the dejecta to the water, and to the subsequent cases of cholera. It should here be mentioned, however, that Klein, who does not believe in the specific nature of Koch's cholera organism, in order to prove his position, drank a quantity of fluid which was said to contain cholera material without feeling any ill effects; and that Bochfontaine swallowed cholera dejecta in pills without suffering any incovenience; but in both cases there is ample evidence that the persons experimenting upon themselves were suffering from no gastric or intestinal derangement of any kind, and that the gastric juice was sufficiently active to prevent the multiplication of the cholera organisms, and it is probable that the fourteen men of the nineteen not attacked in Macnamara's case were in a similar impregnable condition. As positive evidence to set against the above is a most striking case that occurred in connection with Koch's cholera courses, carried on in the Hygienic Institute in Berlin. A doctor who had been for eight days engaged on work in the cholera course became affected with a slight disturbance of digestion, which was accompanied by diarrhœa; he continued to get worse, and was at length so ill that he started for home, where he was almost immediately attacked with symptoms of true

cholera, rice-water stools, great weakness, unquenchable thirst, diminished excretion of water, except by the bowel, and spasmodic contraction of the feet and toes. He was very anxious to have this rice-water material examined for the comma bacillus, and a quantity was despatched to Koch, who was able to demonstrate in and cultivate from it, true comma bacilli ; the patient recovered. There were no other cases of cholera in Germany at the time, but the doctor had been examining and making cultivations of the cholera bacillus shortly before he was attacked, and there can be little doubt that he had, through inattention to the rules of the laboratory, in some way or other, ingested some of the bacilli with which he had been working.

The description of these cases of infection or non-infection of individuals by cholera material introduced into the alimentary canal, naturally opens up the way for an account of the results obtained experimentally.

Thiersch was the first to make experiments on animals with cholera dejecta. He fed white mice with scraps of filter paper, impregnated with decomposing cholera dejecta, but he found that plain filter paper was equally efficacious in causing the illness of the white mice. He was quickly followed by Burdon Sanderson, who carried on a similar series of experiments, and obtained much the same results. Dr. Richards, of Goolando, also attempted to produce cholera in pigs by feeding them with large quantities of cholera material, but he found that the animals died in from fifteen minutes to two and a half hours after the administration of the poisonous material, and the contents of the intestine of the first pig that died, when given to another, produced absolutely no symptoms. It was argued from this that the poison had not multiplied, but had been destroyed or absorbed, or rendered inactive in the stomach of the first host, so that none was left to act on the second pig ; death in the first case being produced by rapid intoxication through the introduction of a large quantity of some active poison, and not by a poison developed in the intestinal canal as in the case of true cholera. This poison, whatever its nature, had no effect on dogs.

Following on these came a series of experiments by Koch, who attempted to produce symptoms of cholera by feeding and inoculating in various ways, mice, monkeys, cats,

dogs, poultry and other animals with cholera dejecta and with comma bacilli; in no case could he produce cholera, and on search being made for the bacilli in the stomach and intestine, they were invariably absent, apparently having been destroyed in the stomach, as in only a few instances did they reach the intestine at all, and then in very small numbers. To demonstrate the difference between these and certain other bacteria, a mouse was fed with a red micro-organism that had been isolated in a pure condition; and after a time its intestinal contents were disseminated on potatoes, on which small red colonies of the same organism shortly made their appearance, showing that their vitality was not appreciably affected by their passage through the stomach. Having found that the comma bacillus was so destroyed, Koch thought that if the organism could be introduced directly into the large intestine, so as to escape the action of the gastric juice, it might be able to retain its vitality and multiply; the results obtained, however, were in all cases negative, even when purgatives were previously given to induce an altered condition of the intestine. The only cases in which partially successful results were obtained were those of a series of rabbits into which pure cultivations of the bacillus were injected directly into the circulation, and of a number of mice, in which the cultivations were injected into the abdominal cavity; the rabbits recovered in one or two days, after suffering from marked symptoms of intoxication; but the mice died at the end of the first or second day, bacilli in these experiments being found in the blood. Koch argued from these observations (1) that the gastric juice of the healthy stomach killed the micro-organism; (2) that even when it found its way into the intestine, it was passed along it so rapidly that it could not take any effect on the healthy or even slightly irritated mucous membrane, or that it was actually very rapidly destroyed in the intestine itself. In guinea pigs, in which he attempted to produce cholera, he found that there was great acidity of the gastric juice, and that the peristaltic movements of the intestine were very strong and rapid.

It will have been concluded from what has been stated that both naturally and in artificial cultivations there is some poison developed during the growth of the bacillus, which, when introduced in considerable quantities, produces

a condition of intoxication, which would account for the death of those animals in which partially successful results were first obtained. Further, it is evidently quite possible that this intoxication may account for the very rapid deaths which occur during certain epidemics, and also for the preliminary diarrhœas by which the intestine, in some cases at any rate, is prepared for the reception and multiplication of the cholera organism itself. It must, indeed, be looked upon as one of the common causes of this diarrhœa. If these facts be borne in mind it is possible, even without analyzing the further experiments on animals, to understand the immunity against cholera experienced by Bochfontaine and Klein, and the susceptibility of the doctor who was attending the cholera course, whilst he was suffering from indigestion and diarrhœa. It may also be understood how infection occurs by milk and through water after what has been described, and in accordance with these facts is the experience of all those who have had to deal with cholera epidemics.

Utilizing the experience gained by Koch in his experiments, Nicati and Rietsch performed a series of operations, by means of which they claimed they were able in a certain proportion of cases to produce typical cholera symptoms. They introduced pure cultures of the comma bacillus into the upper part of the intestinal canal, having previously tied the bile duct; but as Koch pointed out later they were successful in producing true cholera, probably, only in those cases in which the intestine was somewhat injured during the manipulation to which it was necessarily subjected, and its peristaltic action interfered with, and it is very naturally objected that death might be due not to the action of the cholera bacillus at all but to this rough manipulation. The observers themselves believed that it was the presence or absence of bile in the small intestine which determined the success or failure of their experiments.

In order to avoid the operation of opening into the duodenum, Koch thought that the two great factors in bringing about the destruction of the bacilli might be thrown out of court, first by neutralizing the acid reaction of the stomach by means of 5 c.c. of a 5 per cent. solution of carbonate of soda; and, secondly, by interfering with the peristaltic action of the bowel. He found in test experiments that the intestinal contents remained distinctly alkaline for six hours after the introduction of such a solution. But he also found that the organism still passed through the stomach alive, and that it failed to produce any changes in the small intestine, the food and the bacilli passing from the

stomach to the cæcum in a few minutes; the peristaltic action of the intestine causing such rapid passage of the bacillus that it was completely powerless to produce any characteristic symptoms of cholera, a fact the importance of which was accentuated when it was found that the only guinea pig in which any choleraic symptoms were observed, and in which there was any increase in the number of bacilli in the small intestine, was probably suffering from an attack of peritonitis, due to the animal having aborted just previous to the experiment—a condition in which, as has long been recognized, there is invariably interference with the peristaltic action of the intestine. It was possible, then, that this second factor might be neutralized by the introduction into the peritoneal cavity of some reagent, which would by its action cause partial or complete paralysis of the small intestine. For this purpose he first used opium, but he afterwards found that alcohol was equally efficacious. After administering the soda solution, and injecting into the stomach of the guinea pig 10 c.c. of broth, to which one or several drops of a pure cultivation of bacilli had been added, he injected into the abdominal cavity tincture of opium, in the proportion of 1 c.c. to every 200 grammes of the animal's weight; the results he obtained were indeed startling.

Of course objections were raised to the method, and it was argued that some of the animals died from an overdose of opium, some from blood poisoning, and so on. Subsequent observers found, indeed, that the dose of opium was somewhat too large, as some animals experimented on never awoke from the opium sleep. Macleod, of Shanghai, found that he obtained the best results by giving "repeated doses of 1 c.c. or less of the tincture till the animal was stupefied sufficiently to lie on the side or back for ten minutes when placed in that position." "Several times," he says, "the full dose recommended by Koch had to be given, but usually a smaller one sufficed." Control-animals treated, according to Macleod's method, and in all respects in the same way, with the exception that they received sterilized broth instead of cholera material, always recovered.

The contents of the bowel or dejecta from cholera patients passed into the stomachs of guinea pigs so prepared, produced cholera symptoms and death. Giving the results of of his experiments, Macleod states that from one of the animals that died after a dose of cholera material, the small gut contents were collected into a sterilized vessel, and injected (by means of a fine indiarubber flexible catheter) in doses of 2 c.c. into the stomach of two other animals.

These two animals died, and the contents of their small intestine were used in the same way, and so on through ten generations. Of twenty-one animals thus treated, two recovered, nineteen died. The doses varied from 0.5 to 2.5 c.c.

It is sometimes argued that in consequence of the difference of the symptoms in animals (especially guinea pigs), and in man, under these conditions of infection by the stomach, the disease cannot be the same, and it is pointed out that vomiting and profuse diarrhœa are entirely absent in cholera, experimentally produced. Against this objection may be put the fact that there is usually in the guinea pig a large accumulation of fluid transudation in the small intestine and even in the stomach; whilst in the very acute forms of cholera the pathological appearances presented in the intestines are almost identical with those found in man. In exceedingly acute cases the peritoneum presents a dark colour and a peculiar glistening mucous appearance, which is very characteristic, and seems to be associated with the tarry condition of the blood. The mucous surface of the intestine is usually congested, and in the intestinal canal is a considerable quantity of serous fluid, in which are floating the small white rice-like bodies which are merely portions of desquamated epithelium and mucus. There is usually, at this stage, marked injection of the vessels of the solitary glands, and at the periphery of the Peyers patches, and the longer the patient remains alive the more accentuated become these appearances. It is only in the later stages of the disease, or during the period of reaction, that the swelling of the follicles is very marked, and that more or less extensive ulceration of the mucous membrane may be observed. The dejecta, of course, are similar in character to the contents of the bowel, are watery owing to the great amount of serous effusion, and contain the rice-like bodies in which are found the comma bacilli. Bacilli are also found lying free in the watery fluid. The dejecta have little or no odour, and when they are allowed to stand they separate into two layers—an upper slightly grumous layer, and a lower grey deposit. "Neutral, or slightly alkaline, they contain a very small proportion of organic or inorganic salts—one to two per cent.—which consists of chloride of calcium, carbonate of ammonia, potash, salts, and a small quantity of urea. They contain little or no albumen, and during the first day or two no bile pigments."

If microscopic sections of the lower part of the small intestine be made, and stained according to Löffler's method,[1] the bacilli may be seen with the aid of a high

[1] First stain in a solution of Leonhardi's Dresden methyl violet ink, and then in an alkaline solution of fuchsin, which is made up as follows:

100 c.c. aniline water.

1 c.c. of a solution of one per cent. of caustic soda.

2 grammes of solid fuchsin.

The whole is well shaken, after which the specimens may be left in it for twenty-four hours; the sections are then washed in distilled water acidulated with a drop of acetic acid, dehydrated in absolute alcohol, cleared up in cedar oil, and mounted in Xylol balsam.

magnifying power (× 800) beneath the loosened epithelium of the villi, in the follicles, and in the surrounding connective tissue. They are especially numerous near the ileo-cæcal valve, and are distributed, not in masses, but usually in ones, twos, and threes, scattered thickly through these tissues.[1]

On post-mortem examination of the guinea pigs, in which cholera had been experimentally produced, "the blood was fluid, thicker and darker than natural, the tissues of the thoracic and abdominal walls were remarkably dry, the small intestine was throughout distended, congested, and paralyzed-looking, and occupied a much larger proportion of the abdominal cavity than usual. The cæcum was distended with fluid or semi-fluid contents. If the animal died early the fluid was not quite clear in the small gut, there being present traces of food; still the watery character was very manifest, and mucous flakes were abundant. If the animal died on the second or third day no food remains were to be seen, and the fluid in the small gut was the counterpart of the typical cholera stool of man. In either case the comma bacilli were demonstrated microscopically, and by cultivation as in man."

While the organisms in the broth injected could be frequently counted in a microscope field, in a drop of the small bowel contents from an animal having received such broth, the bacilli might be so numerous that counting them, without dilution of the fluid examined, was an impossibility. On floating the bowel in water, the stripping of the epithelium could be well demonstrated "immediately after death," a fact which appears to dispose of Macnamara's assertion that this separation of the membrane is always a post-mortem appearance. In animals which died about the eighth day the appearances were very similar to those met with in cases of Asiatic cholera, when the patients have succumbed during the stage of reaction, and no bacilli can be found in the intestinal canal or in the surrounding tissues. In the earlier stages the bacilli can be demonstrated in sections lying under the partially-detached epithelium, or in the lumina of Lieberkuhn's follicles.

In fluid cultivations, as has already been mentioned, the comma bacilli, or little groups of them, assume various forms, all of which, however, may be looked upon as made up of more or less perfect spirals. At the point of division, just before division take places, there is a small clear space which, by some authorities, has been looked upon as a spore. In some of Macleod's preparations this appearance was so marked that it was difficult to convince oneself that it was not due to actual spore formation, but as drying for twenty-four hours completely destroyed the activity of the bacillus in which these clear spaces were most marked, and as they could not

[1] It is not my intention to give the complete pathological anatomy of cholera, except so far as it is associated with the presence of the cholera bacilli.

be stained by any of the ordinary spore-staining methods he was convinced that he was not dealing with true endospores.

On three occasions Hueppe was able to demonstrate in moist chamber cholera cultivations on agar-agar small brilliant spore-like bodies which he called "Arthrospores." These were placed sometimes at the extremities, sometimes in the middle of the comma bacillus. They appear to develop where the conditions of nutriment, temperature, or moisture, are somewhat unfavourable to the development of or to the very existence of the bacillus. This is proved by the fact that the protoplasm in the bacilli, in which these arthrospores are present, is stained very imperfectly with methyl blue; the clear bodies themselves taking on a brownish-red tinge. Hueppe maintains that he has observed these bodies germinate out into comma bacilli, and he holds that they are much more resistant to various germicidal reagents and unfavourable conditions than the vegetative form of bacillus. Several observers have tried to repeat these experiments, but, as any apparent spores that have been formed invariably failed to resist the action of drying, such "Arthrospore" containing bacilli, must, for the present, be looked upon as involution forms, similar to those described below.

When these are formed the ordinary dimensions and forms of the comma bacillus as previously given are sometimes departed from, and in old agar-agar cultivations, and in old gelatine cultivations in which the surface has become somewhat dried, spirals are frequently found of which the constituent bacilli are considerably thicker than ordinary comma bacilli, the large curved forms remaining attached by delicate terminal filaments. In most cultivations there are slight modifications in both form and size of the cholera bacillus, which appear to be associated with the difficulty or facility with which they obtain food, water, and oxygen, and also with the temperature at which they are grown. For example, if ten per cent. of alcohol be mixed with the gelatine, or if the gelatine medium contains but little beef extract or peptone, and if the temperature at which the media are kept be very high or very low, say 45° C. on the one hand or 20° C. at the opposite extreme, a large proportion of spiral filaments is formed; whilst if the medium be well adapted to the nutrition of the organism, and the cultivation be kept at a temperature of 36° C., short comma bacilli are almost exclusively developed. It is a curious fact, however, that, once developed, these various forms may persist or pass on their characters unchanged for one or two generations, even though they are now inoculated into ordinary fresh peptonized gelatine medium, and Cornil and Babes say that if there are inoculated simultaneously, on peptonized gelatine, a fresh culture of filaments, another of ordinary comma bacilli, and a third with the short and only slightly curved

bacteria which are formed under various conditions, the same typical naked-eye appearances of the cholera bacillus are always obtained in each case, but on microscopical examination it is found that the first still develops in the form of spiral filaments, the second as comma bacilli, and the third as short bacteria, and that these characteristics are carried on to the fourth generation.

With all these modifications in form they still produce typical cholera symptoms when injected into the alimentary canal of animals. In addition to these changes there frequently appear at one extremity of the bacillus small cystic dilatations which are due, according to Virchow, to a kind of œdematous degeneration. As these dilatations make their appearance in certain bacilli, others of them shrivel up, and tadpole and spindle-shaped organisms are formed which may break down into small granules and fragments. In the later stages of degeneration these various involution forms are completely sterile. In the earlier stages, so long as they take on the aniline colouring matter pretty freely, successful inoculations may usually be made, but if the unfavourable conditions are continued in the new cultivations, the organisms soon lose both their power of taking up the staining reagents and of reproduction.

It has been stated that the comma bacillus is entirely an ærobic organism, and there can be little doubt that its vegetative activity is much more marked when the organism is grown in contact with the oxygen of the air. It is certainly more resistant to the action of germicidal agents, and exhibits movements in a much more marked degree. It had been observed, however, that comma bacilli do not cease to multiply even when their supply of free oxygen is entirely cut off, but under these conditions, as Wood has pointed out, the bacteria are much more sensitive to external influences, and are very readily destroyed by acids and other germicidal agents. He has found, however, that where free oxygen is cut off, as Pasteur had observed in the case of other ferments, the bacilli produce a relatively much larger quantity of the specific toxine, or poison, than when oxygen is present, and by a number of ingenious experiments he showed that when they were grown on albuminous substances with complete exclusion of oxygen, and of substances from which oxygen could be easily derived, the cholera poison was produced more energetically and more rapidly than under the ordinary conditions of ærobic cultivation ; probably because, under these conditions, much larger quantities of albumen must be split up to meet the energy requirements of the organism.

Wood and Hueppe from this argue that the bacillus gives rise to such important changes in the intestine because there

we have quantities of albuminoid material which can be rapidly broken up by the action of the bacilli even in the complete, or almost complete, absence of oxygen, which is supposed to rule in the intestinal canal, as a result of which we have the formation of large quantities of toxine and rapid intoxication. But as they are voided in the dejecta after growth under these conditions, the cholera organisms are more easily destroyed than at any other time or under any other conditions.

It is very significant that in cholera, yellow fever, and typhoid fever, three diseases in which the manifestations are chiefly in the intestinal canal, and in which the evacuations apparently contain the living poison, *direct* infection from the stools appears to be the exception rather than the rule, and Wood explains this as due, at any rate in part, to the state in which the specific organisms associated with these diseases are present in the stools, and are dependent upon the conditions present in the intestinal canal. Should they find their way during this stage into the stomach of the living person they, being more susceptible, would be much more liable to be destroyed by the acid gastric juice, or to undergo attenuation ; but if they are allowed to live outside the body, even for a short time, they become more resistant in character, they multiply more readily, they are less particular about the nature of their food, and consequently they are much more dangerous.

CHAPTER IX.

CHOLERA (*continued*).

Pettenkofer's researches—Saprophytic and Parasitic Stages of Cholera Bacillus—Temperature Conditions—Relation to Epidemics—Moisture—Ground Water—Flushing—Cholera in Shanghai Endemic but Intermittent—Cholera Endemic at the Mouth of the Ganges—Vitality of Cholera in Old Cultures—Relation of this to Quiescent Periods during Parts of the Year—Gastro-intestinal Irritations prepare for Cholera—Chinese Vegetables—Cholera Poison Formed in the Intestine absorbed into Body—Inoculation against Cholera—Gamaleia's Experiments—Germicides useful in attacking the Cholera Bacilli—Water Supply, Pilgrimages, Feasts predisposing to Cholera—Quarantine except in Harbours Useless—Time and Place Dispositions.

PETTENKOFER'S indefatigable researches on the relation of ground water and the drying zone to cholera epidemics have thrown much light on many obscure points, and have opened up the way for further work. He holds that the increase of cholera is due entirely to the increase of the "drying zone" near the surface of the soil—*i.e.*, the lowering of the level of the "ground water," and that the rise and fall in the level of this ground water is the principal factor in the production of conditions necessary for the outbreak of epidemics of various kinds. On these grounds he has taken up a very strong position against the spread of cholera directly from patient to patient. There can be little doubt that many of Pettenkofer's observations are entirely in accordance with this view, but equally can there be little doubt that all his interpretations of the facts he has collected are not entirely accurate, although, as Hueppe points out, Wood's observations offer solutions of questions that have hitherto been unanswerable. The instances are almost innumerable in which there has been a most remarkable immunity against the passage of cholera from individual to individual; where the dead bodies have been buried immediately the disease has afterwards occurred, not in those who have had to carry out the actual burying of

the dead cholera patient, but in those to whom has fallen the duty, a day or two later, of washing the soiled linen used by the patient during life. In the first case the attendant is exposed to the action of the bacillus as it comes from the patient, but at a time, it is argued, during which the organisms are more readily destroyed, either in the dejecta, or by drying ; but the attendant who has to wash the soiled linen may be attacked by the bacillus after it has had time and opportunity to develop on the damp sheets, in the presence of air, and when it has acquired a greater power of resistance, and has become much more dangerous. It has now, in fact, lost its anærobic habit, and has become adapted to its new surroundings, with the result that it is much more resistant and is better qualified to live outside the body and to resist the action of ordinary germicidal reagents, or of the acid gastric juice, should it find its way into the stomach of a fresh host.

In similar fashion may be explained the fact that when the drying zone becomes limited, the cholera bacillus appears to die out more readily. It appears that the micro-organism passing directly from the fæces into very damp soil containing insufficient oxygen to satisfy its saprophytic requirements is, on account of its feeble resisting powers, "suffocated" at once, or within a very short period. Where, however, the depth of the drying zone increases, there is more air (oxygen) in the soil, the organisms are more able to multiply in its presence, and, taking the field against other putrefactive organisms, gradually become more and more hardy, acquire the ærobic and saprophytic habit, and thus become more dangerous to the inhabitants of the locality in which all this occurs. It must be remembered, however, that the term "drying zone" is entirely a relative one, and that it may still contain, as it usually does, sufficient moisture for the wants of the cholera organism. That the organism takes some little time to pass from the ærobic to an anærobic condition is evident from Koch's early experiments, in which he made plate cultivations of cholera bacillus, and then, before the gelatine was perfectly set, covered about one-third of the surface with exceedingly thin glass—cover glass thickness—or split mica. He then found that colonies grew as usual, and became visible to the unassisted eye in the uncovered portion of the gelatine and for a very short distance under the covering plate (2 mm.). He observed, however, that where the air (oxygen) was cut off, the colonies did not

increase in size sufficiently to allow of their being distinguished without the aid of a lens; the power of growth to the size of a colony visible to the naked eye being attributed by Koch to the presence of the small quantity of available oxygen that remained in the nutrient gelatine,—growth ceasing as soon as this oxygen is exhausted,—the colony remains of small size.

A second experiment was made by inoculating nutrient jelly contained in a small glass with comma bacilli; this "was placed under the receiver of an air pump, another glass prepared in a similar way being placed outside the air pump as a control experiment. It then appeared that those bacilli under the air pump did not grow, while those outside the receiver did well. But if those which had been under the receiver were afterwards exposed to the action of the air, they then began to grow. They therefore had not been destroyed; they only wanted the necessary oxygen to be able to continue their growth. A similar result is obtained when cultivations are placed in an atmosphere of carbonic acid; whilst those cultivations placed for control purposes outside the carbonic atmosphere grow in the usual manner, those subjected to the action of a stream of carbonic acid remain inactive and undergo no development. Here also, however, they do not die; for after they have been exposed to the carbonic acid for a considerable time, they again begin to grow as soon as they are taken out of it." In place of carbonic acid, hydrogen may be used, and the result is much the same.

It cannot be assumed from these experiments that the organisms require to obtain free oxygen from the air in order that they may continue their growth, for, as has been pointed out by Hueppe and Wood, if they are grown on a suitable medium, such as unchanged normal albumen, they are able not only to exist, but to produce unusually large quantities of their specific poison, as they are able by the dissociation of such a medium to obtain all that they require for their growth and development.

All that can be argued from Koch's experiments is that the bacillus has a saprophytic stage (although this was apparently not at first recognized by Koch), during which it grows and multiplies, but only under *ordinary conditions* where free oxygen can be obtained from the atmosphere. During its parasitic existence it becomes so far modified, that, still growing with great vigour, it produces its toxine more easily or in greater quantities in the absence of oxygen, other conditions being favourable. During this stage, however, it is much more readily affected injuriously by acids and other germicidal reagents, and is therefore much more easily destroyed than when it has had time to acquire its full saprophytic faculties.

Mention has been made of the conditions as regards temperature that are necessary for the growth of the cholera bacillus when other conditions are slightly adverse. As the result of an extensive series of experiments, first carried on by Koch, and afterwards repeated by other observers, it has been found that the comma bacillus flourishes most luxuriantly, and is most productive, at a temperature ranging between 30° and 40° C., although it can grow at a much lower temperature, and even at several degrees above the higher point mentioned. At 20° C. it flourishes luxuriantly upon a peptonized gelatine medium.

As early as 1884 Koch described the growth of the cholera bacillus at a temperature of 17° C., but as might be expected the growth is not nearly so prolific, and it is certainly considerably less rapid than at a slightly higher temperature. A temperature below 17° C. seems to be inimical to the growth of the bacillus, as at 16° C. development may be said to have almost ceased. It is, however, remarkable that an intense degree of cold does not deprive the organism of its power of growing, for, if after being exposed to great cold for some time, it is again placed under favourable conditions, it appears to regain its powers of rapid multiplication. Koch, to test this, submitted a cultivation of the bacillus for one hour to a temperature of 10° C. (10° C. below freezing point), with the result that the nutrient medium was completely frozen. This was now thawed, and a cultivation made at a higher temperature and under favourable conditions; the bacillus began to grow again almost immediately, and appeared, indeed, to have lost none of its vitality. These experiments accord well with, and verify the observations that have been made on, the appearance and spread of cholera epidemics in the hot and cold seasons. Most of the cholera epidemics seem to have attained their maximum virulence during or at the end of the hot season of the year. In those regions that are periodically visited it usually breaks out in the autumn, when the external temperature is most favourable to the growth of the bacillus as a saprophyte, and when in consequence the micro-organism is enabled to live for a longer period outside the body, to give rise to numerous progeny and thus to multiply the possible sources of infection. On the other hand epidemics are of frequent occurrence, even in the

coldest seasons of the year ; nay, in some cases the appearance of the cold damp season is the signal for the outbreak of a cholera epidemic, because not until the return of the cold weather, after the hot dry summer and autumn months, is there that dampness of soil and atmosphere which is essential for the existence of the organism, in addition to which the soil remains at a comparatively high temperature for some time after that of the atmosphere has fallen. Again, the external cold, though it may paralyse the organism for a time, does not destroy its vitality, but, keeping the organism in a passive condition, actually enables it to retain this vitality for a considerable period, so that, when it finds its way into dwellings, in which the atmosphere, owing to bad ventilation, is not only raised to a high temperature, but also contains a considerable quantity of moisture and organic matter, it is at once introduced to conditions that are essentially favourable to its saprophytic existence ; it again begins to thrive luxuriantly, and becomes a possible source of infection.

The fact must not be ignored, also, that seasonal temperature plays not only a most important part in the determination of the amount of moisture in the air and in the soil, but also in the production of currents of air by which the organisms may be carried from point to point (though these currents, when the air is dry, or in hot, clear weather, play but a small part in the dissemination of the disease), and by its effects on certain kinds of vegetation, which indirectly have been proved to play an important part in the distribution of the cholera organism.

On the effect of heat on the amount of moisture in the atmosphere, and consequently on the depth of the ground water, it is scarcely necessary to speak at any great length, but there can be little doubt, from Pettenkofer's observations, that such factors do play a most important part in conditioning the growth and multiplication of the cholera organism. The rapid removal of stagnant and upper ground water (if complete) is inimical to the saprophytic growth of the cholera organism, as even organic matter in a state of dust is absolutely useless as a nutrient material for the cholera bacillus. Where, on the other hand, there is a considerable quantity of moisture in the atmosphere, even though the temperature be high, stagnant water is often found.

Koch says: "On the surface or in the ground, in marshes, in docks, which have no outlet, in places where the ground is formed like a trough, in sluggish rivers and the like . . . there a constant nutrient solution can be formed and

may accumulate in the neighbourhood of animal and vegetable decaying matters most readily and give the micro-organisms opportunities for growth;" and he further says, "that whenever the water has a swift current, or is in a constant state of change, both on the surface and in the ground, these conditions occur less easily, or sometimes not at all, for the continuous current prevents a localized concentration of nourishment in the fluid sufficient for the pathogenic bacteria. The connection between the sinking of the ground water, and the increase of many infective diseases, we might explain thus : that with the sinking of the ground water, the current which exists in it becomes very much lessened. Besides, the mass of water lying superficially at disposal will be considerably diminished, and therefore such a concentration as I have described as necessary for the growth of the bacteria will be produced much sooner."

These conditions are necessarily closely associated with temperature, with the nature of the sub-soil, and the prevailing winds and air currents, and the consequent affection of the quantity of moisture in the atmosphere, and the production of rain must also be taken into account, as even the transmission of the cholera organism is affected or interfered with by great dryness of the atmosphere. Where there is great dryness of the atmosphere the small quantities of cholera dejecta, which are likely to be left unnoticed, and therefore left disinfected by the attendants of the patient are so rapidly dried that they are speedily rendered inert, and cannot convey the infection further ; whilst the materials on which, in ordinary circumstances, they would thrive, are so dried on the surface that although the organisms may find their way on to them in a living condition, there is not sufficient moisture left to allow of the development of the probably somewhat weakened organism.

As Flügge points out, such conditions "only occur where there is a very great deficiency in the saturation of the air with moisture—for example, in a desert . . . It is conceivable that in a desert climate such as is present in Mooltan and Lahore during the greater part of the year, and where everything dries up, as it were, under one's eyes, the conditions favourable for the spread of the cholera may only be present at most during the somewhat moister or so-called 'rainy' season (July to October)."

Extreme wet may, however, exert an influence in interfering with the saprophytic growth of the organism, especially if the rains be heavy and continuous. The conditions mentioned by Koch, as inimical to the collection of organic material on which the organism may grow, are brought into play, infective dejecta are removed from the surface soil, from the surface drains and from the sewers, rapid and con-

tinuous currents are created, organic matter of all kinds is washed away, air is driven out from the subsoil owing to the rising of the ground water, and all the conditions become unfavourable for the growth of the organism. The fact that vegetation has been mentioned, as associated with climate in the production of cholera, requires some explanation, which will be given later. Dr. Macleod, writing on the subject of climate itself, says :

"Cholera makes its appearance in Shanghai every summer with startling regularity, Before the end of July it is hardly met with, by the end of August it is well marked, in September it is in full swing, not quite so virulent in October, and in the beginning of November an occasional case may be heard of, after which time it disappears entirely till the following late summer. For twenty years this has gone on with unfailing regularity under the observation of medical men now resident, and for how long previously no one can estimate." He then goes on to describe the weather in June as damp and hot, in September as hot, damp, and muggy, in October as cool and wet during the first part, and as frosty towards the end, "at Christmas there is usually ice, there may be snow." He then says, "so far as temperature of the air is concerned, we enjoy tropical heat for nearly a couple of months before the disease breaks out; it is most virulent in the hot, damp September, and does not disappear until after the hoar-frosty mornings are experienced" (the end of October).

Dealing with the same subject Koch has stated that in no part of the world, of which the climate, the geology, and the epidemiology are known, does cholera occur all the year round except in the province of Bengal, and he says :

"All authors are agreed, that the delta of the Ganges is the true home of cholera, and I have come to the conclusion that this is the case, and that there are no other places of origin of cholera in India. For the only district in India, where cholera prevails continually year after year in a uniform manner, is the delta of the Ganges. In all other places it shows marked variations, or it may even disappear altogether for a shorter or a longer time. In certain places, for example in Bombay, it never entirely disappears, but it is highly probable, that on account of the unusually active trade with the rest of India it is constantly being imported there afresh." He then describes this tract as a perfectly flat country only slightly raised above the sea level, which, during rainy seasons, is almost entirely under water in the lower part of the delta, where the "Ganges and the Brahmapootra break up into a network of water courses, in which the sea water, mixing itself with the river water, flows hither and thither with the tide, and at flood time places large tracts of the Sunderbunds under water. A luxuriant vegetation and an abundant animal life have developed in this uninhabited region, which is inaccessible to man not only on account of the floods and the numerous tigers, but is avoided principally on account of the pernicious fever which attacks everybody who remains there even for quite a short time. One can easily imagine how dense the vegetable and animal matter

is which is given up to decomposition in the marshy districts of the Sunderbunds, and that here an opportunity is afforded for the development of micro-organisms, such as exists in scarcely any other place on the globe. Peculiarly favourable for this are the regions between the inhabited and the uninhabited parts of the delta, where the excrements of an unusually thickly populated country are washed away by the current, and, flowing here and there, are mixed with the brackish water of the Sunderbunds, already teeming with decaying matter. Under these peculiar conditions quite a distinct fauna and flora of micro-organisms must be developed there, to which in all probability the cholera bacillus belongs. For everything points to the cholera having its origin in this district. All the greater epidemics have begun with an increase of cholera in the southern portion of Bengal. Jessore, from which the first intimation of the epidemic of 1817 came, lies on the borders of the Sunderbunds; and Calcutta, which is now the fixed home of the cholera, is connected with the neighbouring Sunderbunds by a marshy and sparsely inhabited tract of land. Now the comma bacillus finds in this district, contiguous to its presumptive home, the most favourable conditions imaginable to implant itself and to spread from one individual to another."

In the large towns in this cholera region there appears to have been no diminution in the mortality from the disease during the British occupation; and until a new water supply was obtained and it was no longer necessary for the natives and others to take their water from tanks, from the Ganges, or from the Hoogley, even a better sanitary and drainage system appeared to have little effect. Once this supply was obtained, the mortality fell to about one third, and even a considerable portion of that third is to be accounted for by the fact that it was, and is, very difficult to impress on the natives the necessity of avoiding the old contaminated sources of water supply. Although Koch brought out these remarkable statistics he is still, as he says, "not a supporter of the exclusive drinking-water theory," and he considers "that the ways in which cholera can spread itself are extremely varied, and that almost every place has its own peculiarities which have to be thoroughly investigated, and the regulations which are to serve for the prevention of infection in the place in question must be drawn up accordingly." It is a remarkable fact that Koch never speaks or appears to think of the possibility of the existence of the cholera bacillus as a saprophyte. If it enters food or water it is the result of an accident and the organism can remain there, capable of development only for a short time and under exceptionally favourable conditions. Thus he contends that cholera is endemic in the Ganges only because there is a complete

chain of cases from one year's end to the other by which the infection is handed on. Hueppe, on the other hand, maintains, as above stated, that the bacillus can have a distinct saprophytic existence, and points to the fact that in places analogous to the delta of the Ganges it has a marked tendency to become endemic.

As an example of this we may summarize the results of Macleod's observations on cholera in Shanghai. He says that "the remarkable regularity of the time of outbreak, period of duration, and time of cessation of the disease, so far as I am aware, has no parallel on record." The country round Shanghai is strikingly like that of the deltas of the Ganges and the Nile; "in each there is the alluvial deposit, rich in organic matter, and the high ground-water level, yet in the Ganges delta the disease is prevalent all the year round; at the mouth of the Yang-Tsze it occurs with the regularity of a crop at the same season yearly; in the delta of the Nile it occurs only occasionally but at the same season as at the mouth of the Yang-Tsze. The two latter regions have at least weekly communication with India. Some causes or combination of causes are prevalent all the year round at the mouth of the Ganges; at one season every year, viz, late summer and autumn at the mouth of the Yang-Tsze; and at the same season, but not every year at the mouth of the Nile."

There must then in these three regions be perfectly distinct but local conditions which must determine the difference in the behaviour of cholera in these various regions. The general characters of the soil, the ground water, and position are much the same, but there are well-marked differences as regards temperature, population and methods of cultivation. In the delta of the Ganges cholera is absolutely endemic, it is never absent; the poison, whatever may be its nature, is therefore always present. "From the position and climate, the temperature of the soil varies little throughout the year, and is never so low that some vegetable growth is not active. At the mouth of the Yang-Tsze (where cholera is prevalent only during certain parts of the year), the poison is endemic or is introduced shortly before the time that cholera breaks out each year, in which case it is curious that it should always be at the same time, there being no means of communication with a cholera infected country opened up specially at that time, communication wlth India being weekly. Here the climate admits of a greater range in the soil temperature than at the mouth of the Ganges, there being great extremes of both heat and cold. The soil heats more slowly than the air, hence perhaps it is the determining cause for the spread of the disease in the late summer and autumn, as already described, and of its absence in winter and spring." It is evident from this statement

that cholera does not show itself during the summer when the air temperature is at its maximum, but only when sufficient time has elapsed to allow of the rising of the ground temperature or that of the earth, and as a matter of fact the disease occurs in Europe during the autumn months often when the general or atmospheric temperature has actually begun to fall, but when the earth temperature has attained its most equable maximum, when of course we have the optimum temperature conditions for the growth of the cholera organism in the soil. "At the mouth of the Nile the conditions are much the same as near Shanghai, except that the extremes of heat and cold are greater at the latter. Both have at least weekly communication with India, both communicate through the tropics; whilst Shanghai is at a distance of at least three weeks in time, Egypt is but two. The regularity of the yearly outbreak at Shanghai points to an endemic poison, the irregularity at the mouth of the Nile to the occasional introduction of the poison. Late summer is also the period for the Egyptian epidemics of any extent."

Bearing on this, we have the fact that a cholera epidemic may remain dormant for months or a whole winter; especially as the organisms retain their capacity of reproduction if they are kept moist and are supplied with oxygen. According to Nicati and Reitsch, cholera bacilli were found alive eighty-one days after they had been placed in the harbour water of Marseilles. Koch found cholera bacilli capable of reproduction after they had grown one hundred and forty-four days on agar, but in one hundred and seventy-five days these cultivations were found to be dead. Macleod found that pure cultivations renewed but once a month retained their virulence for a year. Taking all these facts into consideration, it is easy to understand how in Shanghai the organisms may remain dormant for a certain period, and then under favourable conditions begin to grow again with increased activity. As Koch puts it—"One can easily imagine that in superficial layers of earth, in marshes, and so forth, the cholera bacilli may find conditions in which they can exist preserved from death for five months or even longer, just as well or even better than on our moist agar jelly." Here then are three places which, although they have certain features in common, are characterized by certain differences, all of which appear to depend on climatic conditions. In the one case the cholera is prevalent during the whole year; it is in fact endemic and continuous. In the second case cholera appears with the utmost regularity at certain seasons of the year, and then as regularly disappears. In this case the disease appears

to be endemic, but on account of some local circumstances connected with temperature and vegetation it is developed only in crops—at harvest time, as it were. In the third case, in the Nile delta, the disease is extremely intermittent in its outbreak, and occurs only in those years in which the conditions as regards temperature, moisture, &c., are specially favourable for its development. In connection with local peculiarities as favouring the growth of the bacillus and the spread of the cholera, Macleod makes several very interesting observations, some of which bear out in a most remarkable manner Koch's statement as to the general laws that govern the spread of the disease, and also as to the minor local factors that determine individual outbreaks. The population in Shanghai he divides into three classes : resident foreigners, amongst whom deaths from cholera average less than 2 per 3000 per annum ; amongst the seafaring foreign population the average is very much higher, as of the sailors who come to port there die from cholera from 15 to 30 per 1000 yearly ; whilst among the Chinese, even in the settlement, there are rarely fewer than from 200 to 300 deaths in a single season. What part then do local peculiarities and customs play amongst these three sets of people ? It is a fact, generally recognized, that any disturbance of the digestive function is the principal predisposing cause of the disease in a cholera outbreak—a fact that is explained by the absence of the ordinary amount of acid from the gastric juice in these cases ; such gastric derangement is specially met with after a bout of drinking. Macleod says, "Among the sailors a night ashore is the usual precursor of the disease, and commonly that indicates a large consumption of liquor." This, however, does not account for all the cases that occur, and he says, "Occasionally men who have not been ashore are attacked. A sailor belonging to an American ship was attacked two or three days after arrival ; he had not been ashore, the ship had touched at no port for weeks before, and the water supplied on board had been in use during the voyage. The captain reported that he had specially encouraged the men to eat fresh vegetables largely, and that he had seen the man referred to sitting on deck munching lettuce the day before he became ill (three of this ship's crew died of cholera). These vegetables had been supplied by Chinese bumboats."

The custom amongst the Chinese "is to carefully collect human excreta and add these to water; this mixture is then used for watering vegetables, &c., so that the leaves come in for a fair share. All European and Chinese night soil is collected daily and carried away to be used for agricultural purposes. No night soil finds its way into drains, and there are no water closets," and all refuse not used directly for watering vegetables is carried into the country and used for agricultural purposes, so that if the poison be in the excreta, in China at least, its deposition in the ground is secured, and it has certainly been discovered empirically by European residents that vegetables cannot be taken with impunity. "Chinese vegetables are regarded with suspicion during the cholera season, more especially such as are uncooked."

When the relation of the comma bacillus to the dejecta from cholera patients is borne in mind, there can be little wonder that the consumption of Chinese lettuces should be followed by an attack of cholera. In connection with the conveyance of cholera by means of drinking water, it may be objected that the Chinese do not drink water as a rule, but indulge in weak tea; but here again it is stated that water for household use is stored in an earthenware or wooden vessel placed in or near the kitchen, and into this vessel others are dipped from time to time. The water is obtained from creeks or wells. Vegetables may be seen hanging over these vessels or lying on tables, so that though the vegetables are cooked, tables, dishes, cloths, and all that come in contact with uncooked vegetables furnish abundant opportunity for contamination of food after it has been cooked, where such filthy habits prevail as amongst the Chinese. They do not use milk as do foreigners, but they supply it, and from what has been said of vegetables and Chinese habits, unless the supply is known to be well cared for, it cannot be regarded as beyond suspicion of forming a vehicle for the distribution of cholera, typhoid, and other poisons. It is evident, then, that although no one set of factors alone can be looked upon as determining or explaining all outbreaks of cholera, the bacillus, so far as our present knowledge goes, must be looked upon as the essential factor in the causation of the disease; this bacillus, like every other organism that we know throughout the whole animal and plant kingdoms, being, to a great extent, dependent on its environments for its very existence. In considering this question let it be understood most distinctly that though the bacillus is proved to be the cause of the disease, it is not necessary to assume, as some people seem to suppose, that the careful, and in

themselves complete, observations of the great epidemiologists of this and other countries are valueless ; and as in time a more perfect knowledge of the conditions favourable or unfavourable to the growth of the organism is obtained, it may be expected that all observations made with care by those whose only desire is to get at the truth, will fit in and take their place in a large and comprehensive scheme through which there will ultimately be a possibility not only of understanding the disease more thoroughly, but of removing or combating its causes.

It has already been pointed out how Pettenkofer's observations may be made to agree with Koch's ; it has been seen that the apparently contradictory statements on the subject of the spread of cholera along the lines of pilgrimage and trade have been more or less satisfactorily explained and reconciled on the assumption that the bacillus is the cause of the disease. Then, too, the well-known facts of the association between cholera and gastric and intestinal disturbances of various kinds ; between it and consumption of various articles of food and of water from sources which are probably contaminated, or in which the presence of the cholera organism has been demonstrated, and the facts connected with climatic conditions, ground water and subsoil drainage, temperature and special local conditions, may all be satisfactorily explained on Koch's comma bacillus theory. These explanations, taken with the fact that the comma bacillus is invariably found during certain stages of the disease, that it has never yet been found in any but typical cases of cholera, that with it the disease has been produced in animals, experimentally, and one might also say in man, though accidentally (at the cholera course in the Hygienic Institute in Berlin), make it impossible for us to shut our eyes to the fact that in the cholera bacillus we have the only suggested causal agent that will allow of a satisfactory explanation of the mass of observations made up to the present. It had already been demonstrated that the bacillus was found only in the intestinal canal, when it naturally suggested itself to Koch that the symptoms of cholera were to be explained by the theory that in this disease there was absorption from the intestine of some soluble poison produced by the bacillus *in situ* ; that there was, in fact, a local formation of the poison, but a general absorption into the system (the " Intoxication " theory). This

theory was at once accepted as being from every point of view more far-reaching and more satisfactory than the older one which explained all the symptoms by referring them to loss of water through the excessive intestinal discharges ; a symptom or effect was, in fact, looked upon as the cause of other symptoms. The earlier observations on ptomaines and sepsines, and Pasteur's and Hansen's later observations on ferments, naturally led to a search being made with the object of finding out any specific products of the cholera organism. Koch himself records the fact that he succeeded in preparing cultures of the comma bacillus which were so intensely poisonous, that when injected into animals, either subcutaneously or into a peritoneal cavity, there were set up in a few minutes all the symptoms which occurred in animals suffering from cholera a day or two after infection—"paralytic weakness of the hinder extremities, coldness of the head and legs, and prolonged respiration, a condition which usually leads after some hours to death." Buchner, who demonstrated, as he believed, the formation of butyric acid during the growth of pure cultivations, was unable to corroborate by any experiments of his own this view of Koch's, but Pouchet and Villiers were both able to extract from the dejecta or from the organs of cholera patients certain products which they deemed to be characteristic.

The former, with the aid of chloroform, extracted from cholera dejecta an extremely toxic oily liquid which, as it becomes oxydized in the presence of air and light, takes on, first a rose, and then a brown colour. It readily combines with hydrochloric acid to form a chloride, but again breaks down, *in vacuo*, or when the temperature is raised, or on the addition of an alkali. It gives the reactions characteristic of the alkaloids, and gives the blue reduction coloration with ferro-cyanide of iron and perchloride of iron. Villiers succeeded in separating from the organs of a single cholera patient a couple of centigrammes of a peculiar alkaloid. On treating this with hydrochloric acid, there separated out a number of acicular crystals: these crystals have since been described by various observers, and it is stated that in this form the basic substance, whatever it may be, exerts comparatively little poisonous action, but that as soon as it is again set free from the acid combination, by the addition of an alkali, such as soda or potash, it exerts not only an extremely caustic local action, but also when injected into a guinea pig produces muscular tremblings and very great irregularity of the heart's action, the animal dying at the end of about four days. It is impossible, however, to be quite certain that these reactions were obtained with Villiers' pure acicular crystals, and not with one or other of the sepsines or toxines, as there is some little doubt as to the exact nature of the poison obtained in several of the series of experiments. Brieger, going beyond

these researches, was able to isolate, especially from old cultures, substances analogous to, or identical with, cadavarine, putrescine, and choline. Carrying his experiments further, and cultivating the comma bacillus in media containing creatine, he produced a toxine or specific poisonous product which produced, when injected into the animals, dyspnœa, muscular tremors, cramp, and death. To this he gave the somewhat formidable name of Methylguanidin. Carrying his observation still further, he succeeded in separating from a precipitate obtained by the addition of a mercurial salt, two other toxines, both of which appeared to be more or less characteristic of the cholera growth.

It will be noted that in all the experiments made, in which the toxines were obtained even from pure cultures of Koch's comma bacillus, the time required for their production and the quantities separated are out of all proportion to what occurs in actual cases of cholera, where, from the rapidity of the course of the disease and the severity of the symptoms, a large quantity of the poison must be developed in a very short time. Most of the artificial experiments, however, have been made on different media (usually bouillon containing peptone) and under very different conditions from those which obtain in an animal. In a series of investigations carried out by Wood in Hueppe's laboratory, an attempt was made to grow cultures under precisely those conditions that are met with in the human intestine, with the result that a very rapid toxine production, and an additional proof of the bacillary origin of the cholera virus were obtained. He took for his nutrient medium normal albumen, and thus obtained the earlier products such as the albumoses that occur in the breaking down of albuminoids. The presence of these would of course account for the greater toxicity of his cultures under such conditions.

One of the earliest observations made by epidemiologists was that one attack of cholera protects for a certain time and to a certain degree against a second, and from this it was now argued that it should be possible to obtain a system of preventive inoculation ; and Gamaleia, basing his work on what was already known of preventive inoculation in other diseases, commenced a series of experiments by means of which he hoped to construct such a method of inoculation against cholera. In this he was ultimately successful. He found that if, from a culture of the *vibrio Metschnikovi*, an organism almost identical with the comma bacillus, both morphologically and physiologically, in beef broth, he took

the pellicle which formed on the surface at the end of five days, sterilized it in an autoclave at 120° C., and then injected from two to six c.c. of the fluid expressed from the sterilized mass into the muscles of a guinea pig, the animal was rapidly and completely protected against the action of Koch's comma bacillus, introduced in the ordinary fashion. By further experiments he found that by simply keeping for several days the sterilized mass in which the dead bacilli still remained a much larger quantity of the poison could be obtained—sufficient, in fact, to kill the animal when given in the same dose as before, but still protecting the animal when exhibited in smaller doses. (Here the organisms could no longer be producing the poison, but it appears as though there was stored up in their body a considerable quantity which could only diffuse out into the fluid after a certain lapse of time.)

To Gamaleia also we owe the knowledge that this organism may become very much modified in various ways, and some of the experiments he carried out provide us with an explanation of some of the most important facts connected with the increase and decrease in virulence of type, in cases that occur during the "rise and fall" of an epidemic.

He was able to increase the virulence of the special comma bacillus in a most remarkable manner. After obtaining a growth of the organism in broth he introduced a small quantity of the culture into the lung of a white rat; this was followed by an acute form of croupus pneumonia accompanied by marked pleurisy, the animal rapidly succumbing. With the fluid that accumulated in the chest a second animal was inoculated in a similar fashion, with the result that this animal died more rapidly than the first. This inoculation was continued through a whole series of animals, until finally the rats succumbed very rapidly indeed, and an organism was found not in the intestine, but in very large numbers in the blood.[1]

In addition to drying, acid, and the other destructive reagents already mentioned, it has been found that a very considerable number of chemical reagents arrest or prevent the growth of the cholera bacillus.

Koch's list contains alcohol, 10 per cent., sulphate of iron, 2 per cent. (this latter acts first as an acid, and secondly as a precipitant of the

[1] That this may have a bearing on Wood's observations as to the virulence of the organism in the ærobic and anærobic conditions is most evident, and is well worth further investigation.

albuminoid materials on which the organism lives), alum, 1 per cent., camphor, .33 per cent., carbolic acid, .25 per cent., essence of peppermint, .5 per mille, sulphate of copper, 4 per mille, quinine, .2 per mille, corrosive sublimate, 1 per cent.

Babes has found that the organisms will not develop in a gelatine nutrient medium which contains .5 per mille of corrosive sublimate, .1 per mille of carbolic acid, .2 per mille of sulphate of copper, .8 per mille of salycilic acid, .1 per mille of thymol, 2 per mille of iodine, 2.2 per mille of bromine, 7 per cent. of alcohol, .8 per mille of sulphate of quinine, .5 per mille of acetic acid (the action of which, however, depends to a certain extent upon its interference with the degree of alkalinity of the original gelatine, as the bacillus cannot grow in a medium in which there is more than a merely appreciable trace of acidity). He also tried a very interesting experiment in which he inverted a gelatine plate on which was placed a cholera culture over a watch glass or shallow vessel containing a drop of essential oil of mustard; no development of the organism took place, and if the plate was left for twenty-four hours the organisms were all killed, as there was no subsequent development; so that oil of mustard is an excellent disinfectant. Other active volatile principles act in very much the same manner.

The experiments with gelatine cultures are of special value because they allow demonstration of the effect that disinfectants have on the bacillus as it grows on specially favourable nutrient media. Reference has already been made to the fact that putrefactive organisms and their products exert a prejudicial effect on the cholera organism, but it should be remembered that when the conditions are favourable there is usually such rapid proliferation of the cholera organism for the first day or two, that the others are left behind in the race for existence. It is, however, a case of the hare and the tortoise, for after a time the cholera organisms die out, and never again obtain a permanent footing unless a fresh supply of nutrient material becomes accessible. It is important to remember this in connection with the spread of cholera by water supply, as it explains the immunity enjoyed, after a time, even in the most cholera-stricken regions. In India, in the regions in which the cholera is endemic, the wells, as a rule, are merely surface tanks into which sewage and surface water may be drained, and which are frequently on the same level as, and connected with, the cesspools, so that even the water supply contains a considerable quantity of organic matter in which organisms of all kinds can flourish most luxuriantly; whilst these same wells, being merely dug-out pits beneath the slightly raised houses, are open for the reception of sewage and excreta

of all kinds, especially in times of illness, when neither patients nor nurses have strength or time to see that these are properly removed. This source of danger is so evident, and is so in accordance with what one would expect, that efforts have been made to remove, as far as possible, all organic material which might serve as nutrient material for infective organisms from the soil and ground water, and also to remove as rapidly and as completely as possible not only dejecta, but also the water employed for cleansing linen, clothing, and utensils "without allowing them to come in contact with the surface of the soil, with wells," with vegetables, and the like.

Most hygienists are agreed that it is necessary not only to have a pure water supply—*i.e.*, a supply free from all possible contamination, derived from wells so deep, or from reservoirs so far from human habitations, that there is no possibility of contamination by sewage, dejecta, or surface drainage, and so carefully conveyed by conduits and pipes that no such contamination can take place during distribution—but that a water supply should be so ample that dirtiness is heavily discounted. With all the improvements that have been made in the drainage system and water supply of Lower Bengal, cholera has only diminished about 60 per cent., so that there still remain certain factors that favour the spread of cholera, and every now and again such a spread or outbreak may take place with extreme rapidity, and may involve a very wide area. Cleanliness, however, both general and personal, may be said to be the most important factor in the prophylaxis of cholera. Flügge, for example, states most emphatically and explicitly that "the average cleanliness of the population has the greatest influence in this respect. The more cleanly the method of handling the sick and the infected clothes, the more carefully contamination of the soil, of the water, and of various other objects with the dejecta is avoided, the fewer will be the sources of infection. The more carefully the hands are cleansed and the articles of food prepared, the more will the paths of spread from existing sources of infection be diminished. It is evident that in this respect marked differences must exist between more and less civilized countries; between new and well-built, and old and cramped cities; between poor and wealthy neighbourhoods; between the

portion of a city inhabited by the poor and that in which the better classes dwell." The habits of people, then, especially as regards their food, play a most important part in the propagation or restriction of cholera. The organism is almost invariably introduced by the mouth, so that in addition to passing into the alimentary canal by contaminated water directly, it may also be introduced by utensils, or food washed or sprinkled with such water, and Koch gives an excellent example of this when he describes the market women of Marseilles as being in the habit of sprinkling the vegetables exposed for sale with water from the street gutter, into which he proved that comma bacilli were constantly making their way, so that any one partaking of these vegetables uncooked, just as in the case of the American sailor at Shanghai, was taking in an unknown quantity, but a very appreciable and deadly dose, of the cholera organism and its poisonous products. Not only do these uncooked vegetables offer a nidus and an excellent substratum for the growth of cholera organisms, but they often produce that condition of slight indigestion which along with an overloaded stomach is one of the most favourable for the development of the comma bacillus in the human alimentary canal.

For the same reason feasts, fasts, Saturday night carousals and Sunday dyspepsias, pilgrim festivals, arrivals in port, and similar events are all predisposing causes of cholera, as in all cases there is a disturbance of the digestive function and a lowering of the system, due either to excess in drinking or in eating, or to gastric disturbance and lowered vitality resulting from abstinence from the use of food for too long a period. It is found, too, that wherever people assemble in large numbers in excess of the ordinary population, the strain on the sanitary arrangements is always excessive, and further, is necessarily accompanied by carelessness in the selection and preparation of food. Cholera depends for its existence, outside those places in which it is endemic, on these fairs and pilgrimages, and only by controlling them and by attending most thoroughly to the sanitary conditions at the points where people are massed together can there be any hope of preventing the outbreak and spread of this insidious and deadly disease.

It is not necessary here to do more than mention the pre-

disposing effect of autumnal gastric disturbances and slight diarrhœa, which have, in many epidemics, been the invariable precursors of the true Asiatic or Indian cholera. It is known that during an epidemic one attack of cholera, especially a severe one, exerts a great protective influence on those who survive, and as these consist almost exclusively of the people who under ordinary circumstances are again most exposed to infection, there may be natural breaks in the line of extension of an advancing epidemic; breaks which have been used, by some authorities, as evidence of the sporadic outbreak of the disease.

So much has the study of Koch's comma bacillus tinged and affected all our ways of looking at cholera epidemics that we now consider the conditions under which the bacillus can multiply and be carried from point to point, and the conditions that favour its development and multiplication, instead of dealing with cholera itself as the entity with which we have to contend. The epidemiologist has now assumed the *rôle* of biologist in the widest sense of the term. The bacteriological hygienist agrees with the "localist" that it is necessary to get rid of all conditions in which the cholera poison can be propagated and distributed, and that to this end the ground water should be kept as free as possible from sewage, that all areas should be properly drained, and that everything should be done to render the "drying zone" as little congenial to the bacillus as possible; but he goes further, and insists that the poison should not be allowed to be introduced into localities in which it does not already exist, for he believes that however favourable the conditions may be, there will be no outbreak of cholera until the bacillus is introduced and gains a foothold. He therefore insists on careful inspection of all ships coming from India or from other cholera-stricken regions, though he is firmly convinced that it is only in seaport towns that inspection or quarantine can be of any value; that if cholera makes its way beyond the seaport no quarantine or sanitary cordon can stop its spread; that once beyond port the most careful isolation of all patients and disinfection of every article of clothing, feeding utensils, &c., should be rigidly carried out and insisted upon; that the dejecta should be mixed with large quantities of carbolic acid, concentrated hydrochloric acid, or strong cor-

rosive sublimate solution; that the comma bacilli in rooms should be thoroughly dried and aired by throwing open the windows for several days, and that instructions should be issued as to the necessity for thoroughly personal, culinary, and household cleanliness, as to the avoidance of all water except that known to be pure, as to careful boiling and cooking of drinking water and food, as to the necessity of paying attention to the slightest gastric derangement, and as to the avoidance of all excesses in both eating and drinking. He pays attention then, first, to all relating to the power of the individual to resist any attack of the organisms. All place dispositions favouring the production of conditions of gastro-intestinal disturbance are to be carefully neutralized, and in the same way all time dispositions, such as autumn diarrhœa, determined by the unripe or overripe fruits so abundant in the latter half of the year are to be met and counteracted; and second, to everything relating to the rendering of all the environments unfit for the development of the comma bacillus, so that the number of centres from which infection may spread may be kept down as much as possible.

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CHAPTER X.

Typhoid Fever.

Typhoid Fever a Bacterial Disease — Recklinghausen's Observations—Klein—Eberth—Klebs—Coats—The Bacillus—Method of Staining—Position in Tissues—Gaffky's Observations—Pure Cultures—Excretory Products—Experiments on Animals—Mixed Infections—Action of Light and Heat on Typhoid Bacilli—Pseudo-typhoid Bacilli.

From the curious nature of the symptoms of typhoid fever, and from the fact that after complete recovery from an attack there appears to be a certain immunity (for a certain period at any rate) against a second, although relapses are of comparatively frequent occurrence, it was early supposed to be the result of the presence of some specific micro-organism within the body, probably in the deeper tissues of the wall of certain parts of the intestinal canal, and in those organs, such as the spleen and lymphatic glands, that are specially connected or associated with that canal. Although, as early as 1871, Recklinghausen described in abscesses that were formed during the course of an attack of typhoid fever, microbes which he considered to be specific, and although Klein in this country found several varieties of micro-organisms in typhoid lesions, the specific organism was first accurately described, and distinguished from others, by Eberth and Klebs abroad and by Coats in this country, all of whom give very exact descriptions of the typhoid bacillus. These bacilli are short, somewhat thick rods, about 2 to 3μ in length and .3 to .5μ in breadth; they are usually distinctly rounded at the ends, where the protoplasm is always rather more deeply coloured by aniline dyes than the central portion, which was at one time supposed to be a spore, though more recently this lighter coloured portion has been looked upon as evidence of a process of degeneration. These bacilli are said to be stained with difficulty, but I have found that if the sections in which they are present are first

allowed to remain for about ten minutes in a 1-5th per cent. solution of corrosive sublimate and then stained by Gram's method, the bacilli are most deeply stained, although Fraenkel and others state that the colour is invariably discharged if Gram's method be used. They may also be prepared by Kühne's method of first allowing them to remain in a concentrated watery solution of oxalic acid, washing them carefully and afterwards staining with methyl blue dissolved in a 1 per cent. solution of ammonium carbonate. Sections may also be stained for twenty-four hours in Löffler's alkaline methylene blue, after which they are rinsed in water, which removes sufficient of the colour ; the water is driven out with aniline oil, the sections are allowed to dry on the slide and mounted in Xylol Balsam.

The bacilli are found in the adenoid follicles, or lymphatic tissue of the intestine, in the mesenteric glands, in the spleen, and in the liver, and more rarely in the kidneys. They are usually collected in little clumps, and single bacilli are seldom if ever met with. These clumps, although readily enough recognized when seen, are as a rule so sparsely scattered through the tissues that it is often a difficult matter to find them, even in characteristic cases, and as Flügge says, " It is only after the examination of a large number of sections that one or several of these deposits can be found." Gaffky, working in the Hygienic Institute in Berlin, was first able to make pure cultivations of this bacillus, and in 1884 he gave a very complete description of the bacilli that he was able to examine or to cultivate in twenty out of twenty-two cases of typhoid fever of which the examination was committed to his charge. The bacilli, when obtained pure, and cultivated in fluid, grew out into very long threads, both threads and short bacilli apparently being motile, having a peculiar wavy motion ; quite recently this motion has been found to be due to the presence of groups of lateral flagella which, waving backwards and forwards, impart to the organism its peculiar snake-like movement. The bacillus can grow perfectly well both in the presence of free oxygen and also when oxygen is cut off, but, as in the case of the cholera organism, it appears to have somewhat different functions and different powers under the two sets of conditions ; outside the body in the presence of oxygen it appears to develop great " resistant " power and a saprophytic habit, whilst in the anærobic con-

dition, especially in the intestine, although its power of breaking up the albuminoid substances presented to it and of developing its specific toxines is greatly increased, its capability of resisting antiseptic substances is considerably diminished. In plate cultivations made from the organs of typhoid patients, Gaffky found that the bacilli developed in the deeper part of the gelatine as small white points, whilst

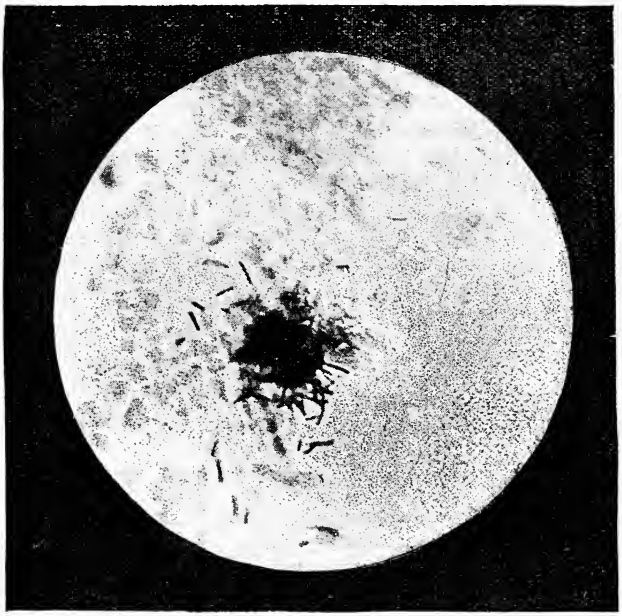

Photo-micrograph of Typhoid bacilli in Lymph follicle of Intestine of a child in which the Typhoid lesions were very characteristic. × 500.

on the surface they grow as moist-looking greyish colonies with irregular margins. Under a low magnification the small rounded points are seen to be slightly granular ; they have a sharply-defined margin and are of a dirty yellow colour ; the superficial growths, although spreading somewhat rapidly, are thin, sometimes almost transparent, and have a yellowish tinge when seen in the sunlight ; the margin is

irregular and is marked by large and small indentations. There is no liquefaction of the gelatine around the growth. In a test tube cultivation the growth appears all along the line of the puncture and also on the surface. The surface culture has a peculiar mother-of-pearl look, it gradually spreads over the whole of the gelatine, forming a kind of bluish-grey film, whilst down each side of the needle track there is a delicate zone of the same bluish-grey colour, surrounded in turn by a peculiar opalescent milkiness. The most characteristic growth, however, occurs on sterilized potatoes. It is characteristic in that, even when there is a most luxuriant growth of the typhoid bacillus, it cannot be recognized by the naked eye, even at the end of three or four days, except by a peculiar moist appearance of the potato, which, taken along with the appearances in milk and on gelatine so far as is at present known, distinguishes the growth of this organism from all others. It will be remembered, however, that the potato is slightly acid, and it appears that this acidity is necessary for this typical growth, for on potatoes rendered slightly alkaline there appears a yellowish or dirty grey growth with sharply defined margins —a growth quite different from that above described. Chantemesse and Widal utilized the power of the micro-organism to grow in acid to help them to obtain pure cultivations. They prepared special tubes of gelatine by adding to each 10 c.c. of the nutrient medium, 4 or 5 drops of 1 to 20 per cent. solution of carbolic or phenic acid ; they did this in order to prevent the development of those microbes that bring about liquefaction of gelatine. They were perfectly successful in their attempts, although, as we have seen elsewhere, it does not follow that their explanation was absolutely correct ; at any rate they were able to separate pure typhoid bacilli which had all the characteristic appearances both on the gelatine plates and under the microscope.

The organism itself, unlike many other bacteria, seems to form an acid and not an alkaline excretory product. It grows in a variety of media, and as I have already said, it appears under certain conditions to become quite saprophytic in habit, this being evidenced by the ease with which it may be cultivated outside the body, and quite recently Wolffhügel found that it may develop readily in both milk and water to

which it has gained access. This bacillus, then, has been found in the body ; it has also been found by Pfeiffer in the dejecta of typhoid patients, and now it has been found in the water supply, so that the chain of evidence is, so far, pretty conclusive. It is a somewhat important fact that the typhoid bacilli may remain active for a considerable length of time in the stools, for the bacilli in fæces kept in a sterilized tube for fifteen days remain alive at the end of that period, and vigorous cultivations could be made from such material. Experimenters were now confronted with the difficulty that typhoid fever is seldom met with except in the human subject. Experiments were made on animals by injecting cultures of the typhoid bacillus into the aural vein of rabbits, with the result that about 50 per cent. of the animals died, and on post-mortem examination it was found that the spleen and those glands usually affected in typhoid were somewhat swollen. A. Fraenkel was able to kill monkeys with injections of the bacillus, and Chantemesse and Widal point out that they can produce a septicæmia by injecting a considerable quantity of a culture of the bacillus into the peritoneal cavity of a mouse. They also found, repeating Fraenkel and Simmonds' experiments of injecting cultivations into the vein of the ear, that this was followed by diarrhœa and rapid emaciation at the end of several days, although the animals frequently recovered. When they were killed at the height of the disease, lesions corresponding to those met with in typhoid fever were found in the intestine, and bacilli were also found in the organs.

The method that was used in the inoculation of cholera was afterwards resorted to, the contents of the stomach were rendered alkaline, the peristaltic movement of the intestine was paralyzed by means of full doses of opium, and the typhoid bacilli were injected into the alimentary canal ; most of the animals died, and numerous bacilli were found in the intestines and even in the glands, but none could be demonstrated in the blood. It was observed, however, that it was not necessary to have an active bacillus present in order to cause very serious symptoms, and even death, with typhoid cultivations ; and it soon came to be recognized that these symptoms were due to regular poisoning or intoxication by toxines and toxalbumens, both of which were described by Brieger as being present in typhoid discharges and in pure cultures of the

typhoid bacillus. It was in typhoid fever, in fact, that this investigator carried on some of his earliest experiments on the poisonous metabolic products of pathogenic organisms, growing in albuminoid substances. As regards the relation of this special bacillus to the disease, it stands on exactly the same footing as that between the cholera organism and cholera, and it follows that most of the points that have been accentuated when we were considering cholera may also be accentuated in this instance. Klein pointed out that in typhoid lesions, especially in the intestinal canal, several organisms were usually associated, and other observers have agreed that in typhoid there is, very frequently, what is known as a "mixed infection"—*i.e.*, in addition to typhoid bacillus other organisms appear to be present and to play an important part. Streptococci, and septic organisms, are frequently found in the tissue of the spleen, liver, and wall of the intestine. It is supposed that some of these organisms play their part in preparing the intestine for the reception of the typhoid bacillus, and it is maintained that a condition of irritation and a removal of the epithelium, brought about by the action of other micro-organisms on the wall of the intestine, may be necessary to prepare the way for the entrance of the typhoid bacillus. The intestine is, in fact, prepared just as a field is prepared by the farmer by ploughing and manuring for the reception of the seed that he intends to sow. The fact that the bacilli can grow on potatoes without becoming evident to the naked eye indicates the possibility of a similar growth occurring on other articles of diet, which, taken into an alimentary canal that has been previously prepared by gastro-intestinal disturbances—diarrhœa and similar conditions—may set up the disease.

It may be appropriate here to consider the action of light on typhoid bacilli, as although the first observations on the germicidal action of light were made on other organisms, in this country by Downes and Blunt, and these experiments were continued by Tyndall and a number of other workers both in this country and abroad, the more recent experiments on the action of light on bacteria have been carried out on typhoid bacilli. That bacteria are influenced by the action of light either to their advantage or their harm is very evident. In the one case it will be found that certain of the colour-producing organisms cannot exert this function

unless they are very well supplied with both air and light, whilst on the other hand such organisms as usually grow in the body appear to become markedly weaker as regards their power of growing and of giving rise to their special deleterious products if they are freely exposed to the light. Recently Dr. Janowski has made a number of experiments by exposing growths of the typhoid bacillus to the action of light, and has found that it exerts a distinctly depressing action on the typhoid organism, an action entirely independent of any oxidation of the food material that might occur under the action of the chemical rays, these chemical rays acting directly upon the protoplasm and rendering it incapable not only of further development but of continuing alive. In order to prove his thesis he took a gelatine tube in which typhoid bacilli had been sown and exposed it to the action of the light on a cold winter day; a similar tube inoculated with the same bacillus was wrapped up in a layer of black paper and then in one of white paper, this also was exposed in the same position. The light in this case delayed the development and the multiplication of the organism in a somewhat marked manner, as in the two protected from the light, growth took place in three days, whilst in that exposed to the light, it did not commence for five days. Of course the growth here referred to is measured by the size of the colony that can be seen with the naked eye and although both were probably growing during the whole time, the rate of multiplication in the one was very considerably greater than in the other. In order that there might be no doubt as to the identity of the organism and the quantity sown in each tube, a U-shaped tube (a double Pasteur tube) was taken and the inoculation was made; the fluid was thoroughly mixed by passing from one limb of the tube to the other, then one limb was protected as above and the other was exposed to the light; similar results were obtained, the bacilli in the limb that was exposed to the light being considerably delayed in their development. He found that direct sunlight acting on fluid cultures of the typhoid bacillus kills the organisms in the short space of from four to seven hours, but diffused light requires a considerably longer period to entirely arrest the development and multiplication of the organisms. Instead of analysing the rays of light by means of a prism, as Englemann and others had done, Janowski

made use of solutions of alum, bichromate of potash, Bismarck brown, fuchsine, methyl blue, gentian violet, &c. He found that the yellow and brown solutions filtered out the chemical rays and prevented the action of light upon the organisms almost as efficaciously as the black and white papers, but found that the other fluids—fuchsine, methyl blue, gentian violet, &c.—had little more effect in preventing the injurious action of light on the bacilli than distilled water or alum solution. He therefore comes to the conclusion that the hurtful action of both diffused light and direct sunlight is due in very great measure to the chemical rays of the solar spectrum—the rays at the other end of the spectrum exerting comparatively little influence on the organism. This entirely accords with Englemann's observations; he found that certain chromogenic bacteria when examined in a drop of water and illuminated by the rays from a micro-spectral objective, invariably made their way to that part of the spectrum furthest away from the violet end, thus indicating that they were attempting to evade the chemical rays which appeared to be hurtful to them. This question of the action of light, especially on pathogenic bacteria, is one of very great importance, and Duclaux's dictum that fresh air and sunlight are two of the most powerful agents that we have with which to combat the onslaught of the bacteria of disease cannot be too strongly insisted upon. Bacteria, especially those of disease, seek out the dark places for their habitation, and as the exclusion of light to a very great extent necessitates the exclusion of fresh air, they find in these holes and corners places of rest whence they may go out to do all the harm of which they are capable.

Janowski also made an elaborate series of experiments on the effect of high temperature on the typhoid bacillus. He found that a temperature of 55° C. continued for ten minutes was quite sufficient to render sterile cultivations of this bacillus, but if this same temperature were continued for only five minutes he could not rely upon obtaining complete destruction of the organism. He also came to the conclusion that an extreme degree of cold, especially when continued for some time or where frequently repeated, had a moderately injurious effect upon the vitality of the typhoid bacillus; a temperature of 14° C. being sufficient to kill the bacilli when

growing in a fluid medium. When they were allowed to dry, however, this did not appear to hold good to nearly the same degree.

These experiments are interesting in their bearing on the outbreaks of typhoid fever at certain parts of the year, especially in countries where the cold appears to be exceedingly intense, and where one would naturally expect the development of the bacilli to be interfered with, but where as a matter of fact such is found not to be the case.

Quite recently Cassedebat, examining the drinking water supplied to Marseilles, which is a very hotbed of typhoid fever, was not able to find the characteristic bacillus in any one of 250 cultivations made of seventy specimens of water, but curiously enough he found three other bacilli which in many respects resembled the true typhoid bacillus most remarkably, although they differed in certain essential characteristics. He points out that they all grow in the phenic acid gelatine, and he further states that several other organisms offer quite as great resistance to this acid as the typhoid bacillus itself. They all present clear spaces or deeply stained masses of protoplasm which may easily be mistaken for spores, but these, like those in the true typhoid bacillus in which as we have seen similar bodies occur, are all killed at a temperature of a little over 45° C. The pseudo-bacilli are very imperfectly stained by Gram's method. They exhibit a lateral and oscillatory motion as well as a forward motion. The plate cultivations are so much alike, that unless all four can be examined simultaneously, it is a very difficult matter to distinguish one from the other ; their growths on potatoes, in broth and in milk resemble one another in a most remarkable manner, except that they develop with different degrees of rapidity, and vary somewhat as regards the alkalinity and acidity of their products at the end of about thirty days, and also as to the degree and time of appearance of turbidity that is produced when these organisms grow in broth. In consequence of these slight differences the use of the various aniline staining reagents, added to such culture media as broth or milk in which the colours undergo changes under the different reactions, has been resorted to and described by Cassedebat, who was able by their use to distinguish one organism from the other. The ordinary cultivation methods are quite sufficient to distinguish these

four forms as a group from most others, for which the typhoid bacillus itself has at different times been mistaken, whilst in addition to the differences above mentioned none of the pseudo forms are quite so toxic to white mice as the Eberth-Gaffky bacillus, and one of them is quite innocuous. Although Cassedebat was not able to find the true form in water taken from a supply that was open to contamination, he found that this was not because the bacilli could not live in water, as in distilled water to which a cultivation was purposely added he could easily distinguish its presence at the end of forty-four days, and when added along with half a-dozen other forms he still found it living and active at the end of seventeen days. As the result of his observations he comes to the conclusion that the true typhoid bacillus does not occur in water so frequently as is sometimes represented, and that one or other of the pseudo-typhoid bacilli has in certain cases been mistaken for it.

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CHAPTER XI.

Tuberculosis.

Tuberculosis a widespread Disease—The Tubercle Bacillus—Koch—Baumgarten—Spores seen by Watson Cheyne—Relation of Organisms to Tissues—Bacillus in Tuberculosis of Animals—Tubercle Bacilli as Saprophytes—Bacilli cultivated outside the Body by Koch—Methods—Temperature Relations—Cultivation on Different Media—Channels of Infection—Ransome—Williams—Cornet—Conditions of Infection—Methods of Disinfection—Tuberculosis at Different Ages—Tubercle in Milk—Diagnosis of Tuberculosis in Cattle—Tuberculous Meat—Koch's Method of Treatment—Nature of Virus and Mode of Action Is Immunity conferred?—Koch's Method a New Departure—Sterile Products cause Marasmus Maffucci—Indications for Treatment.

Tuberculosis, one of the most widespread and deadly diseases with which we have to deal, not in this country only, but in the whole of Northern Europe, has now certainly been proved to demonstration to be due to the presence of a specific micro-organism. Almost innumerable researches have been carried on with the object of finding out and piecing together the various facts in the life-history of this organism, the products to which it gives rise, the conditions under which it can multiply in the human body and in the animal body, the nature of the very grave changes produced in the tissues, the mode of transmission directly and indirectly from one body to another, and, last, but not least, the possibility of combating the ravages made on the body by this organism, by interfering with its growth or retarding its development, either outside the body or after its introduction into the tissues.

Tuberculosis and Phthisis or Consumption account for such an enormous percentage of deaths in our colder northern latitudes, that the subject has come to be one of intense interest, not only to physicians and surgeons, but to all well-educated people, and the subject of the treatment of tuberculosis is—unfortunately perhaps for patients—taken up

almost as exhaustively in the daily papers as it is in special treatises and in the medical journals. It has long been known that tuberculosis was an inoculable disease, but it was only quite recently (1883) that Koch and his pupils were able to demonstrate that a specific organism could be separated from tuberculous tissue and cultivated outside the body—the cultivated organism having all the characters of the organism found in the tissues—and that when introduced into certain animals, this organism was capable of producing tubercular disease, the organism in turn being again demonstrable in the new tubercular growth.

It was for long found to be an exceedingly difficult matter to demonstrate any specific micro-organisms in tubercular tissues by means of aniline or other nuclear stains and Baumgarten's method [1] was introduced after he had failed to attain his object by any of the ordinary methods. The difficulty he had, however, was that, although the tubercle bacilli undoubtedly resisted the potash solution, other organisms were also more resistant than were the animal tissues, so that there was no great differentiation except in size and form between the tubercle bacillus and other bacteria. While Baumgarten was working out his method, Koch had completed a series of investigations, the outcome of which was that he proved that by the addition of a small quantity of an alkali to the aniline stain the dye was rendered capable of penetrating the resistant outer membrane of the tubercle bacillus. It was afterwards found that aniline, thymol, turpentine, or carbolic acid, added to the stain, bring about the same results; these substances, acting, apparently, as mordants on the tissues. The next step in the process of demonstration of the tubercle bacillus was taken when it was found that this organism differed from others in the fact that it retained the staining reagent most tenaciously, even the strong mineral acids, which readily discharge the colouring matter from nuclei and other bacteria having little effect, if acting for a short time only, in taking out the stain from tubercle bacilli; in sections stained in an aniline colour mixed with one of these substances and then treated with an acid, a most beautiful differential staining was obtained, the stained bacilli standing out most prominently from the unstained tissues.

Tubercle bacilli when stained are seen as delicate rods or threads 1.5 to 3.5μ in length and about .2μ in thickness, though these dimensions are by no means constant even in the same preparation, and under different conditions the variations in size are sometimes very marked. As in the case of anthrax and cholera bacilli, the methods of staining and preparation exert a marked influence in determining the apparent size

[1] The sputum, after being dried on a cover glass and then heated to coagulate the albumen, was simply soaked in a solution of caustic potash. Sections were treated in the same manner.

of the organism. The length is sometimes given at 2.6μ and the breadth as from .2 to .5μ. They are usually described as from a quarter to a half the diameter of a red blood corpuscle and longer than the bacilli of mouse septicæmia, or 0.0015 to 0.0035 m.m. in length. These bacilli always appear to have the larger dimensions when treated by Baumgarten's liquor potassæ method. The bacilli are

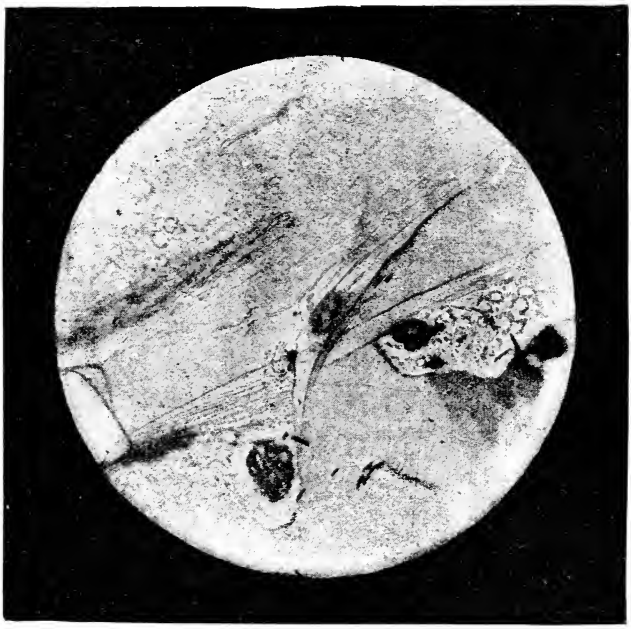


Photo-micrograph of Sputum, from a phthisical patient, containing large nucleated epithelial cells and characteristic tubercle bacilli. × 1000.

usually slightly curved, or two are arranged end to end so as to contain an angle. At first they were described as not containing spores, but it was demonstrated by Watson Cheyne that from two to six spores may frequently be seen in these rods; the spores occurring as small ovoid or rounded clear spaces placed at intervals in the stained thread. In some cases they are so prominent that they appear to

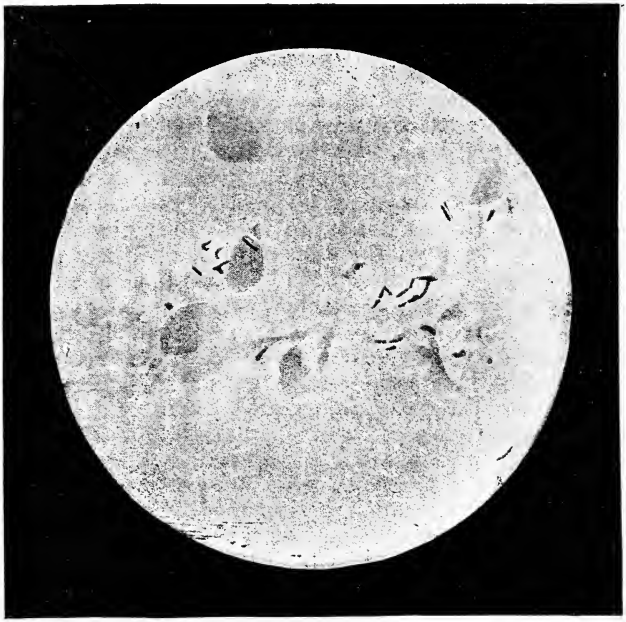
project beyond the straight outline of the bacillus ; sometimes this is so much the case and the spores are packed so closely together, that when examined under a sufficiently high power the spore-bearing thread has been described as a chain of cocci ; by some it is maintained that this appearance is present only if the specimen is imperfectly stained, too much heated, or too long treated with a strong acid ; whilst, on the other hand, certain observers assert that the tubercle bacilli also occur in the form of regular chains of cocci. The bacillus is non-motile.

Flügge holds, however, that it is "always possible, in carefully prepared specimens and with the aid of good lenses, to convince one's self that the supposed chain of cocci does not exist, but that the delicate contour of the bacillus can be for the most part traced through its whole length, and that it is only within this contour that the alternation of stained and unstained zones gives the deceptive appearance of stained cocci separated by narrow intermittent spaces."

The association of this organism with tubercular disease is undoubted ; it is found in the lungs and sputum in various forms of consumption, it is found also in tubercular ulcers of the intestine, around the vessels in tubercular inflammation of the membranes of the brain, a condition which occurs frequently in children, in tubercle of the liver and of all other organs of the body, and in tubercular eruptions of the skin such as lupus. In all these cases the bacilli are found most abundantly at those points where the disease appears to be spreading into the surrounding tissues, and especially where there is the formation of large multinucleated epithelioid cells. If these bacilli are present in considerable numbers in such an area, there will usually be found in the immediate neighbourhood a small portion of tissue that has undergone marked degenerative changes, the cell protoplasm is somewhat hyaline or glassy looking, and takes on any staining reagent, except perhaps picric acid, very badly ; the nucleus is also considerably altered, especially in that it no longer stains, or is stained very imperfectly, with carmine or the aniline dyes. At such a stage there can frequently be demonstrated, in these cells, imperfectly stained tubercle bacilli, though in some cases the bacilli stand out sharply and very brilliantly from the glassy or homogeneous looking cell. After a time the cells lose their outlines; they become more and more

indistinct, until eventually nothing but the ghost of a cell is left. This occurs where we have what is known as the cheesy or caseous degeneration, in which condition nothing but broken-down nuclei and very rarely a tubercle bacillus can be distinguished. It would at first sight appear as though the tubercle bacilli had disappeared, root and branch, from this caseous centre, but if a small portion of the cheesy material be inoculated into an animal susceptible to tubercle, flourishing groups of tubercle nodules all containing tubercle bacilli are produced, first near the seat of inoculation, and then at points situated at some distance from the point of primary infection. The results of such experiments naturally led the opponents of the bacillary theory of tubercle to assume that the poison of tubercular virus was not associated with the bacillus, which they contended was merely of accidental or sequential and not of causal occurrence, as it could not be found in material which undoubtedly produced the disease ; but after the demonstration of spores in the bacilli, and bearing in mind what was known of spore formation in other organisms, most observers were very naturally led to the conclusion that, although the bacilli are not to be demonstrated in these infective caseous masses, spores in enormous numbers are probably present, and that from these, bacilli are developed as soon as the surrounding conditions of nutrition, moisture, and heat are again sufficiently favourable. The fact that it is so difficult to stain spores made it no easy task to demonstrate the accuracy of this theory, but it may now be held, as the outcome of inoculation and other experiments, that there can be no reasonable doubt on the subject. It is a curious fact that whilst in the human subject tubercle bacilli appear in many cases to be actually contained within the giant or other large cells, in some herbivora, and in fowls, the bacilli sometimes occur single or in small masses, apparently outside the cells. There are several explanations given for this, but the most rational appears to be that where the bacilli are few in number, and where they are being rapidly destroyed by the tissue cells, by far the larger proportion are taken up by, and are seen in, these cells ; this being specially observable in cases of chronic tuberculosis, whilst in those cases where the tubercle bacilli are relatively numerous, as in cattle, and even more markedly in the fowl and in the horse, the

destruction of the tissue cells takes place so rapidly, in consequence of the invasion of each cell by a large number of bacilli, that nucleus and protoplasm break down completely and the little group of bacilli is set free, or at any rate is not embedded in a mass of protoplasm, but is merely mixed up with the granular *débris* of the cell, from which,



Micro-photograph of Tubercle bacilli, found in the scraping from the lung of a cow suffering from Perlsucht. × 1000.

or along with which, it may be carried to other parts of the body.

Having found the tubercle bacillus almost invariably accompanying tuberculous disease, Koch, to complete his proof, wished to separate the organism in the form of what is called a pure cultivation, in order that he might study its life-history, and that he might determine whether the organism when introduced alone into the animal body,

could give rise to tuberculosis. With all the ordinary nutrient media then at his command he entirely failed to obtain any growth of the organism outside the animals in which it led its parasitic life. It depended so much upon these conditions of parasitic life that when removed from them it was no longer able to grow and multiply : it might still remain alive ; in fact, Cornil was able to demonstrate that at the ordinary temperature of the room the tubercle bacillus, kept in ordinary Seine water, continued to exist, but not to multiply, for seventy days. At length, however, Koch overcame the difficulties with which he had to contend, in a most ingenious manner, and he succeeded in growing as a saprophyte what had hitherto been demonstrated only as a parasitic organism. He argued that as the tubercular process developed but slowly, he would have to obtain a medium which would remain unaltered for a considerable length of time when placed in a temperature at which the organism could grow and multiply. He was satisfied too, from his early experiments, that only special substances would serve as nutrient material for this fastidious organism, and he ultimately found that solidified blood serum was by far the best medium on which to cultivate it, as it alone of the many substances which he had then tried supplied all the requirements of the organism. This blood serum contains all the elements necessary for the nourishment of the organism ; it remains solid at the normal temperature of the body, at which temperature it may be kept for a long enough time to allow of the development of the slowly growing bacillus, whilst a small amount of water might be left in the test tube along with the serum without dissolving it, thus serving to supply the moisture requisite for the perfect growth of the bacillus. In consequence of the slowness of the growth above referred to, it is an exceedingly difficult matter to obtain pure cultivations of the tubercle bacillus should it once become mixed with putrefactive bacteria, and it was for long deemed almost an impossibility to separate it from these other forms : this difficulty has now, however, been overcome. Most of Koch's earlier pure cultivations were obtained by taking as seed material, tubercular lymphatic glands from freshly-killed guinea pigs, which had been inoculated, some three or four weeks before, with tuberculous material.

As this method may be looked upon as almost classical it will be well to give the description in Koch's own words. "A number of knives, scissors and forceps are heated in a flame sufficiently to free them from any adherent bacteria. They are then laid ready in such a manner that no further contamination of the instruments can take place. Meanwhile the animal immediately after being killed is fastened to a dissecting board. In order to avoid the flying off of particles of dirt, hairs, &c., when the skin is incised, the fur of the animal is freely moistened with a 1 to 1000th solution of corrosive sublimate. With a pair of scissors and forceps, both still hot, the skin is now divided and turned back on each side sufficiently to free the lymphatic glands of the axilla and groin; but the glands, if they are to be used to start cultivations, must not be touched with the instruments employed for cutting through the skin. With another pair of scissors, also heated, a piece 1 to 2 c.c. cube is cut out of the side wall of the thorax, and the surface of the lung laid bare. A number of tubercular nodules are thus rendered accessible, and a few are removed as quickly as possible with fresh instruments, which must, however, be cooled for this purpose. In order to set free the bacilli contained in the nodules, the latter are cut in pieces or crushed with the scissors, or, better still, between two scalpels that have just been heated and allowed to cool. The substance thus subdivided and crushed is removed by means of a platinum wire fused into a glass rod (which, immediately before use, has been heated and allowed to cool), introduced into the test tube, spread out on the surface and well rubbed about. During this operation the test tube must be held obliquely or almost horizontally between the thumb and forefinger, and the cotton wool plug held meantime between the other fingers of the same hand in such a way that no contamination of it by other objects can take place. The transference of the substance into the solidified serum, which may for brevity be designated inoculation, must take place as quickly as possible in order that no germs of extraneous organisms from the air may alight on the inoculation material or enter the test tube. It is desirable also to conduct the experiment in a room where no dust is flying about, and in the same way all unnecessary movements by which dust from the clothing, &c., is mingled with the air are to be avoided, as experience has shown that it is to particles of dust that the germs of micro-organisms suspended in the air adhere.

"In spite of all these precautions we cannot be perfectly sure of preventing the entrance of a few solitary germs, and it is necessary in each case to inoculate several (five to ten) test tubes, so that if we fail to obtain a pure cultivation in one or two tubes, we shall yet have others that are free from impurity.

"The process is the same as that above described for obtaining seed from a pulmonary tubercle, when lymphatic glands, tubercles from the spleen, &c., are to be used to start a culture. The process must always be carried out with heated instruments, which must be changed every time a fresh stratum is laid bare. All preparatory incisions which do not come in contact with the inoculation substance itself are to be made with hot instruments, but the inoculation material is to be cut out with a cool pair of scissors and forceps. It is necessary to change the instruments constantly in order that impurities adhering to them after the division of the skin and superficial layers, may not be carried into the cultures.

"When the organs of a recently killed or dead animal could be obtained,

and the inoculation with substances containing tubercle bacilli was done in the way just given, I invariably succeeded in obtaining pure cultures. The result was uncertain, on the contrary, when material from human corpses or from cattle with *perlsucht* was used, as it was always impure on the surface, and, moreover, was not always quite fresh when it reached me. In these cases I first rinsed the surface of the object repeatedly with a solution of corrosive sublimate (1 to 1000) and then cut away the upper parts in layers with red-hot instruments, which were changed repeatedly; finally, I took the material for inoculation from a depth which justified me in concluding that it would be free from the bacteria which had entered the tissue after the death of the animal. In this way I generally succeeded in obtaining pure cultures even from this kind of material, particularly from small superficial pulmonary cavities, the outer wall of which, after treatment with solution of corrosive sublimate, was removed with hot instruments.

"After the inoculation of the solidified serum with material containing bacilli has been accomplished, the vessels are placed in the incubator and kept constantly at a temperature of about 37° C. Every incubator is not suitable for the culture of tubercle bacilli. Growth takes place but very slowly, and the vessels must therefore remain in the incubator for weeks. So that if the incubator is so constructed as to favour rapid evaporation of liquids from the culture vessels, the serum gets dry before visible colonies of tubercle bacilli have developed. For example, an apparatus cannot be used in which the heat is unequally distributed, so that the vapour constantly present condenses in the cooler parts, *e.g.*, on the glass cover, and has to be continually replaced by moisture given off from the culture glasses. D'Arsonval's thermostat is very convenient; the warmth is equally distributed in it, and the blood serum remains almost unchanged.

"For the first few days no alteration is to be observed in the cultures in the incubator. If, however, there is a change, and drops or spots of white or other colour form on the surface of the serum, increase more or less rapidly in size, render the fluid at the bottom of the glass turbid or cause the serum to liquefy, it is a sign that the culture is not pure, and that foreign bacteria have entered and choked the growth of the tubercle bacilli. If these drops or spots are examined they are found to consist of bacilli or micrococci which, by Ehrlich's method of staining, assume a different colour from the tubercle bacilli, and are distinct from them also in size and shape. In the tubes free from these impurities, the first signs of the growing colonies of tubercle bacilli are not visible to the naked eye for ten to fifteen days. They then appear as whitish points and small spots lying on the surface of the serum; they have no lustre, and consequently stand out clearly from their moist surroundings. They are best compared to tiny dry scales adhering loosely to the surface of the serum. The number of the scales and the extent of surface covered by them vary with the richness of the infecting material in bacilli, and with the extent of the surface over which it was rubbed or spread out.

"The individual scales attain only a limited size, so that if few are present they remain distinct; but when numerous and closely packed, they coalesce finally and form a very thin, greyish-white, lustreless covering on the serum. After a fragment of the tubercular lung of a guinea-pig has been rubbed on serum, small whitish colonies of tubercle bacilli appear close to the greyish-red bit of lung, and also in its neighbourhood wherever it has been pushed over or pressed on to the surface of the serum by means of the platinum

wire used to distribute the bacilli as widely as possible. The colonies in some cases are relatively few in number, because only a few bacilli were present in the pulmonary tubercles, as the examination of sections shows. In other cases the little colonies are much more numerous; in many, as especially after inoculation with the contents of cavities very rich in bacilli, they soon coalesce and form a coherent membranous mass."

From the cultivations so made other tubes were inoculated by lifting off the small scales with a needle bent at right angles, breaking the scale up somewhat so as to cover the point of the needle with the bacilli, and then drawing it along the surface of the solidified blood serum. At the end of ten or eleven days there became visible to the naked eye, and very much earlier if examined with a lens, a regular thin superficial layer spreading along each side of the track of the needle. This organism does not bring about the slightest liquefaction of the blood serum; the scales are somewhat dry; they are exceedingly thin, and spread only over the surface, no growth making its appearance in the deeper layers of the nutrient medium. Tubercle bacilli cultivated in this manner through seventy generations still continue to act on animals in the same way as the original or first culture. Similarly the bacillus grows only on the surface of a fluid, forming a very delicate, thin film, the organism being essentially ærobic in its habit. The film growing on solidified serum is loosely attached to the surface, and any fluid introduced, however gently, floats off a considerable portion in larger or smaller flakes, which do not break up, but gradually sink to the bottom. Koch observed, from the behaviour of the films when growing on the surface of fluids, or broken down and introduced into fluid media, that the tubercle bacilli were non-motile. He described the appearances of these colonies under the microscope, and came to the conclusion that the growth was perfectly characteristic, as compared with any other organisms known up to that time. They have the appearance of lines or short threads thrown into curves like worms, or snakes, or flourishes of a pen, thinner or thicker according to the age of the colony, each thread being composed of a large number of individual bacilli, arranged with their axes in the long axis of the thread, running parallel to one another, but with a small space between each, these being apparently occupied by some zoogloœa medium. This arrangement can be best brought out by pressing down a

cover glass on to the surface of a colony as it grows on the serum, and then removing it without sliding it in any way and staining by any of the usually recognized methods.

As regards the conditions under which this organism exists, we have already seen that a certain amount of moisture is absolutely necessary for its growth, and Pawlowsky was able to cultivate it, even on potato, when he took the precaution to keep a considerable quantity of moisture in contact with the growing organism, and watched the potato for a considerable length of time, the growth not being visible to the naked eye for three weeks or a month. Koch's great difficulty in his earlier experiments was, as we have already seen, to obtain a substance which, in addition to containing all the other elements necessary for the nutrition of the bacillus, would remain sufficiently moist for the requirements of the bacillus when exposed to a pretty high temperature for a considerable length of time. As regards temperature, it was found that, although there was an actual cessation of growth and development below 28° or 29° C., on blood serum the organism might be exposed to very low temperatures for a considerable length of time without losing its power of again becoming active when returned to favourable environments. It grows best at about 37° C., but as soon as the temperature rises beyond 38° C. the development of the bacillus in the cultivation tubes begins to diminish in activity, and at 40° C. (Koch originally gave this limit as 42° C.) it ceases entirely to grow and multiply. It is a remarkable fact that the temperature at which the bacillus develops best is exactly that of the human body (37.8° C.). In the cow and the horse, where the other conditions must also be favourable, the temperature is about 38.3° C., in the calf a little over 39° C., whilst in the hen we have the maximum temperature of 40° C.

Klein, in a series of experiments reported in 1886, finds that it is possible to inoculate successfully with tubercle taken from the human subject, also that it is possible to inoculate from a guinea-pig to a cow, but when inoculation is made from a cow to a fowl the experiment breaks down, and there is no tubercle produced, so that, not only can one modify the activity, and the power of growth of these bacilli outside the body by altering the temperature at which they grow, but it is also within the range of possibility

to modify the bacilli by introducing them into different animals whose normal temperatures and other general metabolic conditions are different. This modification has more than nominal value, for it has been proved experimentally that, although the organisms in human and in bovine tuberculosis are morphologically identical, they are not absolutely the same in all their vital and pathogenic characteristics. For instance, tubercle bacilli taken from a phthisical patient and introduced into the tissues of a cow will soon set up an acute general tuberculosis, whilst bacilli taken from a case of *perlsucht*, or ordinary bovine tuberculosis, almost invariably give rise to the *perlsucht* form of tuberculous disease, and rarely, or never, to the acute generalized form. It would appear that in these cases the microbe becomes adapted to the special conditions present in each host, and consequently becomes less suited to the conditions in others. It might be objected that in the case of the fowl the bacilli are present in enormous numbers, and that, therefore, their action should be more virulent; but, in answer to this, it may be pointed out that increased activity of growth is not necessarily always associated with increased virulence. In illustration of this fact, it may be pointed out that, after many experiments, Nocard and Roux were able to obtain most luxuriant cultivations of the tubercle bacillus on agar-agar or on blood serum, to which 6–8 per cent. of glycerine had been added. The organisms on these media grow so rapidly that they are quite visible at the end of four days, and at the end of twenty days, and not four weeks, as on ordinary blood serum, the growth seems to have reached its maximum, when it appears as a pale grey, thick, mamillated or reticulated, mass. In the same way luxuriant growths may be obtained in bouillon to which a similar proportion of glycerine has been added, small opaque flakes or flocculi first making their appearance at the surface, and then sinking to the bottom, where they remain. This growth in glycerine broth may take place at a comparatively low temperature, 18°–20° C., though it then goes on very slowly. Earlier generations of such cultivations produce typical tubercle nodules that grow with extreme and characteristic rapidity when inoculated, but after several generations of such pure cultivations have been made in these glycerine media, the virulence may become distinctly diminished,

although the growths are as luxuriant as, or are actually more luxuriant than ever. We have in fact a kind of reversion to the saprophytic condition of the culture, a condition accompanied by diminished parasitic virulence. It is possible, therefore, that the higher temperature that is met with in cattle along with other conditions there present may have a distinct effect in diminishing the virulence of the organism, whilst at the same time it may play an important rôle in causing its parasitic and vegetative activity to be increased within the body of these animals, though this is not necessarily accompanied by increased vegetative activity outside the body. As Koch pointed out at the International Medical Congress, of 1890, the tubercle cultures from fowls were quite distinct and could not be passed on as such from animals to animals of different species or by growth at different temperatures, and he concludes that although nearly related to the ordinary tubercle bacillus they are specifically distinct. It should be noted, too, that tubercle bacilli grown on glycerine agar are, according to Nocard and Roux, somewhat shorter than the bacilli met with in tubercular sputum, that they also contain numerous ovoid spores, but that otherwise they are exactly like those described by other observers in various animals and on other media. As has been already mentioned, there was necessarily a doubt whether any disease could be tubercular if it was not possible by special methods to demonstrate in histological preparations the presence of the bacillus. This histological demonstration is, however, after all, a clumsy method, and in many cases it has been found possible to obtain demonstrations of the tuberculous nature of a disease by inoculation experiments when the organisms have been so few that they have escaped the notice of the most careful observers. It may therefore be confidently anticipated that more and more proof of the tuberculous nature of lupus, scrofula, cold abscesses, and bone disease will be gradually accumulated.

As early as 1843 it had been demonstrated that tubercular material from dead subjects when inoculated into rabbits produced tuberculosis; in 1865 these experiments were repeated and extended by Villemin, and other observers have from time to time confirmed the results that were then obtained. Koch's observations and experiments have now,

however, placed the matter on a much surer footing ; he has, as we have seen, succeeded in separating a specific bacillus from tuberculous material, with which he has been able to produce tuberculosis with the utmost certainty, so that, instead of dealing with comparatively large fragments of diseased tissue, he has worked with particles so small that they can only be distinguished with the aid of the best microscope and the use of special methods of preparation. This, of course, has cleared up many points which hitherto have been very obscure, and, most important of all, it has enabled us to determine the channels by which human beings may become affected with the disease, especially in connection with the respiratory passages and by way of the alimentary canal, and through wounds or damaged tissues.

It has been demonstrated that in the sputum of patients suffering from phthisis, in those cases where the softened lung tissue is breaking down and is being expectorated, an enormous number of tubercle bacilli may often be met with, though they may be present in comparatively small numbers ; so frequently is this the case, that the presence (in different numbers from day to day) of tubercle bacilli in the sputum, or their absence, is relied upon as a diagnostic feature by attention to which the physician is enabled not only to determine the rapidity with which breaking down is going on, but even to obtain very considerable assistance in arriving at a decision as to whether a disease is tubercular or not.[1]

Until quite recently not the slightest attempt has been made either to prevent the diffusion of such sputum, or to disinfect it in any way, and it has been said, with some considerable degree of truth, that a very large quantity of virulent tuberculous material has been allowed to be freely disseminated, with the result that it must have been the lot of many individuals to contract tuberculosis by means of the inhalation of particles of dried tuberculous sputum in which active tubercle bacilli or their spores were necessarily entangled. That the sputum contained the elements which

[1] In making an examination of sputa for tubercle bacilli, the fact that the bacilli are most numerous in the small yellow caseous points should always be borne in mind. Such points should be carefully searched for, crushed between two cover glasses, dried, stained, and examined. Single bacilli may be found elsewhere, but masses can, as a rule, be found in these disintegrating points only. It is only where disintegration has commenced that any reliance can be placed on the numbers of tubercle bacilli as affording any assistance in forming a diagnosis and prognosis.

were the exciting causal agents of the disease had been experimentally proved, even before the actual discovery of the bacillus was made, and dogs, which had been in the habit of taking up the sputum of tuberculous persons, had been known to contract the disease, an observation that was fully corroborated by further experiment. Similarly it had been related how barn door fowls in a country district, which for a long time were perfectly healthy, were suddenly attacked by an outbreak of tuberculosis after a phthisical patient had come to live at the farm. The expectorations of this patient were voraciously devoured by the fowls, with the result that tuberculosis of a most virulent nature broke out in a most extraordinary fashion amongst the brood. Feeding experiments were also made to corroborate this accidental experiment; and more recently similar accidental experiments have been recorded both in France and in this country.

In the case of certain micro-organisms the products of putrefaction exert such a deleterious influence on them that they are destroyed very readily and rapidly. Again, in the case of the cholera bacillus, desiccation at once proves fatal, not only to its growth, but also to its actual virulence and power of infection. In the case of the tubercle bacillus, however, observers, both in France and in Germany, very early pointed out that putrefaction and drying could exert but little influence on the number of the bacilli, whilst drying alone interfered only slightly with their virulence, as it was quite easy to inoculate rabbits with sputum that had been dried at a temperature of 30° C. Later, Galtier found that maceration and putrefaction for a period of five days, and even intermittent freezing and melting, did not interfere with the transmission of the disease by means of the bacillus. Other observers have demonstrated that the bacillus remains virulent after it has been exposed, in sputum, for forty days, and even after 186 days if it is carefully protected from the action of the air. It is, of course, concluded from these experiments that, difficult as it is to cultivate the specific micro-organism of tuberculosis outside the body as a saprophyte, the parasitic form (or its spores) still retains its vitality and power of development for a considerable length of time—and under what would appear to be very unfavourable circumstances—even when removed from its host. The way was thus being thoroughly prepared for Dr. Georg Cornet's researches on infection in hospitals and rooms where phthisical patients were treated. Dr. Ransome had, early in the controversy, demonstrated the presence of tubercle bacilli in the air respired by tuberculous patients; and Dr. Williams, in 1883, suspended glass plates smeared with glycerine for a

period of five days in one of the ventilating shafts of the Brompton Hospital for Consumption. Washing off the glycerine "with distilled water, the fluid was mixed with a little mucilage and evaporated down to one-half, and then examined for the bacilli," and in this he was able to demonstrate bacilli in fair numbers. In a thoroughly purified ward a number of non-phthisical patients were placed, with the result that no bacilli were found in the air carried off by the extraction shaft; whilst in another ward, filled with consumptive patients, the washings from a plate exposed for fourteen days in the extraction shaft contained numerous bacilli. Similar experiments have been made by other observers, but it was left for Cornet to make a systematic examination of the dust in rooms were phthisical and non-phthisical patients were treated. By numerous careful inoculation experiments, he demonstrated the fact that the expectorations from phthisical patients are a source of a very real and appreciable danger. The bacilli are not only exhaled, in small numbers no doubt, but he finds that they are also contained in very considerable numbers in the dried sputum obtained from handkerchiefs, bed linen used by phthisical patients, and in the sputum that has made its way on to the floor and walls through the dirty habits of many of the patients. His experiments extended over a very considerable period, and to the rooms of private patients in hospitals, in lunatic asylums, &c.; he even found bacilli in the streets and open spaces in a certain proportion of cases where tuberculous patients were collected together. These results have the greater value from the fact that in no case did he consider his experiments complete unless the dust with which he was experimenting, when inoculated into animals, produced the disease. It follows from all this that infection, as the result of inhalation of the dried virus, is one of the most common forms with which we have to contend, this being especially the case in older people, amongst whom pulmonary tuberculosis is most commonly met with, but in whom, as in children, pulmonary catarrh seems invariably to precede the tubercular disease. In children, the catarrhal inflammation of the lungs that so frequently accompanies such conditions as measles, scarlatina, diphtheria, whooping-cough, and other similar conditions, may very frequently become tubercular in character.

There seems, in these cases, to be, first, a weakened condition and impaired power of resistance of the epithelial cells lining the small bronchi, the smaller air passages and the air vesicles making up the spongy tissue of the lung. The bacilli in the air and dust then finding their way to a surface already weakened and specially prepared as it were for their reception, recommence their parasitic life, multiply, and make their way further into the tissues, where they set up the changes associated with tubercular disease. It is evident from all this that much work still lies ready to our hand in connection with the spread of tubercle, and that if we could only persuade people to look upon tubercle as an infectious disease similar in character to scarlet fever, though not so rapidly developed, much would have been done to prevent its spread, and a great advance in preventive medicine would have been made. Through the work of Koch, Cornet, and others, the Germans have come to look upon perfect cleanliness in the treatment of phthisical patients as absolutely essential. Pocket handkerchiefs and bed linen used by phthisical patients are most carefully sterilized by means of bichloride of mercury, hot air, steam, or other germicidal agents; patients are strongly enjoined not to expectorate except into receptacles specially made for the purpose, receptacles that can be carried about, can be most readily cleaned, and in which expectorations can be easily disinfected. Of course, the results of all this are not yet manifest, but it may be confidently anticipated that within a comparatively short time a considerable diminution in the number of phthisical patients in Germany will have to be recorded; not to be compared, perhaps, with the diminution of cases of other diseases, but still a very appreciable one.

As a single example we may take the Grand Duchy of Baden, where there was a diminution of deaths from tuberculosis from 3.08 per 1,000 inhabitants in 1882 to 2.80 per 1,000 in 1887, or no less than .28 per 1,000. Were this to be equalled in the British Isles, and the patients were not carried off by other diseases, the saving to our community would be nearly 10,000 lives per annum.

We may here mention the various disinfectants used. By far the best is heat, especially moist heat. Sunlight, or even ordinary daylight, will, according to Koch, kill tubercle bacilli in from a few minutes to five or six days. Koch has also proved that a number of ethereal oils, some of the so-called aniline or tar dyes, mercury in the form of vapour and silver and gold com-

pounds, all exert an inhibitory effect on the growth of tubercle bacillus; the compounds of cyanogen and gold, especially, even in a solution diluted to one part of cyanide of gold to two millions of the solvent substance, checking the growth of tubercle bacilli. Carbolic acid also kills the bacilli if it is allowed to act in considerable strength and for a lengthened period; but none of these can, for a moment, be compared as regards not only efficiency and cheapness, but for facility of application, with steam or boiling water. All disinfection should be just as completely carried out when a phthisical patient leaves a room or ward as if a case of scarlet fever had been treated there; the walls, floor, and even the roof should be thoroughly washed and disinfected by means of hot water or lime. During treatment in such apartments no patient should be allowed to expectorate on the floor; and glass or porcelain spitoons, which can easily and safely be boiled in water, should be placed for the reception of all sputa. During the time that corridors, steps, and rooms are being cleaned and disinfected, they should be kept quite moist, in order that as little dust as possible may arise from the cleaning operations. No room that has been occupied by a phthisical patient should be used until it has been thoroughly disinfected; the bedding and curtains should be well boiled, the blankets steamed, the mattresses disinfected, all the furniture washed with soap and water, the carpets and upholstering thoroughly beaten in the open air, the dust of which should be caught in straw (such straw and the paper taken from under the carpets should always be burned), the floor thoroughly washed with soap and hot water, and the wall paper rubbed down with freshly baked bread. In San Remo all these methods of procedure have been brought under the notice of the hotel-keepers, and they are advised to carry them out in connection with the whole of their rooms at the end of each season, not only the sleeping rooms, but also the public rooms.

The alimentary canal is probably the next most important channel of infection. Evidence of infection by this channel was first obtained by the feeding experiments already referred to, but further evidence of it has been noted in the ulceration of the intestine that is so frequently met with in phthisical patients, and which appears to be due to the action of bacilli contained in the sputum passing down the gullet through the stomach in cases where the gastric juice is not very active, and so to the intestine in which ulceration occurs in the course of the tubercular process as the result of the pathogenic activity of the bacilli. In children this mode of infection is comparatively common, and tubercular ulceration of the intestine, or tubercle of the glands connected with the intestinal tract, is of frequent occurrence.

In 127 cases of tuberculosis in children that I examined this tubercular ulceration was found in 43; whilst in 100 cases, or nearly 79 per cent. of the whole, the glands were in some stage or other of tubercular degeneration. It would thus appear that tuberculosis connected with the intestine is of frequent occurrence in children, and we should therefore argue that

the infection, in these cases at any rate, frequently takes place by the alimentary canal. The age at which these tubercular glands were found is very significant; during the first year of life there were 4 cases; from 1 to 2½ years, 33; from 3 to 5½ years, 29; from 6 to 7½ years, 12; from 8 to 10 years, 13; and from 11 to 15 years 9 cases. In 14 cases these glands only were affected.

Bolitz (Inaugural Dissertation, Kiel) gives similar figures, but on a more extensive scale.

Out of 2,576 children whose bodies were submitted to a *post mortem* examination in Kiel during the years 1873–1889, there were 424 cases of tuberculosis, or 16.4 per cent. of the whole mortality.

The following shows the percentages of the whole of the deaths from tuberculosis at each of the different ages:—

Still-born children .	0.0 per cent.	Up to 2–3 years old	33.0 per cent.
Up to 4 weeks old	0.0 ,,	,, 3–4 ,, ,,	29.6 ,,
,, 5–10 ,,	0.9 ,,	,, 4–5 ,, ,,	31.8 ,,
,, 3–5 months old	8.6 ,,	,, 5–10 ,, ,,	34.3 ,,
,, 6–12 ,, ,,	18.3 ,,	,, 10–15 ,, ,,	30.1 ,,
,, 1–2 years ,,	26.8 ,,		

Here, again, as where the lung is attacked, we must look upon the bacillus as the exciting cause, but tissue weakness as the predisposing cause. These conditions may be summed up as follows:—

(*a*) The presence of the bacillus tuberculosis in such a position and for such a length of time that it obtains a coign of vantage, so to speak, from which to attack the tissues of the body.

(*b*) Some weak point in the epithelial surface "made by disease, or due to irritation or bad food," by which the organisms may attack the deeper tissues in sufficient numbers to ensure their being able to hold their own in the struggle for supremacy that ensues.

(*c*) The comparatively low vitality of these deeper tissues brought about by imperfect nutrition or irritation; the cells of which they are composed being no longer able to deal successfully with any large number of bacilli that can under ordinary circumstances find their way thus far.

As regards the possibility of the bacillus tuberculosis being present in the intestinal canal, it should be remembered that the class of patients amongst whom abdominal tuberculosis is most rife, consists of infants, which during the first year of their life, and sometimes for a longer period, are suckled at the breast; after this, however, the diet is extremely mixed, and as a rule it is extremely unsuitable; but it is in by far the

larger proportion of cases, even amongst the poorer classes, partially, at any rate, composed of cows' milk. During this first year of their life, children with tuberculosis of the mesenteric glands, or of those glands connected with the intestine, form a very small proportion of the cases of infantile tuberculosis. Whilst the child is suckled by its mother there is little tubercle, but after this first year there is a very rapid rise in the mortality from tubercle. It is a somewhat singular fact that although tuberculosis is frequently met with in young married women, tubercular disease of the breast is extremely rare, so rare, indeed, that one observer, Dr. Hubermaas, who took great interest in this subject, was able to collect the records of only some eight cases. In cattle, on the other hand, where the mammary gland carries on its functions when the animals are placed under conditions which are far from healthy, or at any rate far from normal, this tubercular disease of the milk gland is not by any means of infrequent occurrence.

Some of the earliest experiments from which actual proof of the infective nature of tuberculous material was obtained were those made by Gerlach and Chauveau, who used the milk of tuberculous cows to feed young animals ; though tuberculosis was not produced in every case, the former was successful in a sufficient number to justify his conclusion that there was some specific virus in the milk of tuberculous cows which could, when ingested, produce tuberculosis of the alimentary tract, or of the glands associated with it. Numerous experiments on young pigs, some of them accidental, others designed, and others on calves and hens, have been recorded, in which tuberculosis has evidently followed their being fed with tuberculous milk. At the International Medical Congress held in Copenhagen in 1884, Professor Bang, of the Royal Veterinary School in that city, gave the results of a careful examination of twenty-seven cases of tubercular disease of the udder in cattle, in the milk of which he was able to demonstrate the presence of tubercle bacilli, both directly under the microscope and in the sediment obtained by means of the use of a centrifugal separator. With this milk, or with the separated sediment, he was able to produce tuberculosis both by inoculation and by ingestion. Another observer, Nocard, was able to demonstrate the presence of the specific bacillus in milk in eleven cases, and Professor M'Fadyean and

I also found tubercle bacilli in the milk from six cows out of six hundred examined. So certainly are the bacilli found in cases of tubercular disease of the udder that certain authorities maintain that it is possible to differentiate between the simple and the tubercular inflammation of the udder in the cow merely by means of a microscopic examination of the milk. (See Appendix).

This question of tubercle in milk has now assumed such importance that much attention has been given to it abroad, and in Denmark a most complete system of inspection has been instituted in connection with one of the largest milk supply associations in the world; and private enterprise, guided by Prof. Panum and Mr. Busck of Copenhagen, has indicated a way in which the State might tread with very great advantage. In connection with this association, six special veterinary surgeons, in addition to local practitioners, are constantly employed in keeping watch over the cattle that supply the milk to it. One of these veterinary surgeons alone, specially retained, examines eight hundred cattle fortnightly, and makes most careful notes (notes that I had an opportunity of examining) of the condition of every animal. Bang, to whose energy and observations the opening up of the tuberculosis question is very greatly due, contends that a diagnosis of tubercular disease of the udder can generally be made without difficulty during life and in the very early stages of the disease. With this conclusion it is somewhat difficult to agree, but much more may be done in the way of careful and systematic examination than is done at present, and in Denmark they have certainly systematized the examination of cattle to a far greater extent than we have succeeded in doing. As it may be of interest to mention the points on which the veterinary surgeon should depend in making his diagnosis, they may, so far as I was able to follow the routine of a number of inspectors, all of whom however differed slightly in their methods, be briefly summed up in the following:—

(*a*) First of all the sub-maxillary glands are examined; these are easily felt, and any change is readily made out.

(*b*) The glands at the root of the neck and those in front of the haunch bones are always carefully examined. The glands in the flank should be equal in size, about the size of the middle finger, and not hard. Mere enlargement, even when considerable, is, however, not looked upon as of great importance if it is perfectly equal on the two sides.

(*c*) The animal is made to cough by means of pressure on the trachea, and the lungs are carefully examined during and after the coughing.

The condition of the skin over the flanks is carefully observed; it should, in a healthy animal, be "loose," like that of a dog, soft and pliable; any adhesion, hardness, or harshness, should be carefully noted.

(*d*) The udder is carefully examined for inequality of size and for any induration. It is a somewhat curious fact that tuberculous disease usually affects the hind quarters of the udder, which become hard and knotty, but not painful; whilst in acute inflammation of the udder, the anterior quarters are quite as much affected as the posterior; the pain is usually very acute, and the process is accompanied by much more marked febrile symptoms.

(*e*) Then the glands above the udder, high up between the quarters, are

most carefully examined. In cases of tubercular disease of the udder these glands are invariably affected, are unequal in size, and the larger one, corresponding to the affected quarter, is usually considerably indurated.

(*f*) Careful auscultation is carried out at least once a month. The fore-foot of the side that is being examined being always well advanced. The normal expiration sound lasts half as long as the normal inspiration, and if this rhythm is deviated from in any way, a further and thorough examination of the lungs should always be made.

(*g*) The examination is continued still further if the slightest suspicion of tubercular disease is aroused by the above investigation, and an examination per rectum is made, with the object of determining whether there is any tubercle of the peritoneum or not. As the onset of the disease in the udder is so rapid, and as, as yet, it is held by most observers that the bacilli may make their appearance in the milk, even where the udder is not directly affected, it follows that if there is the slightest suspicion of the existence of tubercular disease in a cow, the milk from that animal should not be put into the milk supply, and as a matter of fact, on the Danish farms above referred to, it is not sent to town, but it is either thrown out, or after being most thoroughly disinfected by prolonged boiling, is given to the pigs.

(*h*) The farmer keeps a record of the quantity of milk given by each cow, and a note of what is done with it; and any milk that is put out of the supply by the veterinary surgeon or by the farmer himself, on account of *suspected* disease, is paid for by the company, or the difference between the full value and the value as pig food.

Any other inflammatory condition of the udder is carefully noted, and even then the milk is withdrawn from the regular supply.

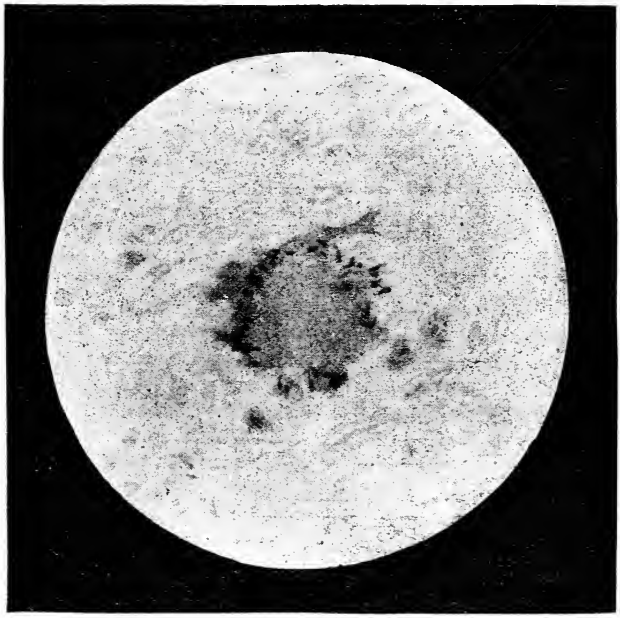
(*i*) A small quantity of milk is always drawn off by the veterinary surgeon, who carefully notes its colour. If it is too thin and watery looking he immediately condemns it; whilst if it loses the peculiar blue tinge that freshly-drawn milk from a healthy cow almost invariably has, and takes on even a slight yellow tinge instead, the milk from the infected quarter is not used for any purposes, although the milk from the other quarters may be used, after being thoroughly boiled, for the feeding of pigs.

The authorities of the association insist rigidly on the fortnightly inspection, because it has been observed that very great swelling may appear as a sign of udder tuberculosis in from ten to fourteen days, as in this position the onset of the tuberculous disease is usually much more rapid than in the lungs, in which the process in a very large majority of cases appears to be far more chronic in character. In all cases, the condition of the glands must be systematically and carefully observed.

In the light of recent events, too, it is to be hoped that Koch's tuberculin may be utilized in the diagnosis of tuberculosis in cattle, and that satisfactory results may thus be obtained.

In consequence of the rapid onset of the disease diagnosis without regular inspection is almost impossible, and it is probable that the swelling of the udder is only one of the later manifestations of the disease, the glands above the udder apparently being a far more reliable index. Professor M'Fadyean and I were so much struck

with this difficulty of diagnosis, that in a paper read at a meeting of the Pathological Section of the British Medical Association, held in Dublin, August, 1887, we stated that it appeared to us "that where, as is very frequently the case with cows kept in towns, a complete history of the diseased condition of the udder is not obtainable, a differential diagnosis of mammitis (inflammation of the milk glands) is by no means easy, except by microscopic demonstration of the



Tubercle bacilli arranged around a closed up milk-duct in a case of Tuberculous disease of the udder of a cow. × 1000.

bacilli in the milk ; which may also fail if a most careful search is not made" ; and Principal Walley, dealing with the same subject, says, "that he could not undertake to diagnose, with accuracy, tubercular mammitis in every case, nor even in a majority of cases" ; and he states that in specimens of the udders of tuberculous cows he has examined, after death, he has found good examples of tuberculosis in the mucous membrane of the milk sinuses without

the occurrence of any induration of the milk gland, "indeed," he adds, "I may say that no veterinary surgeon could, during life, have diagnosed the existence of tubercular mastitis (tubercular inflammation of the udder) without the aid of a microscope." That tubercle bacilli make their way into the milk, when there is tubercular ulceration in the milk-ducts, can be readily understood, and has frequently been shown; but it has also been demonstrated by Professor M'Fadyean that in cases of tuberculosis, bacilli may be found lying free in what are otherwise apparently healthy milk ducts. Fresh evidence is being accumulated every day, but these facts alone, when considered along with the occurrence of bacilli in milk, with the feeding experiments already recorded by so many observers, and taken in connection with the great prevalence of tubercle in certain classes of animals, afford strong presumptive evidence that milk is a source of tubercular infection, especially in children, and in those in whom, on account of imperfect nutrition and impaired digestion, the walls of the alimentary canal are less able to resist the invasion of the organism known as Koch's Bacillus Tuberculosis.

The danger of contracting tuberculosis from taking meat from cattle affected with the disease is perhaps not so great as that associated with the drinking of tuberculous milk; it is nevertheless one with which the sanitary authorities will have to deal, and one to guard against which it is necessary to take very considerable precautions. There can be little doubt that in those cases where the disease is localized to any one of the viscera at the time of the death of the animal, there is little danger to be anticipated from eating the well-cooked flesh from other parts of that animal; and if we could be absolutely certain that the localization was complete, all would be well. Even in cases where the flesh is taken from animals in advanced stages of tuberculous disease there would be a certain proportion of cases in which no evil results would follow, and one observer who made sixty-two experiments with such flesh boiled for ten or fifteen minutes, found that only 35.5 per cent. of the inoculated animals became tuberculous. Even in cases of generalized tuberculosis Nocard failed in thirty-nine out of forty cases to transmit the disease by means of raw muscle juice injected into the peritoneal cavity of guinea pigs, but

he succeeded in the fortieth. Other observers, however, have been more successful, and in two rabbits I was able to produce tuberculosis by injection into the peritoneal cavity of the raw juice expressed from the intercostal muscles of a tuberculous cow after all tuberculous pleura had been carefully "stripped"; whilst the juice taken from the muscle of the thigh injected into two other rabbits was perfectly innocuous. The danger of infection by the consumption of meat from tuberculous cows may have been much exaggerated, but that there is a very appreciable danger must most certainly not be lost sight of by our Medical Officers of Health and the Veterinary Inspectors of the Board of Agriculture.

Koch, as we have seen, had discovered a specific organism which he had been able to cultivate outside the body; he had inoculated and produced tubercle; and he had found certain germicidal agents which were capable of destroying the organism outside the body. He, and the many interested workers who had investigated the subject, had found that it was more difficult to inoculate certain animals successfully than others. It had been observed even that certain individuals of the same species were much more refractory to the action of the virus than others, and it very naturally suggested itself to those workers, that there were two conditions which would have to be determined before any systematic and organized attack could be made on the tubercle bacillus within the body. The tubercle bacillus itself might be developed under such conditions that its virulence might be more or less modified. For example, Koch found that all his cultivations made on blood serum retained their power of growing in animal tissues in a most extraordinary degree, and for long this substance was the only nutrient medium used for the cultivation of the tubercle bacillus. It was found, indeed, that the other combinations used for the cultivation of other organisms up to that time were valueless as nutrient substrata for the tubercle bacillus. Nocard and Roux, however, found, as we have seen, that on the addition of a certain percentage—6–8 per cent.—of glycerine to peptonized broth, solidified by the addition of agar, they could obtain a nutrient medium on which the tubercle bacillus would grow most luxuriantly. After it had passed through several generations of cultivations on this

medium, the virulence of the organism, or its power of growing in tissues, was distinctly diminished—*i.e.*, the organism was in a condition to be much more readily destroyed by the cells of the animal body, which appeared to keep the upper hand throughout the struggle, very few of the cells being destroyed, as is the case when virulent tubercle is used, where although a number of the tubercle bacilli are undoubtedly destroyed, the degeneration of the proliferated cells is so extensive that caseous nodules are formed, and the typical appearance of caseating tubercle are presented in the inoculated area. The second factor, though equally important, and though recognized indirectly by all physicians, could not put itself so fully in evidence as the first, and it was only after Metschnikoff had made his beautiful observations on the daphnia and on the separating tail of the tadpole and the phagocyte action of certain cells in these processes, that any direct evidence of the bearing of the activity of the cells of the tissues of the body on the destruction of micro-organisms within the body could be definitely adduced. Then, indeed, began the narration of the history of the battle between the cells and the bacilli. If the bacilli were weak, or were present in small numbers only, the cells were invariably the victors; if the cells were degenerated, or were badly nourished, or if their vitality was low, the bacilli proved themselves the more powerful. How these results were brought about soon became the subject of most violent controversy, and in the controversy thus raised explanations of facts that had hitherto puzzled scientific workers were projected and forced home. Let us here, however, examine only those facts that appear to have a special bearing on the subject of tuberculosis. A perfectly healthy individual, placed under favourable conditions as regards food, fresh air, and exercise, is never attacked successfully by tubercle bacilli, the active, vigorous tissue cells being perfectly competent to destroy any bacilli that may make their way into the lungs, the pharynx, or the intestine; whilst even in cases of direct inoculation into a wound, if the wound heals rapidly, no tubercular process may result, the tubercle bacilli, as we have said, being destroyed by the cells. It may be, indeed, that certain secretions of the cells—*i.e.*, those substances that bear the same relation to the connective tissue cells that an enzyme bears to the yeast-cells—also exert a deleterious action on the

bacilli in the healthy individual, and in a minor degree even in individuals with weaker tissues ; the activity of the bacilli is so interfered with or modified that they can be readily attacked and devoured by the tissue cells, which, as has long been known, have a most remarkable power of taking up into their substance many effete materials and particles of dead or inorganic matter. On the other hand, certain excretions, by accumulating in the blood and in the lymph spaces, may impair the activity of these tissue cells and so render them less able—(1) to secrete their protective material, and (2) to wage war directly against the bacilli ; whilst, in turn, the bacilli on their side, as we have already seen, secrete a material that has a most injurious action on the tissue cells, causing them to swell up and eventually to become hyaline. This material is only a poison when in large or comparatively large quantities ; in smaller doses it acts in the first instance as an irritant or stimulant, stimulating the protoplasm to exert all its powers against the advancing bacteria, powers that are so strongly exerted (unless the conditions of nutrition and excretion are specially favourable) that they are rapidly exhausted. It has been observed that in those cases where phthisis was curable the cure has been effected only by careful nutrition of the tissues, and that as soon as they have been brought up to a certain standard of health the disease has been checked, in many cases permanently ; on the other hand it has been found that when the tissues have again fallen below *par* there has been a fresh outbreak of the disease. These facts are in themselves sufficiently interesting and suggestive, but as we shall see they have a further important bearing on the question of the curability of the tubercular phthisis.

It has been observed that a process of localization occurs even when large caseous patches have been formed, and it has been found that around these patches, just as around an abscess, there is always erected a kind of barrier, made up of vigorous connective tissue cells, small, round and larger epithelioid cells ; the blood vessels in this cellular zone being comparatively numerous and of considerable size. We have, in fact, in this arrangement of the blood vessels and cells, a making of roads (the blood vessels) for the bringing up and massing of forces (the active cells) around the enemies' camp (the tubercular or caseous mass with the con-

tained bacilli or spores), and by a process of close siege preventing the organisms from making their way outwards, and confining them entirely to their own territory, so that, when they have utilized what food material there is in the degenerated cells, they are no longer able to exist as vegetative bacteria, and only the spores remain—which may, however, remain latent for a very long period awaiting a favourable opportunity for another attack on weakened tissues. These spores or hibernating germs are confined within the same area, and the *débris* with its contained spores is gradually encroached upon by the surrounding tissues until, eventually, if the mass is not large it may be entirely absorbed, though, owing to the amount of fibrous tissue that is formed by the attacking cells after their activity is somewhat diminished, this process of absorption sometimes goes on very slowly.

It was an easy enough matter, when the history of the development of the tubercle bacillus was known, to kill the organism outside the body, and Koch found that a very large number of germicidal substances were capable of interfering with its growth ; but unfortunately most of these germicides also exerted an injurious effect on the tissues, so that what was gained in one direction was lost in another.

In most other diseases in which preventive or curative inoculation by means of vaccines—less virulent cultures of the microbes—to accustom the animal to the action of the poison and so enable it to resist the more virulent, has been attempted, the aim has been to render the bacilli less, and the cells more active, and to develop in these cells a special activity.

By accident, as he tells us, Koch found that by injecting tubercle virus into the subcutaneous tissues of guinea-pigs which had been previously inoculated with tuberculosis, the tissue in which the tubercle bacilli were acting was actually destroyed and an eschar or slough was formed at the point of inoculation ; whilst although the bacilli were not directly destroyed, they remained embedded in a mass of food material that was gradually but surely used up to supply the needs of these bacilli, and eventually they had to go into winter quarters. But the products of the tubercle bacilli appear to act in some way on the tubercles already formed ; they do something more than bring about complete degenera-

tion of the weakened cells. In the immediate neighbourhood of the young tuberculous tissue, *i.e.*, in those cells that have been slightly affected by the products of the tubercle bacillus it sets up a further reaction ; it stimulates these cells, and at the same time causes a dilatation of the vessels and probably also an increase in their number (this latter may be only apparent) ; a larger amount of food material is brought up for the nutrition of these cells, excreted matter is more readily carried away both by vessels and lymphatics, and in consequence of this, cells that have been too far stimulated by the tubercle poison to recover, die off, whilst those that are still capable of living, even under the excessive stimulation, proliferate and help to form the barrier between the dead mass and the surrounding normal tissues. In this way it would appear that in certain cases of tuberculous disease, Koch has produced an imitation of the natural process of cure. By a process of combined reasoning and experimentation, and basing his method of procedure on the one that had been already adopted in connection with the preparation of vaccines for other diseases, Koch succeeded in obtaining a substance with which, in a certain degree, at any rate, he was able to combat the advance or to modify the tuberculous disease in animals.

As this advance in the treatment of tuberculosis marks a most important point in the history of the disease, and in order that there may be no misconception as to Koch's exact position, it may be well to give in his own words the description of the discovery, composition, and probable mode of action of the remedy.

He says, in describing the observations, by the consideration of which he was led to take the lines of experimentation that ultimately led him to success: "If a healthy guinea-pig be inoculated with a pure cultivation of tubercle bacilli, the inoculation wound generally becomes glued over or sealed, and appears to heal up during the next few days. It is only in the course of from ten to fourteen days that a hard nodule is formed, which soon opens, forming an ulcerating spot which persists until the death of the animal ; if an animal that is already tuberculous be inoculated the course of events is very different. The most suitable animals for this experiment are those which have already been successfully inoculated four or six weeks previously. In the case of an animal so

treated the small secondary inoculation wound becomes sealed at first, but no nodule is formed ; a peculiar change takes place at the point of (primary) inoculation. As early as the first or second day the point becomes hard and dark-coloured—a condition that is not confined to the point of inoculation—and spreads around for about 0.5 to 1 centimetre. During the next few days it becomes more and more clear that the epidermis thus changed is necrotic, and finally it is thrown off, and a flat ulcerated surface remains, which generally heals quickly and completely, without infection being carried to the neighbouring lymphatic glands. Thus the inoculated tubercle bacilli act quite differently on the skin of a healthy guinea-pig, and on that of a tuberculous one. But this remarkable action does not belong exclusively to living tubercle bacilli, it also belongs in the same degree to dead ones, whether they be killed by low temperature of long duration, which I at first tried, or by boiling heat, or by the action of certain chemicals.

" This peculiar fact having been ascertained, I followed it up in all directions, and then further found that pure cultivations of tubercle bacilli thus killed, after they are ground down and suspended in water, may be injected under the skin of healthy guinea-pigs in large quantities without producing anything but local suppuration. Tuberculous guinea-pigs, on the other hand, are killed by an injection of very small quantities of suspended cultures within a time varying from six to forty-eight hours, according to the dose ; a dose which is just insufficient to kill the animal being sufficient to produce a widespread necrosis of the skin in the region of the point of (primary) inoculation. If the fluid with its suspended matter be still further diluted, so that it is scarcely turbid to the eye, the animals remain alive ; and if the injections be continued at intervals of one or two days a noticeable improvement in their condition soon sets in ; the ulcer at the point of inoculation becomes smaller, and finally cicatrization takes place. This is never the case when such treatment is not resorted to. The swollen lymphatic glands become smaller, the condition as regards nutrition improves, and the progress of the disease is arrested, if it is not already so far advanced that the animal dies of debility.

"These facts formed the basis of a therapeutic method against tuberculosis. But an obstacle to the practical employment of fluids containing in suspension the dead tubercle bacilli was found in the fact that the tubercle bacilli are not (readily) absorbed; they disappear, and remain for a long time unchanged *in situ*, producing larger or smaller suppurating centres. It was clear, therefore, that in this method the curative effect on the tuberculous process was obtained by a soluble substance, diffused so to speak, into the fluids that surround the tubercle bacilli, and transferred without delay to the circulating fluids of the body; whereas that which has the pus-forming quality seems to remain bound up in the tubercle bacilli, or at any rate to be only very slowly dissolved or washed out. Thus the only important thing that remained to be done was to carry out the process that takes place within the body—outside of it also—and if possible to extract and isolate the curative substance from the tubercle bacilli. This problem required long and continued experimentation, but at last I succeeded, by the help of a 40 to 50 per cent. solution of glycerine, in extracting the active principle from the tubercle bacilli. My further experiments on animals, and finally on human beings, were made with liquid thus obtained; and in this way, also, the liquid which I supplied to physicians and surgeons in order that they might repeat the experiments, was obtained. *The remedy with which the new therapeutic treatment of tuberculosis is carried out, is, therefore, a glycerine extract of pure cultivations of tubercle bacilli.*

"In addition to the active principle there pass from the tubercle bacilli into the simple extract all other substances soluble in 50 per cent. glycerine, and therefore it is found to contain a certain quantity of mineral salts, pigment, and other unknown substances—extractives, &c. Some of these substances can be readily separated, as the active principle is insoluble in absolute alcohol, by which it can be precipitated, not pure certainly, but in combination with such other extractive matters as are also insoluble in alcohol. The colouring matter, too, can be separated out so that it is possible to obtain from the extract a colourless dry substance, which contains the active principle in a much more concentrated form than does the original glycerine solution.

"This purification of the glycerine extract has, however, no advantages as regards practical application, as the substances removed have no action on the human organism, so that the purifying process would only involve unnecessary expense. The constitution of the active principle can, as yet, be only a matter of conjecture.

"It appears to me, indeed, to be a derivative of albuminous bodies, and to be closely related to them, but it does not belong to the group of so-called toxalbumens, as is proved by the fact that it can withstand high temperature, and in the dialyzer it passes quickly and easily through the membrane. The quantity of active principle present in the extract is in all probability very small. I estimate it at a fraction of 1 per cent. Thus, if my assumption be correct, *we have to deal with a substance, the action of which on the tuberculous organism, far surpasses that of the strongest drugs known.*

"Various hypotheses may of course be formed as to the specific mode of action of the remedy on tuberculous tissue. Without in any way affirming that mine is the best possible explanation, I imagine the process to be as follows: The tubercle bacilli in their growth produce in the living tissues—just as in the artificial cultivations—certain substances which exert various but always deleterious influences on the living elements surrounding them, the cells. Amongst these substances is one which, in a certain concentration, destroys living protoplasm, and causes it to undergo a transformation into the condition called by Weigert, 'coagulation-necrosis.' The tissue having thus become necrotic, the conditions are so unfavourable to the nutrition of the bacillus that it is unable to undergo further development, and finally, in some cases, it dies off. In this way I explain the remarkable phenomenon, that in organs freshly attacked by tuberculous disease—for instance, in a guinea-pig's spleen or liver filled with grey nodules—numerous bacilli are found, whilst bacilli are rare or entirely absent when the enormously enlarged spleen is made up of whitish substance in a condition of coagulation-necrosis, such as is often met with in guinea-pigs that die of tuberculosis. A solitary bacillus, however, cannot produce necrosis at any great distance from itself, for, as soon as the necrosis has covered a certain area, the growth of the bacillus—and, in consequence, the production of the necrosis-producing substance—diminishes, and thus a sort

of mutual compensation is set up, and to this is due the fact that the growth of isolated bacilli is so remarkably restricted, as for example, in the case of lupus, in scrofulous glands, &c. In such cases the necrosis extends only over a part of the cell, which then, in its further growth, assumes the peculiar form of a giant cell. I thus follow in this statement of my views the explanation of the growth of giant cells first given by Weigert. Now if the necrosis-producing substance were artificially added to that contained in the tissue surrounding the bacillus, then the necrosis would extend further, and thus the conditions of nutrition of the bacillus would become much more unfavourable than is usually the case. Then, not only would the more completely necrosed tissues disintegrate, slough, and—where this is possible—take with them the enclosed bacilli, carrying them outside the body, but the growth of the bacilli would also be interfered with to such an extent that they would die off much sooner than they do under ordinary conditions. It is in calling forth such changes that, to my mind, the action of the remedy seeems to consist. It contains a certain amount of the necrosis-producing substance, of which a correspondingly large dose has a deleterious influence — even in healthy persons—on certain elements of the tissues, probably on the white blood corpuscles or cells closely related to them, thus giving rise to the fever and the whole peculiar complex of symptoms that supervenes. In tuberculous persons a much smaller quantity suffices to cause, at certain spots—*i.e.*, wherever tubercle bacilli vegetate, and have already impregnated their surroundings with the necrosis-producing substance—a more or less extended necrosis of cells with the production of the accompanying conditions that affect the entire organism. In this way it is possible to explain—at least for the present—in a provisional manner, the specific influence which the remedy, in certain well-recognized doses, exercises on tuberculous tissue, as well as the possibility of increasing the doses in so remarkable a fashion, and, finally, to explain the curative effect which the remedy undoubtedly exerts where the conditions are at all favourable for its exhibition."

The substance to which the name of Tuberculin has been given has been analyzed, and is found to be "a syrupy, slightly foaming liquid (sp. gr. 1015 ?) of brown sherry colour, its aqueous solutions

showing a greenish florescence. In odour it resembles elder yeast or leaven, combined with a sweet aromatic admixture, such as honey. If slowly heated, the smell of yeast gives way to an agreeable odour resembling fruits; on further heating, the smell becomes like that of fresh bread crust," but without the acid fruit odour. If the heating of the material is continued, "the smell assumes the empyreumatic character exhibited by burning albuminous matter and carbonizing horny substances. Only an extremely small quantity of ash (under 1 per cent.) was obtained. The liquid shows a neutral reaction." It was found to contain a small quantity of mucine, indicated by a turbidity on the addition of dilute acetic acid, which is increased on the addition of potassium ferrocyanide, indicating the presence of albumen. Peptones are present in considerable quantities. There is a slight reducing action obtained when the fluid is heated with Fehling's solution; there was no reaction with acid bichromate of potassium, so that acids and ptomaines are absent; but it was assumed, as would now appear incorrectly, that toxalbumens, or albumoses, globulins, or enzymes must be the substances to which the material injected by Koch owes its special properties.

Any description of the lymph other than that given by Koch himself must be the result merely of guess-work, but even guesses will sometimes afford indications as to the lines on which researches are at present being carried out, not only in connection with tuberculosis, but with several other most deadly and wide-spread diseases. The peculiarity of this method of treatment is, that it is not known to protect against an attack of tuberculosis, though Koch states that he hopes his guinea-pigs will be protected from future attacks; it is used solely as a therapeutic agent to check or stop the disease after it has once obtained a foothold in the body. It is thus in principle more like Pasteur's inoculation against hydrophobia, for the inoculation is made after the patient has been inoculated with the disease virus; and it is also similar in certain points, though the principle is different, to the protective inoculation obtained by Hankin, who, by means of albumoses obtained from anthrax cultivations, has been able to produce immunity against anthrax, though not to cure, after that disease was once induced; and that Dr. Cartwright Wood and I carried on with the pyocyanin products in rabbits, to tide them over an attack of anthrax the bacilli of which were introduced into the subcutaneous tissue shortly before, or immediately after, the injection of the pyocyanin. We, however, considered that the pyocyanin had not acted directly on the anthrax bacillus, but that it acted by stimulating the cells; whilst Koch's "lymph" acts (1) by destroying the tuberculous tissue, and rendering it unfit for the

nutrition of the bacillus, and (2) by setting up a localized reaction in the neighbourhood of the bacilli, by means of which the cells are so stimulated that they are able, as we have already seen, to prevent the extension of the bacilli into the surrounding parts.

Koch's fluid may not accomplish all that is expected from it; it may, in fact, be found that Koch has not completed his experiments, but he has made a wonderful advance in our knowledge of the conditions necessary for the combating of micro-organisms; and has extended the observation of the earlier workers at the globulines and albumoses who really opened up the way for the advance of the numerous workers who have recently come into the field. It would, however, take us beyond the scope of the present work to say more about this marvellous discovery of Koch's. Let it always be remembered that tubercle destroys the tissues in which it grows, and that the treatment by Koch's method completes the process of destruction, if this is not already accomplished; so that, under the very best conditions, phthisis can only be stopped, and, although a comparative cure may be obtained, highly differentiated tissues once destroyed can never be restored or replaced.

It will thus be seen that although Koch has selected a special irritant material as that which it was found necessary to separate in order to obtain the results that he wished, and has obtained the sequence of specific (?) stimulation of the cells, he has departed from the usually accepted methods, in that he acts directly on the tissue cells, and leaves the bacilli to die of starvation. It is not now a case of the cells destroying or modifying the activity of the bacilli; it is simply, in the first instance, a cutting off, or rather a rapid exhaustion, of the substances required for the nutrition of the bacilli; the bacilli remain, and although they may eventually undergo retrogressive changes, it appears probable that their spores remain for some time, at any rate, ready to break out should favourable conditions again present themselves. Apart from this local action, however, the fact cannot be ignored that where the tissues are not already too far weakened, or the cells so imperfectly nourished that they cannot react to stimulus, the liquid injected by Koch may exert a general and specific action on the tissue cells through which they are acclimatized, as it were, to resist the poison; so that it is quite possible that a partial immunity

as regards the tubercle bacillus may be acquired. It is probable that the tuberculin acts as a stimulant on all cells, and that to the increased metabolic changes that are set up is due the general reaction that is described as following on the exhibition of this material.

That this is not entirely a fanciful explanation may be argued from an example. It is a fact, well known to those who have charge of tuberculous patients suffering from diseased glands—scrofulous glands, as they are called—that so long as these glands remain uninjured and are subjected to no stimulation, and so long as the nutrition of the patients keeps fairly good, they remain as a rule comparatively free from pulmonary phthisis and other forms of tuberculosis; whilst other members of the same family, existing under the same conditions, both as regards hygiene and nutrition, become affected with the commoner and more fatal forms of the disease.

A further illustration may be taken from the difference that exists between children of the lower classes and those met with in other grades of society—children in a sick children's hospital frequently having every organ crammed with, or almost replaced by, tubercular nodules and cavities, so that it seems marvellous that the unfortunate children could have remained alive at all; whilst children better nourished, but not previously affected by any form of tubercle, appear to succumb very easily, when comparatively localized tubercle has been developed. In these cases, however, it is usually found that some vital organ such as the brain is affected.

It would appear that, in these cases, the tissues may develop greater resistance in consequence of the circulation in the fluids of the body of the soluble products; but that when this acquired resistance is once lost, or is overcome, the stimulation exhausts the cells, and they now more easily fall a prey to the active tubercle bacilli. It must, however, in this connection, be pointed out that the products of the tubercle bacillus, or sterilized cultures of the organism, when introduced subcutaneously into healthy fowls or guinea-pigs, produce a condition which is spoken of as "marasmus;" and as early as 1879, Maffucci, as the result of a series of experiments, concluded that these sterilized cultures when left in the body, exerted such a marked influence on the tissues, that they induced emaciation, atrophy of the liver

cells, and of the cells of the different parts of the spleen, and that they also set up certain changes in the circulation, the result of which was seen in marked congestion of the lungs, kidney, spleen, &c. These experiments were no doubt suggested by the similar changes that are met with in the human subject in the course of tubercular disease, even in those organs that are not directly affected by the tuberculous infiltrations. Recently he has confirmed and extended his former observations ; and it will be interesting to see whether the conditions met with in patients injected according to Koch's method, are similar to those observed in animals experimented on by Maffucci's method.

How far the object of Koch's endeavours will be attained still remains to be seen, and his method has been, and will be, put to most severe tests for it involves the question of life or death to thousands, nay, tens and hundreds of thousands. There appears little doubt that in lupus (a tuberculous skin disease) the process has been checked, and, in some cases, at any rate, it has not again broken out for a considerable period after the treatment had been stopped. There also appears to be pretty reliable evidence in favour of his contention that there is an amelioration of the condition of the patient and an improvement in the disease in certain other forms of tuberculosis ; but the use of the remedy has not been sufficiently prolonged to allow of our arriving at any very definite conclusion, however favourable our opinions may be. Virchow, the greatest pathologist of the age, has found, in a number of cases that have come under his observation—a comparative small number when the enormous number that have been injected is taken into consideration —that the characteristic degeneration of the tissues of the young tubercle is not always brought about, that the localization of the disease is not by any means perfect, that there is a tendency of tubercle material that should be "thrown off" to continue the infection and even increase its rapidity of spreading, especially in the lungs, and that in some cases the bacilli, instead of being rendered inert, appear to take on greater activity, and to be carried in the various currents in the body, even to parts situated at some distance from the original tuberculous focus. We must bear in mind, however, that almost all the cases that up to the present have been brought to the post-mortem

table have been cases in which the disease was far advanced, and in which Koch's inoculative treatment has been sought as a last resource by physicians and patients alike.

A most important point to be remembered in connection with tuberculosis is that under favourable conditions it is an extremely curable disease; such conditions, however, must be very far-reaching and must include a sufficient and suitable supply of food to the patient, perfect oxidation of the tissues, and facilities for the excretion of effete products. The effects of climate, of exercise, of pure mountain air, free ventilation in houses and of generally favourable hygienic conditions, are all due to the promotion of the healthy condition of the tissues and of the increased vitality of the cells; this vitality increasing their power of resistance and enabling them to cope successfully with the bacilli and their products with which they may be brought in contact. It should not be left out of count, however, that we may, by the use of suitable drugs, be able to exert an antidotal influence by means of which, acting directly on or through the cells, these cells may be put in a still better position for resisting the attacks of the tubercle bacilli and their products. This antidotal system of treatment, if it could be carried out by means of drugs, should prove far more efficacious than the use of any means of treatment that we have at present at our disposal, especially if it could be combined with some of these latter.

This is not the place to discuss the medical aspects of the question; but the above facts are mentioned in connection with the biological problems associated with the action and inter-action of the bacilli and of the cells of the body.

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The earlier literature is given very fully in CORNEL and BABES "Les Bactéries."

CHAPTER XII.

Leprosy.

Distribution of Leprosy—Similarity to Tuberculosis—Description of Disease—Tubercular, Anæsthetic, Mixed—The Leprosy Bacillus—Method of Staining—Position—Leprosy Cells—Bacilli Resistant and Grow Slowly—Cultivation Experiments Mostly Negative—Theories of Cause of Leprosy.

Within the last few years leprosy has, metaphorically speaking, returned to life in this country. The occurrence of a case in one of our market attendants has created more commotion, and has put in train a more complicated machinery, than phthisis, with its thousands and tens of thousands of victims was for long able to set going. Nevertheless, we can now afford to think of leprosy as almost a thing of the past, as far as our own country is concerned, although we still come across traces of its sojourn amongst us in our Libertons, or Leper towns, or Leptons, that indicate only too surely that this disease was looked upon with the greatest dread by our ancestors of the Middle Ages, who evidently took pains to keep in their own regions, and within their own asylums, the lepers of that period. With the exception of certain isolated areas along the shores of Spain, and in Portugal, in Norway, some parts of Sweden and Iceland, in Italy, Roumania, and Hungary, in the Balkan Peninsula, and in Greece where the disease is still endemic, leprosy is now rarely seen in Europe; but in certain parts of Asia and Africa it is still frequently met with. It is found doing its fell work in the Sandwich Islands, in Mexico, in Cuba, in some parts of Central America, in the north-east of South America, and in the Argentine Republic, in the north-east and north-west of Africa, in Guinea, and in Cape Colony, and in Madagascar, along the shores of the Black Sea, in Persia, Arabia, India, China, the Malay Archipelago, Japan, in Asia, and in New Zealand.

From the whole nature of the symptoms, and from the course of the disease, it was long considered to be a disease somewhat similar to tuberculosis, and the discovery, by Armauer Hansen, in 1880, of a specific leprosy bacillus, which was found to be present in enormous numbers in the lymph channels of the skin in cases of leprosy, paved the way, for the reception of Koch's discovery of the tubercle bacillus.

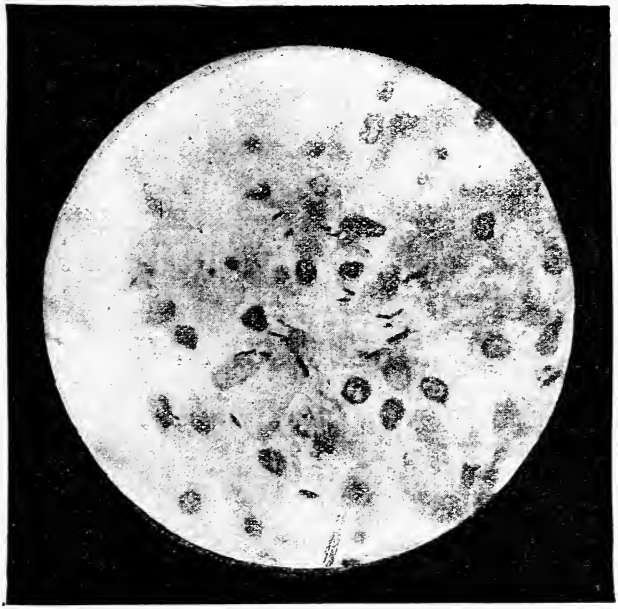
It was for some time supposed that the tubercular and anæsthetic forms of leprosy were different in their origin, and that they might possibly be due to the action of different bacilli, although it was of course known that in some cases there was a kind of mixed leprosy. The fact that the two diseases were the same was, however, strongly accentuated when it became evident that the same kind of bacillus was found in all three forms of the disease—the tubercular, the anæsthetic, and the mixed.

Before a description of the morphological characters of the bacillus is given, the characters of the various forms of the disease may be briefly indicated, in order that the pathological changes produced by the bacillus may be more fully understood, and that the bacilli may be followed and localized in the tissues. In tubercular leprosy there usually appear small irregular spots, sometimes brown, sometimes somewhat purple. These spots occur on the face or on the limbs ; they gradually project so as to form more or less marked tubercles, which vary very considerably in size. There is irregular thickening of the tissues under the skin of the face, which gives rise to a most curious facial expression, the patient appearing melancholy or jubilant according to the folds affected ; the tissues about the various orifices of the face, mouth, eyes, nostrils become swollen, and are often studded with tubercles, the lower lip becomes heavy and hangs down in a curious fashion ; these alterations giving the face what is described as the "leonine" appearance. Sometimes sensibility is interfered with, or it may be altogether abolished. These tubercles are not confined to the tissues immediately under the skin ; they may also be found in the submucous tissue of the mouth, larynx, and pharynx ; and small nodules may even be met with—especially where the swelling is marked—around the eye in the soft tissue of the eyelids. These nodules frequently ulcerate, especially in

the later stages of the disease. On the fingers the thickened patches and ulcerations may extend so far and so deep that part of the finger may be actually separated and thrown off, first, however, becoming dry and black or mummified. The patients may linger on in this condition for a considerable length of time ; or the tubercles may disappear, leaving discoloured patches, in which sensibility is entirely wanting, and which, in consequence, very rapidly undergo ulceration, usually brought on by injury, the patient, unable to feel, allowing the parts to be injured without making any effort to save them. The second form—anæsthetic leprosy—commences in much the same way, except that the discoloured patches are somewhat smaller, and may be red or brown ; in some cases the patches have a peculiar glistening or silvery-white appearance. The pigmented patches, which are often very widely distributed, have, especially where they attain any very great size, a dull, pallid centre, with a line of brown, or brown and red colouration, surrounding it. Some of the smaller spots are anæsthetic, whilst, in addition, there may be large areas in which sensation is markedly diminished or altogether abolished; the hair falls out ; there is marked atrophy of the skin and the cellular tissue immediately beneath, this giving rise to a very wrinkled appearance of the face ; and the patient, as in the other kind of leprosy, becomes dull and heavy-looking, as though he had lost all interest in external affairs. Here also injury to the extremities is of very frequent occurrence, partly owing to the fact that the patient cannot feel, but partly also, because the nutrition of the skin is markedly interfered with. Other skin diseases, the formation of vesicles, and so forth, are often met with. Either form may gradually pass into the other, in which case we have what are known as mixed varieties of the disease.

In order to demonstrate the presence of the bacillus in one of the nodules in a case of tubercular leprosy, it is only necessary to tie an india-rubber ring somewhat firmly around the base of one of them until it becomes pale from the blood supply being cut off, and then, with a needle-shaped lancet or the point of a sharp knife, to make a small puncture, from which a clear fluid exudes. In this may be found an enormous number of bacilli. These bacilli resemble the tubercle bacilli in a most remarkable manner, the only difference being that they are, if anything, slightly shorter.

They may be stained by almost any of the methods that are used for staining the tubercle bacillus, although here any method that is used must be slightly modified, as the bacilli characteristic of leprosy are rather more easily decolorized by acids than are the tubercle bacilli. The Ziehl-Neelsen method, with a contrast stain of methyl blue, gives most admirable results, or Gram's method may be used. The



Bacilli from Juice of a Leprosy Nodule. × 500.

bacilli so stained are seen to be from 4 to 6μ in length and .3μ in breadth ; they are more constant in size than are the tubercle bacilli, and, as a rule, are not marked by the curves that are almost characteristic of that organism ; their ends appear to be slightly pointed. It was at one time considered to be beyond controversy that these bacilli contained spores ; but more recent observations on these and other organisms have led observers to the conclusion that what were at one

time considered to be spores are not spores at all, but are appearances due to the alterations that have taken place in the protoplasm during the processes of preparation and staining. As it is not yet certain that the bacilli can be cultivated, little evidence either for or against the spore theory can at present be adduced. These bacilli, to be seen in their characteristic form, should be examined as they lie in the thickened nodules of the skin or in the mucous membrane of the mouth and larynx, in the thickened nerves, in the lymphatic glands, in the spleen, or in the liver. If a thin section be made of a nodule of the skin, and the specimen be stained with fuchsin, and decolorized with a mineral acid, and a contrast stain of methylene blue be obtained (see Appendix), it will be found that, throughout the section, branching lines or small rounded points of bright red may be seen on a blue background ; these bright red areas are nothing but masses of leprosy bacilli which fill the lymphatics of the skin, and, as one would expect, interfere very seriously with its nutrition. There seems to be a considerable difference of opinion as to whether these bacilli actually invade the cells or whether they lie free in the lymph channels ; there can be no doubt that a very large number of them ultimately are seen as free bacilli lying in these spaces, the difference of opinion being as to the nature of the so-called "lepra" cells. There are usually found in cases of leprosy a number of large protoplasmic masses, which are said to be multi-nucleated, and are spoken of as giant cells, and in these are found numerous bacilli. In younger nodules there are smaller masses of protoplasm, in which nuclei and bacilli may be distinctly seen. At the meeting of the British Medical Association, held in Dublin in 1887, Dr. Unna, of Hamburg, placed before the members his views on many of these so-called lepra cells. He contended that the rounded outline was due in many cases to the form of the lymphatics, and he showed in his specimens that the rounded section was equal in diameter to the longitudinal section of one of these lymphatics filled with bacilli ; that the longitudinal section proved that we have here to deal simply with a lymph channel blocked with leprosy bacilli, and therefore that the rounded masses are merely transverse sections of these stuffed lymph channels. He also maintained that these so-called cells, with their contained bacilli, were merely masses of these organisms

which were held together by gelatinous material ; and that even where there were apparently globular masses in the lymphatic channels, they were nothing but these zoogloea masses, whilst a great number of the organisms were lying entirely free, the nuclei of the so-called lepra cells again being the nuclei of the walls of the lymphatics or of the lymph cells that had become embedded in the gelatinous mass. Many competent observers, however, maintain that the lepra cells have a real existence, and there can be little doubt that bacilli may be found in large cells squeezed from a leprosy nodule.

One of the most marked distinctions between tuberculous and leprous tissue is, that in tubercle the bacilli are comparatively few in number, especially when they are met with in the more chronic cases—the only cases that could possibly be mistaken for leprosy; whilst in leprosy the bacilli are almost invariably present in enormous numbers in the lymph channels of the tissues in which they are growing, on which they seem to exert comparatively little destructive influence, as they remain for a considerable length of time in an almost quiescent condition, setting up little change, but undergoing no retrogressive changes themselves. Cornil and Babes record that in a small fragment of a leprous nodule that had been left in an envelope, forgotten for nearly ten years, they were still able to stain the bacilli very distinctly ; and sections that had been stained in picro carmine and kept mounted in glycerine for a number of years, it was found, might still be stained so as to bring the bacilli into prominence. Then, too, leprosy affects the skin and nerves specially, rarely the lungs and serous membranes. Tuberculosis, on the contrary, affects the latter very frequently, and very rarely the former. From the enormous number of bacilli that are found in the lymph spaces, it can be readily understood that when ulceration of a nodule takes place, these organisms are to be found in large numbers in the blood and serum that is discharged from the ulcerated surfaces. Although this is the case it is a somewhat peculiar fact that this organism is seldom found in the superficial layers of the epithelium covering the nodule, although in the ducts of sebaceous glands, and around the hairs of the skin, as Babes demonstrated, the bacilli may be present in considerable

numbers ; they are seen in the internal sheath of the hair, from which they may, in some few cases, pass through the epithelium, and so to the surface ; this is, however, a very rare condition. As a rule, leprosy bacilli are not met with in the blood ; but in the febrile condition that occurs shortly before death, bacilli have been described as being found in the circulating blood taken at some distance from any nodule. As the leprosy nodules may be found in all parts of the body so also may the bacilli. In cases of anæsthetic leprosy the bacilli may usually be readily demonstrated lying in the dilated lymphatics of the thickened and nodulated nerves. Here, too, as in tubercle, the lymphatic glands are distinctly infiltrated. From its resemblance to tubercle, and from the fact that its specific bacillus is found so constantly associated with the disease, being most numerous in those positions in which the leprous processes are most advanced, but being present from the very commencement of the tubercle formation, it is evident that the bacillus bears a constant—probably a causal—relation to the disease, and it was therefore supposed that leprosy might be carried from one individual to another through its agency ; that, in fact, the disease was a specific infective disease and was inoculable.

Numerous experiments, made with the object of proving this thesis, have, however, failed. (Babes and Klarindero mention, in support of the contagious nature of the disease, the case of a child that developed leprosy on the lips and cheeks during the time that it was being suckled by a leprous mother, and Dr. Castor and many others insist on the communicability of the disease. A patient has recently been reported to be dying from leprosy, the result of inoculation in 1884. The patient was a condemned criminal in Honolulu, who elected to be inoculated with leprosy by Dr. Arning in place of being hanged). Even the inoculation of fragments of leprous tissue gave rise in all recorded experiments to no true leprosy, unless the patients were already the subjects of the disease. The cultivation of the bacillus has also proved to be a most difficult matter. Neisser observed a number of small pellicles that appeared to shoot out from small particles of tissue introduced into consolidated blood serum, kept at a temperature of between 37° C. and 38° C. Bordoni Uffreduzzi obtained growths from the marrow of a bone in which there were a number of free leprosy bacilli ;

these appeared on serum (to which a quantity of glycerine had been added) that was maintained at a temperature of 37° C. These he described as delicate, thin, slightly yellow films with irregular borders; on glycerine agar they are said to have developed as small grey rounded isolated points usually at the end of ten days or a fortnight; secondary cultivations, however, made their appearance at the end of forty-eight hours, and after the first few cultivations the organism could be grown on serum or on ordinary gelatine and agar, but much more slowly than when glycerine had been added. From the general description, and the imperfect staining obtained, some doubt must remain as to the true nature of these bacilli. Babes also was able to obtain cultivations on similar media, even from other organs; he described the growths as being very like those of diphtheria; upon serum they appeared as pale yellow elevated plates, glistening and waxy-looking and surrounded by a transparent indented zone; the cultures emitted a peculiar characteristic odour. They developed all along the track of the needle, especially in glycerine gelose; the rods were elongated, but there appeared to be a number of involution forms, and many of the bacilli were much more plump than usual, and, bearing out Unna's observations, they appeared to be surrounded by a clear capsule somewhat similar to that met with around Friedlander's pneumonia organism. Babes was not successful in making secondary cultivations, nor was he able to produce the disease by inoculating with his cultivations any of the animals that he had in his laboratory. Until these experiments are confirmed by other observers they can scarcely be accepted as conclusive, as it requires a much longer series of successful cultivations and more careful comparison of the organism as it appears in the tissues with that found in the cultures, than either Bordoni Uffreduzzi or Babes have made, to set the matter at rest. Still they have indicated the lines on which future work may be done, and we may anticipate that before long Dr. Bevan Rake, Dr. Castor and others, may continue their experiments with the enormous amount of material which they have at their disposal and give to the world most important results.

None of the numerous non-bacillary theories as yet put forward to account for leprosy appear to be sufficiently well

supported to be able to oust the bacillary theory from its present position. Leprosy is found in all climates ; it is not specially confined to the seashore, it occurs in regions where fish diet cannot be resorted to, and where other articles of diet such as pork and rice (to both of which have been ascribed a causal agency in the production of this disease) are not used at all. The only factor that is common to all forms of the disease, and that is met with in every case, is the leprosy bacillus, and in spite of the fact that we have not yet been able to trace the method of contagion or infection through the agency of this bacillus, we must, from what is known of the presence and action of bacilli in other diseases, assign to it the *rôle* of leprosy producer, until much stronger evidence than we have yet obtained can be adduced in favour of any other cause.

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CHAPTER XIII.

Actinomycosis.

Nature of the Disease—Differences in Cattle, Man, Pig, and Horse—Methods of Preparation and Examination of the Fungus—Microscopic Appearances of Fungus in Different Animals—Nature of Clubs—Cultivation Experiments—History of Actinomyces—The Actinomyces a Streptothrix.

A DISEASE that was for long very imperfectly understood, and which has been described under very different names, is Actinomycosis, which, in 1876, was first recognized by Bollinger as being of parasitic origin. The disease manifests itself in cattle, frequently in the jaws, where it is known in various parts of the country as wen, osteo-sarcoma or bony sarcoma, malignant tumour of the palate, or in the tongue, where it may be recognized as "wooden tongue;" the pharynx or the loose tissues under the skin of the head and neck, or even the trunk, may also be affected. In the pig, abscesses with running sores are most frequently found in the milk gland and in the tissues around the pharynx; whilst in horses it is usually found as "scirrhous cord," a condition well known to veterinary surgeons, though as Professor M'Fadyean points out, it may also be met with in the tongue of the horse. This curious disease has also been known to attack the human subject, in whom the lesion resembles in its characters that met with in the pig, the parasite giving rise, by its presence, to abscesses in the lungs, to pus in the pleural cavity, and to similar conditions in other organs; the bones, especially those of the spinal column, are frequently attacked, and "cold abscesses" or chronic gatherings form in those cases where sinuses from which matter may escape are met with.

It is interesting to note in this connection that even in the pig the lung is sometimes riddled with similar cold abscesses, and that there is frequently breaking down of areas of

bone in the cervical and dorsal vertebræ. Those who had examined the condition only in the human subject and in the pig describe the disease as consisting essentially of a suppurative process, whilst those who have examined "wooden tongue" in cattle, and "scirrhous cord" of the horse, maintain that the process, though sometimes leading to softening, is essentially of the granuloma or new young tissue formation type. These differences, however, appear to be due rather to the nature of the tissues in which the growths occur than to any difference in the character of the organism that produces them, for it is found that in many cases in which the bones are attacked in the cow the lesion takes on a suppurative character. It was, however, the non-suppurative form that was first described, and it will therefore be better to examine it in the first instance. In "wooden tongue" of the ox, on superficial examination, firm hard points may be seen scattered over the surface, varying in size from that of a millet seed to that of a split pea. On incising one of these, it is found to be firm and fibrous at the periphery and especially in the small nodules; the centre may be soft but not purulent, or, rarely, gritty and almost calcareous; in the larger nodules little fibrous bands running through them form a kind of net-work, and in the centre of each mesh is a similar soft point. If this softened caseous point be removed with the point of a knife and examined under the low power of the microscope (× 50) it will usually be found to contain, embedded in a small mass of cells, a small core, which by transmitted light is yellow or brownish in colour, dashed with a tinge of green. This little mass is seen to be composed of a kind of star made up of a number of wedge-shaped rays, the apices of the wedges meeting in the centre, the bases being rounded; looking down on the centre of this cord it appears as though there are rounded instead of wedge-shaped bodies (this is simply because we are looking down on the rounded ends of the wedges). If now one of the "bony" tumours, or one of the tumours in the fibrous covering of the bone, where the tumours are usually of larger size, be examined, it will be observed that the fibrous net-work is exceedingly well marked, especially as on section the softened caseous centre gives way very readily: in the softened material similar "rayed" or star-like organisms may be found. In the material that is

discharged from the "cold abscesses" which are formed in the pig or in the human subject, similar small yellow points (which appear as green or greenish yellow grains, even when examined with the naked eye) may be found on examination under the microscope. The pus discharged from these abscesses has an exceedingly characteristic appearance; it is usually yellow or brownish yellow in colour, is extremely granular, of a peculiar slimy consistence, and contains the green points that may be said to be specially diagnostic of the actinomycotic condition. The small green points when taken from the cow are very frequently somewhat gritty, this being due apparently to the deposition of particles of lime in the core; but the particles that come from the pus in the human subject are usually soft and tallow-like, so that they can be readily flattened out between two cover glasses. The appearance of the actinomyces or "ray fungus" in cattle, as first described, was so exceedingly characteristic that it was thought it could not be mistaken for anything else, and the corresponding condition in the human subject was for some time overlooked, simply because the same typical appearances were not always developed; and it was only after some time that, transition stages being found, first in cattle and then in the human subject, the real nature of this fungus was thoroughly understood.

On examination of the fungus under a high magnifying power, when the sections have been properly stained, the organism is found to be like the capitulum of a daisy, the sterile flowers in the centre corresponding to the club-shaped rays, and if we conceive of two of these heads of flowers as placed base to base, or stalk to stalk, we may obtain an idea of the appearance of the ray fungus as a whole; the organism in sections of course having the appearance of sections through the little ball formed by the two heads. The clubs, however, are not all simple, but in some cases branch, sometimes dichotomously, sometimes irregularly, compound clubs being thus formed.

In a tumour examined by Professor M'Fadyean, the transition stages between the forms sometimes found in the human subject, and those most frequently seen in cattle, are met with; and as I have had the privilege of seeing Professor M'Fadyean's specimens I shall follow pretty closely his descriptions, as

I consider that the interpretation he gives of the appearances presented is perhaps the most satisfactory that has yet been published. The tumour from an ox was fleshy in consistence, had a faint pink colour, and was studded with minute softened pink points, and in each of these points was found an actinomyces colony, at the margin of which, however, only a few of the characteristic clubs could be found, the centre being finely granular. On making sections of these tumours, that had been hardened in alcohol, and staining by Gram's method (see Appendix), and examining under a high magnifying power, Professor M'Fadyean observed that the colony consisted of three distinct elements, though, in many instances, the club-like bodies were absent. The first element is a coccus about .5μ in diameter ; these cocci are usually arranged in chains consisting of ten or fifteen elements, a few of them are usually found in the centre of the mass, but immediately outside this they are exceedingly numerous, so numerous indeed that they appear to be more like masses than chains ; as we approach the margin again the chains radiate outwards and are very distinctly seen. Where they are not very numerous, some larger cocci may be seen undergoing regular vegetative division, so giving rise to the formation of diplococci.

The second element is a thread-like leptothrix or cladothrix, a number of which, interlacing freely, form a kind of felted net-work, especially in the centre of the colony. As we pass outwards, however, they gradually assume a more regular radiate arrangement, and near the periphery " they sometimes shoot out in a tendril-like manner beyond the coccus heaps already described." These threads vary very considerably in length ; sometimes they are divided into short bacilli or even into cocci ; in other cases long threads without any sign of division may be seen. The diameter of these threads is usually greater than that of the cocci. They are described " as some nearly straight, others gently curved, and occasionally they show short nodules almost like a spirillum." Near the margin these threads sometimes become branched just as in the case of the club-like forms already mentioned. The club-like forms when met with here appear to bear a definite relation to the threads ; they are only found at the margin of the fungus mass ; they are arranged radiately with the ends of the clubs outermost ;

they vary much in length, and are usually quite simple, "but some carried lateral buds, and occasionally two appear to be carried by a common stalk." These buds are best stained by the Ziehl-Neelsen method; some of them exhibit a very important relation to the leptothrix forms already described, the thread appearing to be continued into the centre of the club, the outer part of which is formed by a homogeneous, somewhat faintly stained material. This axial thread may be divided into longer or shorter segments, corresponding apparently to the bacilli and cocci forms. They are usually most divided and are undergoing most marked changes in the larger clubs, whilst there are also small rounded bodies, which appear to be essentially of the nature of cocci, surrounded by the same material that forms the thickened club. In some of the smaller colonies of the actinomyces Professor M'Fadyean describes cocci only, which are usually arranged in short chains or in little groups. They are embedded in masses of leucocytes, some being actually within the cells, and appearing to be the points from which the larger colonies start, the cocci being carried by the leucocytes from point to point. Other colonies contain only cocci and thread forms, the threads in this case appearing to be developed from cocci, whilst those colonies in which clubs are present appear to be in a still more advanced stage of development.

By some observers the club-like forms have been described as the spore-bearing parts of the organism, and are spoken of as Conidia or Basidia, but it appears from the above case, and from those that have been described in the human subject, that the thickened extremity is due merely to a kind of involution process, and occurs in the older thread-like organisms as the result of a growth and swelling of the outer sheath of the Cladothrix threads, this sheath corresponding, in fact, to the gelatinous material which is formed in zoogloea masses of other organisms, or to the capsule first described by Friedländer which is formed around the bacillus of pneumonia. When these clubs are once developed the central part may be only partially active, whilst the periphery remains passive, but extremely resistant to the attacks of the surrounding cells, which attack the club-shaped masses very vigorously, the large thickened clubs being frequently found in various stages of degeneration

within the large masses of protoplasm found in this region.

In the human subject the felted network, the cocci and the bacilli are usually most numerous; they are, in fact, said to be typical of actinomyces in the human subject. Here again the involution or club-forms are frequently met with, especially in the pus that is discharged from the abscesses of the lungs or of bone. The slimy pus in these cases, however, appears to contain a considerable proportion of the "mucine," that in cattle goes to form the thickened sheaths above described. The process of evolution of the "Ray" fungus is much the same in the human subject as in the abnormal case already described as occurring in the cow.

It is interesting to note that most of the experiments that have been made on the cultivation of this organism have been attended with complete failure—a failure that in some measure, at any rate, appears to be due to to the fact that almost all experimenters have used for their inoculating material only those colonies in which the club-shaped organisms have become well developed. The first attempt that was at all successful was made by Boström, who, throwing aside the club-like processes, took for his inoculating material the central network, selecting as far as possible young growing colonies for his seed material. His method of procedure was as follows: With the utmost care he removed small colonies, which were at once introduced into sterilized gelatine, with which a "plate" cultivation was made. After a few days any points that were found to be pure, *i.e.*, around which other organisms were not growing, were removed from the gelatine with a sterilized platinum needle, and were crushed between sterilized glass plates; with the platinum needle, stroke cultivations were made on ox blood serum, and agar-agar. A finely granular growth first made its appearance along the line of inoculation; this gradually became more marked, then small yellowish red nodules were seen, around which delicate branched processes spread out; these yellowish masses soon began to run together, and at the end of seven or eight days were covered with a delicate fluffy white layer. This growth apparently went on best at the temperature of the body, and on microscopic examination

of the structures, cocci, segmented threads, longer threads and clubs were all found ; threads and clubs alike in most cases being characterized by a branching similar to that met with in the fungus as it grows in the human body. Inoculation of this fungus into the peritoneal cavity of rabbits and other animals was usually attended with positive results. Later, by attending to the same details as regards the nature of the seed material, M. Wolff, J. Israel, and Babes have all succeeded in cultivating this organism on agar-agar, blood serum, and especially on the raw white of egg, according to Hueppe's method (see Appendix), and with the cultivations so obtained actinomycosis has been produced experimentally in animals.

From all these facts it will be gathered that actinomycosis is the result of the activity of a living organism introduced into, and existing as a parasite in, the animal tissues ; that the same organism may be found in animals and in the human subject, that the club-shaped organism is really an involution stage, and that the characteristic growth is the mycelial thread-like mass which appears to develop from the cocci. The fact that we are unable to cultivate from the clubs alone, affords ample evidence that, in place of being spore-bearing masses, they are merely encysted, or thick-walled, involution forms.

As to the mode of infection, it has been pointed out that the actinomyces has been found lodged in the crypts of the tonsils of the pig and of the human subject, and that the parasite evidently leads an epiphytic life on barley and other cereals. It may be introduced from without through wounds, as in cases of scirrhous cord in horses, or through inoculation, by means of accidental scratches of the skin, or of the mouth and pharynx, and it is recorded that a case of primary mediastinal actinomycosis in the human subject was in one case supposed to be traced to perforation of the back of the throat by a barley spikelet swallowed by the patient. In pigs the mammary affection is thought to be due to the entrance of the actinomyces into the teat ducts.

In Denmark the farmers attach so little importance to infection from animal to animal, that cows in which these actinomycotic tumours are well developed are allowed to run with the rest of the herd, at any rate until suppuration

commences; when this occurs, however, the animals are usually slaughtered.

I may here summarize what is known of the history of a colony in the words of M'Fadyean, who has given the subject much careful study, and to whose authority in this matter I attach much weight.

"1. It has its starting point in one or more cocci transported by the plasma currents, or by the agency of a carrier cell (leucocyte).

"2. The cocci multiply by elongation and subsequent fission. When undisturbed by the surrounding leucocytes their growth and multiplication are after the manner of a streptococcus, but frequently they become irregularly grouped together (Staphylococcus heaps).

"3. By elongation, some of the cocci give rise directly to short bacillary forms, and through these to long filaments.

"4. The further extension of the colony is effected by the growth and multiplication of both threads and cocci. The former multiply by segmentation into bacillary elements, which may again elongate to leptothrix forms.

"5. The leptothrix filaments may give rise by close segmentation to coccus forms.

"6. The formation of clubs and similar forms is evidence of diminished vegetative power of the filaments (possibly also cocci), in connection with which they originate.

"7. The growth of a colony may be arrested at any stage by the agency of the animal cells (leucocytes), or by failure in the supply of the necessary pabulum. In that event the majority of the threads tend to develop clubs at their outer ends (involution forms). The central cocci and the remainder of the filaments then disintegrate; but the clubs which offer a greater (passive) resistance to the surrounding cells may persist for an indefinite period."

From what is here stated, and from what I have already said, it will be evident that we have here to deal not with an ordinary "mould" fungus, but rather with a form of streptothrix that undergoes dichotomous division at certain points. It must therefore be looked upon as possibly one of the Cladothrix algæ, or more probably as one of the Schizomycetes in which the Cladothrix formation occurs, and which has been described as closely allied to the streptothrix Försteri.

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CHAPTER XIV.

Glanders.

Glanders—Farcy—Clinical Appearances of the Disease—Chauveau's observations on Glanders Poison—Löffler and Schütz—Method of Demonstrating the Glanders Bacillus—The Bacillus—Methods of Cultivation—Glanders in Various Animals—Farcy in Man—Temperature relations of Bacillus—Desiccation—Germicides.

Although one of the best known, and, from its anatomo-pathological point of view, best described of all diseases with which the veterinary surgeon has to deal, it was long before most observers in the veterinary schools could be brought to look upon glanders as a contagious infective disease. It is found especially in the horse and the ass, but as in the case of tetanus, it may also be encountered in other animals. When it occurs in or under the skin it is known as Farcy, and in this form is usually found in man. The primary disease is usually in the respiratory passages, the lungs and the skin, especially around the orifices of the nostrils and mouth ; the parts secondarily affected may be those in communication with the lymphatics from an infected area, or we may have distinct metastatic abscesses (or abscesses at some distance from the original focus of the disease), the material in such cases being carried apparently by the blood along the course of the blood vessels. In all cases the intensity of the disease appears to be in direct ratio to the number and rapidity of formation and softening of the small nodules, the process, as we shall find, being determined by the presence and activity of a specific bacillary organism that has made its way into the tissues. The small nodules, whether they occur in the skin or on the mucous membrane, say of the septum of the nose, appear as small grey, gelatinous-looking points about the size of a millet seed. In spite of this gelatinous appearance they are usually comparatively firm in consistence. Under

the microscope each of these points is found to be made up of granulation tissue, consisting of small round cells very like leucocytes or lymph corpuscles, a few larger nucleated cells, with here and there fragments of disintegrating connective tissue. After a time, as the nodules become rather larger, the centre assumes a yellowish appearance, or small opaque points may be seen, and ultimately at these points we have the tissue breaking down into soft, pulpy, or caseous, purulent material. The small nodules met with in the early stages of the disease, are usually surrounded by a deeply-congested area, and if they occur in the nostril the mucous membrane covering them is greatly congested, and there is great discharge of water, or of very watery "matter," from the nostril. As the nodules become softened in the centre, ulceration of the tissues near the surface takes place, the softened centre escapes, and a "punched-out" ulcer is left. These ulcers may gradually run into one another, and from the fact that the mucous membrane is congested and thickened, the loss of tissue seems to be very great—much greater than it really is. Along the lines of the lymphatics, and in the lymphatic glands communicating with the ulcerating surfaces, there is great inflammation and induration, what are known as "farcy-pipes" being formed. Here just the same processes are carried on as when the disease occurs in the mucous membrane, in the lungs and in the glands at its root, the lymphatics being of course similarly affected. It will thus be seen that glanders resembles, in a most remarkable manner, certain other infective diseases already described, such as leprosy, tubercle, actinomycosis, &c., and, as a matter of fact, numerous observations were early made with the view of proving, first, that this was really the case; and, secondly, that the contagious element was a *contagium vivum*, and probably a form of micro-organism. Chauveau, as early as 1869, inferred from a series of interesting and most ingenious experiments that the poison was particulate in character, and was, very probably, present in the leucocytes. In December, 1882, Löffler and Schütz made known the results of experiments by which they had been able to demonstrate the presence of a bacillus which appeared to have a distinct causal relation to the disease; and about the same time Bouchard, Capitan, and Charrin also described an organism in glanders—an organism, however, the charac-

ters of which were somewhat different from those ascribed by Löffler and Schütz to their bacillus.

As I have from time to time had opportunity of verifying a number of the points insisted upon by these latter, and as it is now generally accepted that the Löffler-Schütz bacillus is the one really associated with the disease, this organism may be briefly described.

The best method of demonstrating the bacillus is to take a small particle of the softened central part of a nodule and squeeze it out between two cover glasses; all superfluous matter from the edge is carefully removed, and the covers are then treated in the ordinary way, after which they are stained in a mixture of concentrated alcoholic solution of methyl blue, one part to three parts of a 1 in 10,000 liquor potassæ solution; the cover glass is rinsed for about a second in a one per cent. solution of acetic acid which has been tinged to the colour of Rhine wine by the addition of a watery solution of tropæolin. It is then quickly washed with distilled water, then with absolute alcohol, cleared up with cedar-oil and mounted in benzol- or xylol-balsam. Sections of a nodule that has been hardened in absolute alcohol should first be placed for a few minutes in weak caustic potash solution, after which they may be transferred to the stain and treated as above. An even better method of staining is to use Ziehl Neelsen carbolic fuchsin or carbolic methylene blue, and then to decolorize the tissues with distilled water, or with a two per cent. solution of hydrochloric acid. Kühne's carbolic-methylene blue method may also be be used for staining sections of tissues.

After preparation there may be seen minute rods from 2.5 to 5μ in length, which are usually one-fifth to one-eighth of their own length broad. They are always more numerous where cell proliferation is going on most rapidly. From the fact that these organisms do not take on any stain at all readily, and also that they are very easily decolorized, it is often an exceedingly difficult matter to distinguish them from nuclei and nuclear detritus, both of which take on staining material in much the same manner as the bacilli, and it is only by the exercise of the greatest care and by the use of the very best optical appliances that these organisms can with certainty be distinguished in the tissues. It is, therefore, all the more necessary to obtain pure cultivations of the glanders bacillus in order that its characters may be accurately described, and to determine whether it really plays an important etiological part in the production of the disease.

The first successful attempt to cultivate this specific bacillus was made by Schütz in 1882. Adopting the strictest

precautions to prevent the entrance of extraneous organisms, he took small particles of the grey translucent material surrounding the caseous centres of some of the above described nodules from the liver, lung, spleen, and lymphatic glands of a glandered horse. These small particles carefully broken down, were inoculated on fluid and solid blood serum from the horse and from the sheep, and also in broths made from the flesh of the dog, horse, fowl, and ox, and even in various fruit and vegetable infusions. For two days no indication of the presence of any growth could be observed on any of these media, but on the third day the fruit infusion became slightly turbid, and on the gelatinized serum there appeared numerous small, clear, transparent, yellow, slightly elevated drops, like drops of a yellowish fluid that had been splashed on the surface of the serum. In eight to ten days these became slightly cloudy or milky.[1] On examining one of these small drops under the microscope it was found to consist entirely of masses of short, rod-like bacilli, similar to those already described as present in the glanders nodules, giving the same colour reactions, being perfectly distinct in this respect from the tubercle bacilli, which, as regards size and general appearance, they very closely resemble.

Similar bacilli were found in the fluid media, and were usually in the form of pure cultures, that is, only this single kind of organism was present. In some cases there were impurities, but these were so evident to the naked eye that they could be detected at once, such impure cultivations being thrown aside.

The bacillus is usually straight or slightly curved, is rounded at one end, and if any difference at all can be observed between it and the tubercle bacillus, it is slightly shorter and perhaps thicker than that organism, especially

[1] In order to obtain growths on sterilized potatoes some of the nasal discharge from a glandered horse should be mixed with from 100 to 10,000 parts of boiled distilled water, and a few drops of this mixture run over the surface of the potato. Three days after inoculation there appear spots of an amber-like growth; these points, as they increase in size, become redder and more opaque, the colour deepening until it becomes like "copper oxide." The manner of growth, according to Löffler, is quite characteristic; there are only two which are at all like it, the bacillus of blue pus (the growth of which has a yellowish-brown tinge on potato but none of the amber transparency and a peculiar pearly iridescence), and the cholera bacillus.

when it is grown in a fluid medium. It exhibits no movement. With pure cultivations, obtained in this way, and propagated for several generations outside the body, Löffler and Schütz succeeded by careful inoculation in producing typical glanders in various animals, the artificially infected animals presenting on examination exactly the same appearances as the animals that had been naturally infected, and from the nodules artificially produced the typical glanders bacillus could again be cultivated and passed on to other animals, where they, in turn, gave rise to the usual symptoms of the disease.

In making these experiments they came across a most interesting point. In an old horse which they inoculated, and that was apparently quite healthy at the time of the operation, they observed that the disease remained perfectly localized, and that the ulcers very early evinced a marked tendency to heal. The animal, however, was killed, and on making a post-mortem examination it was found that it had already, in all probability, been affected with glanders, and that this had been running its course for some considerable length of time, as not only were there old scars on the septum nasi, but there were old caseous masses scattered through the lungs. We have here an exactly analogous condition to that recently observed by Koch in the case of guinea-pigs already affected with tuberculosis, on which he made the observations that led him to the discovery of his protecting fluid; this animal had been "protected" against the general outbreak of glanders which usually results from an artificial inoculation by a previous chronic attack of the disease.

In order to illustrate the method of procedure in such cases, we may give a short *resumé* of one of Löffler and Schütz' series of experiments. Two horses were inoculated—one twenty years old from the eighth cultivation, and the other two years old from the fifth serum cultivation that had originally been taken from a guinea-pig which had in turn been inoculated from a fourth generation (also grown on serum), originally taken from another animal. Both these horses were inoculated on each side of the neck and on the breast; and the younger animal was also inoculated in the posterior nares. This was purposely omitted in the older animal, in order to see whether the ulcers would occur in the nasal mucous membrane if it were left intact, as far as direct inoculation was concerned. At the end of a few days both horses had been attacked; they ate badly; diffuse boggy swellings made their appearance at the points of inoculation; they were stiff in the joints, the hair was ruffled, and on the eighth day farcy pipes corresponding in their distribution to the course of the lymphatic vessels and glands of the affected area could be distinctly felt under the skin. At this time

the swellings at the points of inoculation had ulcerated, and were discharging an opaque, greenish-yellow fluid. On the twelfth day an ulcer about the size of a shilling appeared on the skin of the forehead; its margins were thickened, and it was so deep that it extended down to the bone; there was a discharge from both nostrils, at the margins of which it dried into thin yellow crusts or scales; then small ulcers with indurated and thickened margins appeared on the nasal mucous membrane in both animals; in fact there were here all the characteristic features usually associated with glanders. These two animals died within twenty-four hours of each other, and typical glanders lesions were found in the tissues after death. Guinea-pigs inoculated with material from these cases exhibited similar characteristic symptoms and lesions; they died in from fifteen to fifty days, and on post-mortem examination it was found that the characteristic macro- and microscopic appearances were present in all of the lesions.

In the study of the specific infective diseases, it has been found that certain animals are especially susceptible to one specific virus, whilst others are but little affected, and that in the case of a second virus, things may be exactly reversed—the non-susceptible animal remaining entirely immune, or, in place of a constitutional disease being set up, only small local lesions making their appearance. It is therefore necessary for experimental purposes to determine in all cases what small and easily-kept animals are susceptible to a given disease, and what are unaffected or are only slightly affected by the virus of that disease.

In the special case of glanders it is a matter of very great importance, from the diagnostic point of view, that the guinea-pig is very susceptible to the disease, the natural virus or pure cultivations of the glanders bacillus setting up a typical disease which eventually brings about the death of the animal. Similar inoculations into a rabbit produce merely a slight rise of temperature, some local irritation accompanied by swelling, and perhaps by slight ulceration, without any further constitutional or general symptoms. Having found that this is the case as regards these two animals where the inoculations are made from animals that are undoubtedly glandered, it is evident that in a doubtful case of glanders, strong proof for or against the specific nature of the disease may be obtained by inoculating rabbits and guinea-pigs with material from the doubtful sources; if the guinea-pig dies with characteristic symptoms, and the lesions remain local in the rabbit, there is strong presumptive evidence (it might almost be said, definite evidence) that the suspected case is one of glanders

In connection with this immunity (complete or partial) or susceptibility of different animals to this disease, it should be pointed out that the human subject may be inoculated either through wounds or scratches or through the application of

the nasal discharge of a glandered animal to the mucous membrane of the nose or mouth. There are, undoubtedly, cases recorded of glanders occurring in the human subject, but these are not so numerous as they might be if it were possible to put all those cases described as acute or chronic blood poisoning under their proper heading. An old friend of mine, the late Dr. Howard Bendall, in a Thesis presented for the degree of M.D. in the University of Edinburgh, 1882, described a case of acute farcy in man, and collected the records of 68 similar cases, a number that might now be very considerably added to. Of all the cases of acute farcy, 47 in number, only 6 were cured ; whilst of 21 more chronic cases no fewer than 15 recovered or were partially cured: the acute cases run a very rapid course, the duration of the disease, however, varying from 4 to 47 days, the average course of the disease being from 2 to 3 weeks. In chronic farcy the patients died in from 50 days to 14 months, whilst of those that recovered, the disease lasted as long as two and a half years. It has now been proved beyond doubt that this disease of farcy in man is due to the action of the same bacillus that is found in the glanders of the horse, this minute organism having been found both in the blood and in the contents of pustules taken from a man affected with farcy.

Cattle are completely immune against glanders as regards spontaneous infection, and only localized ulceration, which rapidly heals, follows inoculation. The goat is somewhat susceptible to the disease, though it appears to occupy a position between cattle and the horse in this respect. Sheep are fairly susceptible, but the disease runs its course very slowly, and appears to resemble the chronic farcy in man. Lions, tigers, and cats may all become affected, the disease in such cases running a very rapid course. Dogs react to the poison very much as do rabbits. So markedly is this the case that it has been suggested that the glanders bacillus might be attenuated by passing it through the dog before it is inoculated into horses and asses. It should be pointed out, however, in connection with all these experiments, that if a very concentrated virus, such as a pure cultivation of the bacillus, especially in considerable quantities, be inoculated even into a rabbit, a generalized disease may be set up, whilst a weaker virus, such as that contained in the discharge from the nasal mucous membrane of a horse, will produce nothing but localized symptoms. (This element of quantity can never be ignored in making experiments with bacteria of any kind.) Field mice are extraordinarily susceptible to the glanders poison, whilst white mice and house mice are quite exempt. The pigeon appears to be the only bird that is at all susceptible to the disease.

We have already spoken of bodies that looked like spores in these bacilli, but from the fact that the glanders virus, both in fluids and in tissues, loses its vitality after fifteen days' drying, it must be assumed that the organism does not form endospores similar to those that are found in bacillus subtilis, for example. The bacillus grows best at a temperature of 37° C., it will not grow at 20° C., nor at 45° C. at the other extreme. At 22° C. it commences to grow slowly, whilst at 25° C. it flourishes most luxuriantly, although it rapidly loses its virulence when cultivated for several generations outside the body. Commenting on this fact, Löffler points out that glanders is essentially a disease of hot countries, where the comparatively high temperature appears to be extremely favourable to the development of the bacillus outside the body, especially in such materials as fodder, manure, and stable refuse generally.

We have interesting evidence of this in statistics collected by Krabbe, who gives the following proportion of horses affected with disease per annum per 100,000 horses in the following countries: Norway, 6; Denmark, 8.5; Great Britain, 14; Sweden, 57; Wurtemburg, 77; Russia, 78; Servia, 95 Belgium, 138; the French Army, 1,130; the Algerian Army, 1,548.

As already mentioned, desiccation for twenty-one days is usually quite sufficient to prevent the multiplication of the bacillus when placed in nutrient media. Consequently it may be possible, by proper ventilation, to diminish the mortality from this disease even in the warmest countries.[1]

Another agent which helps greatly in preventing the multiplication of this bacillus is putrefaction, as the organisms or products developed during that process appear to interfere very markedly with the growth and multiplication of the glanders bacillus. The most satisfactory of all disinfectants, however, is heat, and it has been proved experimentally that a temperature of 55° C., continued for ten minutes only, is quite sufficient to destroy the bacillus, and with it the infective power of the virus, from the fact that no spores, probably, are formed to perpetuate the species. A spray of steam would therefore, in all probability, be the most

[1] Löffler, however, was able to kill with a virus that had been dried on silk threads for eighty-nine days, and Fraenkel states that the organisms or their spores withstand drying.

serviceable and the most available of all disinfecting agents, or perhaps, better still, a thorough washing out of the infected stalls with boiling water. Chlorine and carbolic acid are both capital disinfectants. A 2 per cent. solution of carbolic acid applied for twenty-four hours kills the glanders bacillus, and a solution of 4 per cent. applied to the nasal discharge for a single minute renders it perfectly innocuous. A 1 per cent. solution of permanganate of potash, 0.23 or even a 0.16 solution of chlorine water, or one-fifth per mille solution of corrosive sublimate, will also render the bacillus perfectly innocuous within a couple of minutes. By means of any of these the process of disinfection may be carried on in stables in which glanders has occurred, with the greatest ease and with absolute certainty.

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CHAPTER XV.

Anthrax.

The Bacillus Anthracis—Early Observations—Pollender—Davaine—Koch—Pasteur—Methods of Examination—Appearances of Bacillus under Different Conditions—Spore Formation—Non-spore Bearing Bacilli—The Vitality of the Bacillus and of the Spores—Cultivation Experiments—Cover-glass Preparations—Inoculations into Animals—Methods of Infection—Anatomical Characters of Malignant Pustule—Animals Affected—Spores not formed in the Living Body—The Disposal of Anthrax Carcases—Various Disinfectants—Pathogenic and Saprophytic Anthrax—Buchner's Experiments on Anthrax Bacillus and Bacillus Subtilis—Hueppe and Wood's Experiments.

Anthrax, or splenic fever, is perhaps the best known of all the specific infective bacillary diseases. The Bacillus Anthracis, compared with other pathogenic organisms, is of very considerable size; it is from 5μ to 20μ long, and 1 to1. 5μ broad. It multiplies with very great rapidity in the blood of certain animals, and may be very easily cultivated outside the body; in consequence of these features it was the first organism that was proved definitely to be associated with a specific disease, and it was certainly one of the first to be recognized as occurring in both animals and in man.

In 1849, Pollender, and in 1850, Rayer and Davaine described these organisms as occurring in the blood of animals that had succumbed to splenic fever; then again, in 1857, Brauell, examining the blood of a man affected with anthrax, found this same bacillus.

Later, as already described, Pasteur's wonderful experiments on fermentation were published, and these led Davaine, in 1863, to commence a series of observations on anthrax, which, carried on until 1873, gave everything but absolute proof that the anthrax bacillus was the actual exciting cause of this malignant disease. This proof, however, was not supplied until 1876, when Koch, who had then been working at the subject for

some time, furnished most rigorous proof of Davaine's hypothesis. At the same time he was able to give much additional information as to the development, mode of production, and general life-history of the organism—information that could only have been obtained by a long continued and careful study of the organism outside the body.

The following year Pasteur also succeeded in cultivating

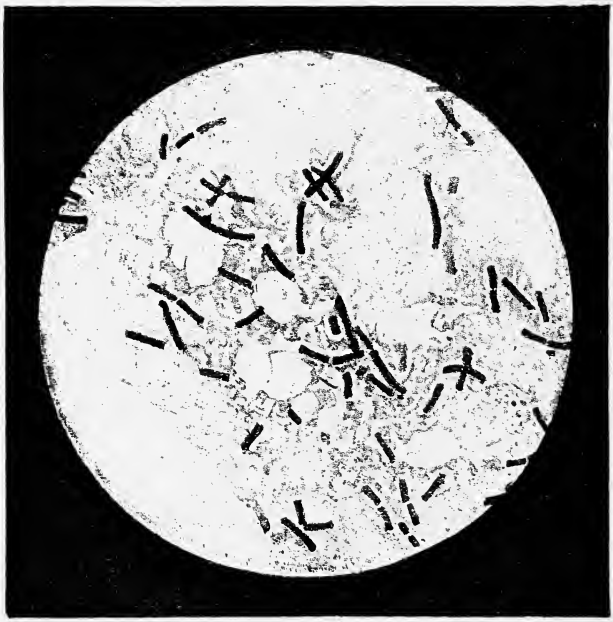

Photo-micrograph of Anthrax bacilli in a preparation of the fresh spleen pulp of a cow that had died from splenic fever. × 1000.

this organism as a saprophyte and from that time up to the present new facts have constantly been garnered, and our knowledge of the biological history of this organism has been greatly extended.

To observe the organism, a drop of blood is taken from the spleen of a cow (or of any other animal that has died of anthrax), and spread out between two cover glasses ; these are

then separated, and one of them is at once lowered on to a drop of three-quarter per cent. salt solution, the other being set aside to dry, after which it may be gently heated in the flame of a spirit lamp and then stained in a water solution of methylene blue, well washed in water and alcohol, and mounted in a drop of water or glycerine. In the unstained specimen there will be found lying between the red blood corpuscles a number of short rods of the size above mentioned, each of which has slightly rounded ends ; sometimes also there may be seen a delicate transverse mark running across the middle, this being especially well marked when the rods are longer than usual. The centre of each rod in the stained specimen appears to be quite homogeneous, and is usually deeply stained ; around this deeply stained portion is a kind of sheath which remains unstained, or is only slightly tinged by the colouring reagent. In some cases there is also at the point of junction, on each side of the transverse line, a somewhat oval area slightly stained, so that when a number of these rods are placed end to end without being separated they have very much the appearance of a finger with the joints slightly enlarged, or of a bamboo cane with its characteristic thickenings placed at almost regular intervals. Both rods and threads are perfectly motionless. In other cases, in place of a mere transverse line, there is the appearance presented of two bacilli that have only recently become separated from one another, still close together, however, and often so disposed that they enclose an angle. In the coloured preparation the same thing is observed. It is now seen that where the rods obtain any considerable length they are distinctly segmented, each chain being divided into a number of short rods, and at regular intervals at the points of segmentation there is usually a slight swelling, although as yet there is no trace or evidence of any spore formation.

In cases of wool-sorters' disease, which is very frequently accompanied by pleurisy, these organisms may be found in the fluid that accumulates in the chest as long threads, which may be grouped together in a kind of network or felt, the individual threads of the bundles often reaching an enormous length without there being any appearance or segmentation.

A similar growth of long threads has also been obtained by cultivating the bacilli, from the blood of the guinea-pig

affected with anthrax, for two or three hours in aqueous humour, the threads then become elongated, the marks of division are not nearly so distinct, and the threads remain homogeneous. It is possible, therefore, that the appearances met with in the fluid in the chest are due to the fact that the organisms have been allowed to remain at rest in the exudation, and that a process of cultivation has been going

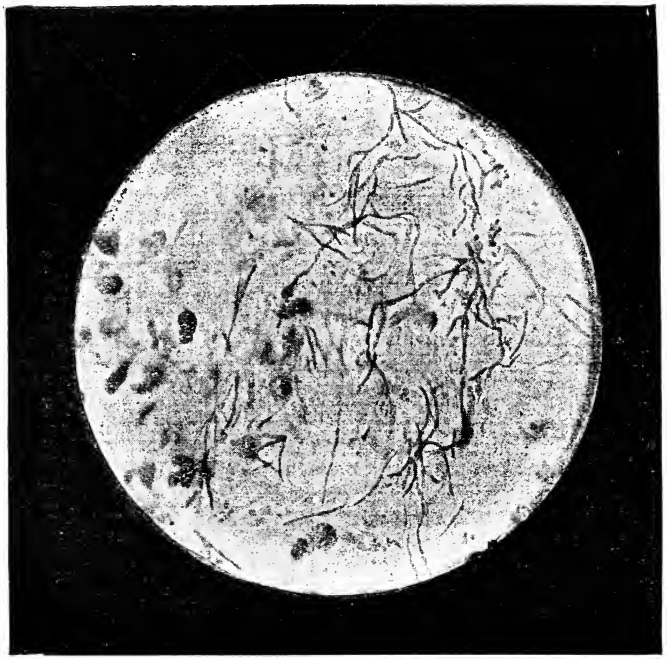

Photo-micrograph—Modified Anthrax bacillus—longer threads leptothrix form. × 1000.

on. In preparations made from the blood it is found that the corpuscles are somewhat irregular in shape, that they are running together in little irregular masses, and that the bacilli are much more numerous than the blood corpuscles. As regards the size of the bacilli it may be observed that the rods vary not only in length (in the same animals), but that they are of different breadths according as they develop in

one species or in another, those found in a guinea-pig being thicker than those seen in the mouse or sheep, whilst those in the rabbit are thinner than any of those above mentioned.

If one or two of these bacilli be placed in a moist cell, in what is called a hanging drop of nutrient broth, and the temperature be kept at about 37° C., the organism may be kept under observation under the microscope during the whole course of its development. First, the short rods so increase in length that they may ultimately cover a whole field of the microscope, the protoplasm at the same time becoming granular; then a large number of very minute points make their appearance, the hyaline appearance is gradually lost, and the threads become quite opaque. A little later, if the observation be continued, it will be found that instead of a single thread, little bundles of the same long threads make their appearance, in which there are found, at pretty regular intervals, the highly refractile bodies with well-defined margins and sheaths which have already been described as spores. When the spores occur in long threads there is usually a slight enlargement at the point at which the spore is situated, and this, with the bright shining point, gives to these spore-bearing threads an appearance that is described as being like a chain of pearls. In many cases the spores make their appearance in the shorter rods.[1] In place of the regular forms above described there are others, or involution forms, which are found to present themselves when the organism is grown under unfavourable conditions; for example, irregular moniliform threads are usually met with where the temperature is too high or too low, the soil is exhausted and so on.

It has been found by Lehmann, Hime, Buchner, Behring, and Roux that anthrax bacilli may be obtained that do not give rise to spores. This appears really to be due to an interference with the vitality of the protoplasm, as asporogenous organisms are best obtained by acting upon them with antiseptics, be these chemical or physical. Roux, for example, was able to obtain asporogenous bacilli, and bacilli that remained asporogenous for several generations, simply

[1] The development of the spore has already been described, as most of the descriptions of the setting free and development of the spores are taken from observations made on this species and described by Koch, Buchner, Prazmowski and others.

by immersing them for some time in 1,000 parts of nutrient fluid, to which one part of carbolic acid had been added. Under these circumstances the growth of the organism is not completely prevented, but no spores appear to be developed. It cannot multiply at a temperature below 16° C. nor above 45° C., 30° to 37° C. being the optimum temperature. It is distinctly ærobic in its growth, and cannot develop unless it can obtain a pretty free supply of oxygen.

It has already been stated that the organisms which occur in the blood are homogeneous, and it is a well-known fact that spores are never developed in the bacillus that grows in the blood, the multiplication there being entirely vegetative in character, and being due to fission or division of the rods as they increase in length. The same thing holds good even in the dead body so long as it remains intact; when once, however, the blood is allowed to come to the surface and in contact with oxygen, spores are very rapidly formed within the bacilli. This spore formation will not take place below from 24° to 26° C. (Koch says 18° C.), and then only in the presence of oxygen, so that they can best be seen in those bacilli that are cultivated on the surface of such nutrient substrata as agar-agar, solidified blood serum, and potato, or in fluid media through which a pretty constant stream of oxygen is allowed to pass.

In this respect the spore formation of anthrax bacilli appears to agree with the sporulation of yeasts, which, it will be remembered, takes place best on a surface of plaster of Paris that is constantly kept moist and well supplied with air.

Anthrax bacilli as distinguished from the spores are very readily killed. The ordinary putrefactive processes that are undergone in the decomposition of carcases in which these organisms have been present during life, especially if the air be excluded, cause the death of these bacilli in about a week. The temperature of boiling water maintained for a few seconds kills the bacilli, but, according to Klein, boiling for ten minutes is not to be relied upon to kill the spores although Koch states that at 100° C. the spores are killed in five minutes. The bacilli are killed by a two minutes' exposure to a one per cent. solution of carbolic acid in water, whilst the spores may remain alive for more than a week in a similar solution. They must be kept for nearly a week in a three per cent. solution, and twenty-four days in a five per

cent. solution if these reagents are to have any lethal effect on them. Carbolic acid in oil—five per cent. solution —had little more, if any, effect than pure olive oil. The spores remain alive, for an indefinite period almost, in a five per cent. solution of olive oil. Sulphurous acid vapour in the proportion of one to one hundred of air kills the bacilli in half an hour, but the spores resist the same antiseptic for seventy-two hours. Corrosive sublimate in one per mille of water suffices to kill spores by simply wetting them.

These tests of the vitality of the spores, however, were made by inoculating them into gelatine after they had been treated with the antiseptic. If, in place of inoculating into a nutrient medium of this nature, the spores are introduced into the circulating blood of a living animal, it has been found by Klein that it requires much longer exposure and much stronger solutions to hinder the development of the spores into bacilli and prevent the production of anthrax.

We have already stated that the organism cannot continue its growth at any temperature above 45° C., but it may still remain alive up to about 60° C. if this temperature be not continued for too long a period, the spore-bearing bacilli, though themselves killed at this temperature, leave their spores, which will withstand a temperature of 100° C. if continued only for a short time, and start into life and into active vegetative growth when again placed under suitable conditions. The only other physical condition that appears to be fatal, or at any rate injurious, to anthrax spores is strong sunlight ; this appears to deprive them in whole or in part of their powers of further development in a most remarkable manner, always causing distinct attenuation of their pathogenic virulence before completely destroying them.

The best way of maintaining cultures of anthrax bacillus is to take a drop of anthrax blood, sow it on a potato or agar-agar, allow it to grow there for several days at about 30° C. until spores are well developed, triturate a small quantity of the growth with some distilled water, place a number of silk threads which have previously been sterilized by heat in this mixture, and then dry them carefully, cut into short lengths, and keep them in a stoppered bottle or in a plugged test tube. From these threads cultivations may be made on almost any artificial nutrient medium. In addition to the media already

mentioned, starchy materials, vegetable infusions, hay and meat infusions, and even sterilized alkaline urine, may be used, the only thing that appears to interfere with their growth being an acid reaction. Even this, however, may be present to a slight degree if other conditions are favourable; for example, the anthrax bacillus grows readily on potato, which gives a slightly acid reaction. On gelatine plates colonies grow and are visible to the naked eye, on the second or third day, as small white points which gradually spread outwards, and as they come to the surface cause a slight liquefaction of the surrounding gelatine; they are then seen as small white masses with wavy margins lying in a clear space, formed by the liquid gelatine. When examined under the low power they appear as round dark-green points with an irregular outline; on the second or third day this irregularity becomes much more marked and, as Flügge describes it, when it reaches the surface of the gelatine "the dark remnant of the deeply-placed colony can only be seen in the middle. Around this centre, however, there is a light-brown or light-grey shimmering mass, which consists of numerous wavy, curling bundles, recalling the appearance of locks of hair or snakes on the head of a Medusa. Ultimately individual threads, or bundles of threads, branch off from the irregular periphery, and grow over the gelatine in various directions. At the same time the gelatine is liquefied over a small area; the colonies, which have now a diameter of 2 to 4 mm., begin to float and break down at their margins under the action of the fluid formed."

An exceedingly good method of obtaining a permanent record of the appearance of these colonies, is to lower a cover glass on to one of them and then to raise it carefully without sliding it over the surface, so as to take an imprint of the colony upon the glass; the surface of the colony adheres to the glass and a thin layer is removed. This, when stained with fuchsine or methyl blue and mounted in Canada balsam, gives an exceedingly faithful "impression" of the appearance of one of these masses.

The puncture cultivations of the bacillus anthracis present most characteristic appearances. Along the track of the inoculating needle there appear delicate feathery rays, which pass for some distance into the gelatine; small lateral rays are given off from these, and the whole of the young growth has a peculiar feathery appearance. These rays are always longer near the surface, and gradually become shorter

and fade off as the lower part of the track is reached. After a time the gelatine begins to liquefy, and slowly the feather-like mass sinks to the bottom of the liquefied medium, eventually forming an opaque white layer, which is found to consist of bacilli in different stages of degeneration. The upper layer of the gelatine, though perfectly clear, is now quite fluid. This process of liquefaction comes on first along the track of the needle, near the surface, and gradually extends downwards until the whole needle track has disappeared. We have then in the tube, the upper liquefied gelatine, then the opaque layer of bacilli, and lastly the clear solid gelatine below. If agar-agar be used in place of gelatine, much the same appearances are presented; there is a smooth glistening greyish-white surface growth, and the feathery rays appear to get more and more solid as the growth continues, but no liquefaction takes place. On solidified blood serum the colonies grow as greyish-white layers on the surface, and the medium is very slowly liquefied. On sterilized cooked potatoes the bacillus grows as a creamy-white somewhat parchment-like granular mass, which rises above the surface for some little distance but never extends far in a lateral direction; it has a peculiar dry appearance. The growths on potatoes, as on other media, take place at the ordinary temperature of the room. This organism can be very readily inoculated into certain animals, with results that may be looked upon as very definite; for example, if with a needle the point of which has been dipped into the spleen of a cow that has died from anthrax a mouse be pricked at the root of the tail, it will die in from seventeen to eighteen hours, enormous numbers of bacilli being found in its blood. It has been observed that in the case of somewhat larger animals, such as the guinea-pig, death does not take place till a rather later period; for instance, a guinea-pig setoned with a silk thread containing spores of anthrax bacillus will die on about the second or third day. If the animal be examined after death, it will be found that near the seat of inoculation there is usually very little to indicate that this was the point at which the infective material was introduced. Should, however, the inoculation be made into the abdominal wall, there is usually marked œdematous swelling of the peritoneum; there may be small hæmorrhages into the subcutaneous tissue, and there is usually

some emphysema ; the abdominal muscles are pale, moist, and are evidently in a condition of cloudy swelling, or in some cases there may be in the muscles, near the point of inoculation, hyaline degeneration. The spleen is enlarged, soft, and pulpy, and appears to contain a very large quantity of blood ; it is dark in colour. The liver also is changed ; it has a half-boiled look, and contains a considerable quantity of blood. The lungs are bright red and the cavities of the heart are distended. As already stated, the blood and lymph from the tissues contain the bacilli in very considerable numbers. If sections of various organs and tissues are afterwards made, it will be found that the bacilli are most numerous near the capillary vessels or in those small venules that arise from the capillaries. They are found in all parts of the spleen and in the small venules of the liver. They may also be found in the small capillaries of the glomeruli of the kidney.

Another method of infection, especially met with in wool-sorters' disease, is by the air passages, a condition that was most carefully described by Greenfield in this country, and has since been made the subject of careful observations by Buchner in Germany. It appears that the spores are inhaled along with dust from hair, wool, &c. ; they develop in the air passages of the lung, make their way through the alveolar membrane or through the walls of the bronchi, and so into the lymph channels and blood vessels of the lung. In some cases, however, they appear to "operate" from the air channels themselves, and, multiplying in these positions, give rise to a kind of pneumonia. The air vessels become filled with sero-fibrinous exudation, in which the bacilli may develop most rapidly, the walls of the alveoli become œdematous ; the bacilli make their way from these points into the circulation, and general anthrax is set up. Pleurisy, with effusion into the thoracic cavity, as already mentioned, may also develop in these cases, and the walls of the bronchi appear to be invaded by the bacillus. It has been found that if the bacillus is taken into the alimentary canal the acid contained in the stomach usually destroys it. If, however, resistant spores make their way into the alimentary canal, they may pass untouched through the stomach and so into the alkaline contents of the intestine. At the body temperature they are then under very suitable conditions

for development into the vegetative forms ; and as they are developed they make their way, especially through damaged epithelial cells which they gradually push to one side, into the deeper layers of the intestinal wall, into the lymph channels, or directly into the blood vessels, so giving rise, in many cases, to a general infection.

Susceptibility of animals to infection through the intes-

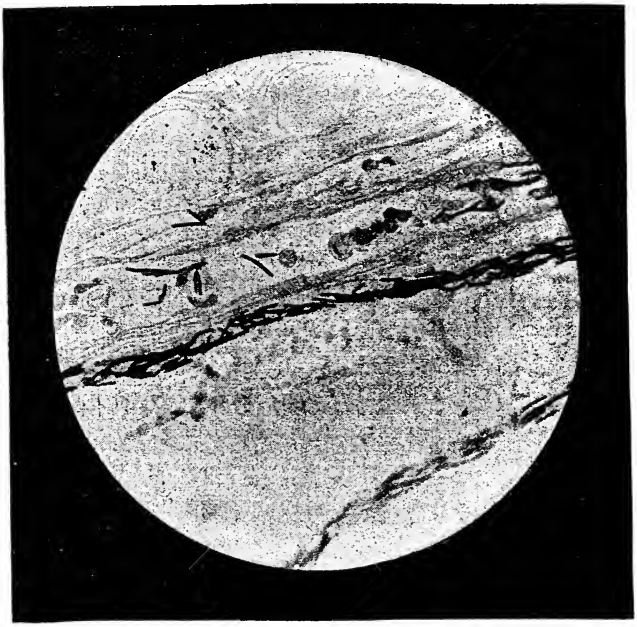

Photo-micrograph of Anthrax bacilli in the blood vessels of the mesentery of a mouse. At one or two points the bacilli may be seen lying in the substance of some of the white blood cells (phagocytes). × 375.

tinal canal varies very greatly, the guinea-pig, which is very susceptible to the disease conveyed by inoculation, withstanding a very large dose of the spores when given by the stomach, whilst sheep and cattle, both of which are more resistant as regards inoculation, are comparatively easily infected through the alimentary canal. I have stated that at the point of inoculation in animals there is usually no evi-

dence at all that it has been the point of entrance of the bacilli, but in the human subject there very frequently occurs at the point where the bacilli enter a wound, a marked local reaction, the result apparently of an effort on the part of the tissues to prevent the further advance of the bacilli. There is first irritation at the point of inoculation, this usually occurring in from one to three days after the inoculation; about a day later a minute vesicle surrounded by a zone of inflammatory redness and swelling makes its appearance. The serum in this vesicle becomes brown, and gradually a kind of mortification is set up; other vesicles, forming a ring, appear around the original point; these in turn become brown or black, until gradually an extensive black scar is formed. If these little pustules, with the surrounding tissue, be freely excised and the wound well cauterized with strong carbolic acid, there may be no return of the disease; in fact, if the removal be free enough, this result is most certainly obtained; but if the inflammation be allowed to go on unchecked, there is gradual extension of the vesication, sloughing, and blackening, until a very considerable area is affected, the bacilli sooner or later making their way into the blood vessels and giving rise to general anthrax. In the vesicles, and also in the tissues around the point of inoculation, anthrax bacilli can usually be found in considerable numbers, the tissues are somewhat œdematous, and there is an increase of leucocytes in the inflamed area. When the blackening commences there are usually found along with the bacilli, or in some cases in the centre of the ulcer replacing them, chains of micrococci; these are seldom seen in the very early stages, but in the later stages they are almost invariably present.

The animals most susceptible to this disease are sheep, mice, rabbits, guinea-pigs, and, according to Flügge, horses, hedgehogs and sparrows, all of which die or are very ill when the organism is inoculated directly into the subcutaneous tissue. Most birds are not readily inoculated, and for a long time it was found impossible to kill fowls by means of anthrax. White rats, old dogs, and amphibians are exceedingly resistant to the disease in almost any form; cattle, too, which are readily infected through the alimentary canal, are but slightly susceptible to anthrax introduced by direct

inoculation into the tissue. It has been found, however, that by reducing the temperature of the fowl and by raising that of a frog, these animals may be infected by inoculation, and they have even under certain conditions been killed. The usual method of infection in man, of course, is by the skin, and it is found that anthrax usually affects those who attend on or slaughter animals that are subject to this disease, so that those usually affected are butchers, grooms, and others engaged in similar employments. During the time that I was acting as physician to the Western Dispensary in Edinburgh, which was situated near the public abattoir, several cases of localized poisoning and two of general anthrax poisoning came under my charge within a comparatively short time. These were all cases of butchers, who having slight scratches on their hands or arms, had been infected by the blood of animals that they had killed which were suffering from splenic fever. Tanners, skin-dressers, and wool-sorters are also specially liable to this form of the disease, though the latter class are also very frequently affected through the lungs. In certain regions where the disease seems to be almost endemic the spores of the bacillus may make their way into the alimentary canal of the human and other subjects, through the medium of food or water.

It has already been stated that the anthrax bacillus within the body of an animal is incapable of forming spores, but in those cases where the bacilli can make their way from the lungs into the saliva, from the mouth, or from the lower part of the alimentary canal, whence they are discharged with the secretions, spores may be readily enough formed. Consequently an affected animal must always remain a centre of infection, and even the dung from a diseased animal may contain a large number of spores which, when scattered over a field, are sufficient to infect whole herds or flocks. The only way in which to get rid of the infection in such a case is to burn the animal at once, or to bury it deep down in the earth. This latter method of disposing of the animal should only be resorted to where it is possible to bury it six or seven feet deep. It is a well-known fact that the organism cannot form spores where oxygen is absent, and at any temperature below 12° C., and below this temperature the bacilli are incapable of existing for any length of time. At a distance of six or seven feet from the surface the temperature of the

ground is usually below 12° C., so that animals put below this depth, even if swarming with bacilli, cannot be looked upon as centres of infection ; the organisms, no longer able to form spores, soon lose their virulence and die out altogether. In order to get rid of any spore-bearing bacilli, all discharges or blood that may have escaped at the place where the animal lay after death should be carefully disinfected with 5 per cent. carbolic acid. It was for long thought that by cultivating the anthrax bacillus through a great number of generations on ordinary nutrient media it could gradually be converted into the hay bacillus or the bacillus subtilis — a very similar organism, but one that has no pathogenic properties—and Buchner, in a published series of experiments, claimed that he had obtained this conversion, and that in the same way he could again turn back the wheel from the hay bacillus to the pathogenic form. These results, however, have not been repeated, although many have tried similar experiments ; but Hueppe and Wood, by using a species of earth bacillus, which, in its morphological characters and method of growth on nutrient media, appears to be almost identical with anthrax bacillus (but possesses no pathogenic properties), have found that it must be very nearly related phylogenetically, from the fact that when inoculated into animals it acts as a kind of vaccine, and renders the animal immune to an attack of anthrax.

It will be well, however, to take up this and other questions relating to anthrax in connection with vaccination and immunity.

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CHAPTER XVI.

TETANUS.

Tetanus a Specific Infective Disease—A Wound Fever—Organism found by Nicolaier in the Soil taken from Streets and Fields—Experiments on Animals—Symptoms of Disease—Pure Cultivations Obtained—Description of Organisms—Characteristic Shape—Spore Formation—Organism Anærobic—Cultivations—Kitasato's Method of Cultivating the Organism—The Bacillus found only at the Seat of Inoculation—Wide Distribution of Spores—Bossano's Examination of Earth—Vaillard and Vincent's Observations—Tetnaus Bacillus a Facultative Saprophyte—Conditions under which Tetanus is Contracted—Poisoned Arrows.

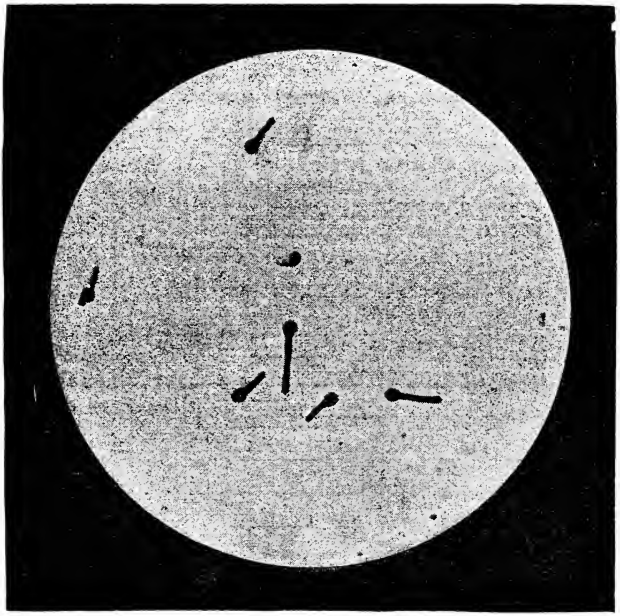
TRAUMATIC tetanus, or convulsions resulting from poisoning associated with an open wound, was for long suspected to be the result of some condition similar to septic or hospital fever ; that it was an infective disease was recognized by many of the older surgeons, and attempts were made at a very early date to treat it as a traumatic infective disease. That this poisoning was the result of the activity of an organism, which made its way to a wound and there flourished and gave rise to the characteristic products and symptoms, was not the result of direct experiment made with the object of finding out such an organism, although attempts were not wanting to demonstrate its presence in the wounds and along the course of the nerves in cases of tetanus. All efforts, however, proved unsuccessful until after an organism obtained from other sources had been obtained and described, and an artificial tetanus had been produced.

In 1884 Nicolaier, working with soils obtained from the streets and from the fields, found that these when inoculated into certain animals produced effects different from those produced by soils taken from cultivated gardens and from woods. The former he found, when a small particle was placed in a little pocket under the skin of mice, rabbits,

and guinea-pigs, gave rise to symptoms which he described as tetanic in character. In from two to four days after the inoculation the hind-quarters of the animal became paralysed, first the one near the seat of inoculation, then the other ; then rigidity came on followed by loss of motion ; the forelegs were in turn affected, then the neck, and at length the whole of the body became rigid, and sometimes curved as in tetanic convulsions occurring in the human subject.[1] On examination after death there was found, at the point of inoculation, a small abscess, in the pus of which were several species of micro-organisms. One of these, when obtained pure or practically pure, if inoculated into another animal produced exactly the same symptoms. Nicolaier was not able to obtain any perfectly pure cultivations, but he described an organism which was afterwards separated by Kitasato and by Tizzoni and Mdlle. Cattani independently. This organism, although never directly demonstrated in the earth that produced the disease, is constantly found in the pus of the abscess, in the walls of this abscess, and even in the immediately surrounding tissues. It occurs as long delicate threads scarcely thicker than the bacilli of mouse septicæmia with slightly rounded ends ; like some other organisms, especially those met with in putrefactive processes, they give rise to spores which are usually developed at the end of the shorter rods into which the long threads break up ; this spore, forming the head of what is called the drum-stick-shaped bacillus, is usually large and may be seen as a clear mass causing enlargement of one end of the bacillus. The spore develops best at the temperature of the blood, and under favourable conditions is completely formed about thirty hours after multiplication has commenced ; at the temperature of the room this does not occur for about a week, although the organism itself developes readily enough at this temperature. The rods are motile. Owing to the fact that the organism is anærobic and that the presence of oxygen interferes very greatly with its development (it is said that oxygen kills it altogether), it proved a somewhat difficult matter to obtain perfectly pure cultivations, although when once it had been recognized that the organism was anærobic, plate cultivations were readily enough obtained

[1] For further description of the disease see "Micro-Organisms," Dr. C. Flügge, New Sydenham Society, 1890.

by keeping the plates on which the nutrient medium was spread in an atmosphere of hydrogen. When puncture inoculations are made in tubes of gelatine to which grape sugar has been added, there is no growth along the part of the track of the needle near the surface, but in the deeper part away from the air, there is a moderately luxuriant growth which appears in the form of a central



Specimen from pure culture of the Tetanus Bacillus, with enlarged spore-bearing "drum-stick" ends. × 1000.

streak, from which numerous spikes pass laterally into the surrounding medium. The culture at this stage has very much the appearance of a spruce-fir, the lower branches (*i.e.*, those in the deeper part of the gelatine where the access of oxygen to the organism is entirely cut off) being longer and more distinctly marked than those nearer the surface. Later this characteristic appearance is lost, the organism invades the whole of the nutrient medium,

forming a kind of cloud; the gelatine becomes softened, and there is emitted a peculiar fusty smell which appears to be almost characteristic of this organism.

Most of the earlier experiments were somewhat unsatisfactory, and considerable doubt was cast on this organism as a producer of tetanus from the fact that in many cases pus that was known to contain this specific bacillus, and even old pure cultivations of the organism, failed to set up the characteristic symptoms when inoculated in the usual manner. More recent observers, however, have pointed out that the tetanus bacillus, like many others of the septicæmia group, is virulent only so long as it is grown under anærobic conditions. This is especially the case in those organisms in which there has not been time for the spores to develop; so that when grown in the presence of free oxygen (when that is possible), or when exposed to the air after the growth has been commenced under favourable conditions, and has gone on for some time, but before there has been time for the formation of spores, the organism rapidly loses its virulence, or, as we have seen, dies off altogether. Experiments made by inoculating the pus from tetanic patients often gave entirely negative results. Here it was evident that the failure was due, in many cases at any rate, to the fact that the pus with its contained organisms had been exposed to the air for some time, and the bacilli had been compelled to grow under conditions unfavourable to the retention of their specific virulence, before they were used for purposes of inoculation. In such organisms, as we should expect, it is found that spores are not seen, or they are very imperfectly developed, and it may be in the case of the older cultures, where these spores are developed into the young bacilli, that these, not having attained their full resisting power, die off very readily in the presence of oxygen. By paying attention to this point it has gradually been proved, almost beyond doubt, that the tetanus that may be produced in white mice or guinea-pigs by the inoculation of small particles of garden earth, is of the same nature as the tetanus that is produced by the inoculation of the pus from a primary wound which has apparently given rise to tetanus in the human subject. Further, now that the conditions under which the organism exists have been studied, and its anærobic character recognized, pure cultivations have been made, and it has

been proved that the disease may be produced without fail if certain definite precautions are taken before the inoculation be made. One great difficulty connected with the obtaining of pure cultivations was, of course, that under the conditions favourable to the growth of the tetanus organism, other anærobic bacteria would also take the opportunity of developing. Some only of these other organisms, however, give rise to the formation of spores, and this occurs at a later date than in the case of the tetanus bacillus. Kitasato, very ingeniously, made this fact the stepping-stone to the cultivation of a pure growth of the spore-bearing tetanus bacillus. As we have already seen, spores of many bacteria can readily withstand a high temperature (in some cases of even 100° C., if this is not continued for too long a time, and they will withstand for a considerable period the action of a temperature of 80° C.), whilst the vegetative or fully developed forms are killed off very rapidly at a comparatively low temperature. Kitasato's method of procedure was as follows : From the immediate neighbourhood of the suppurating wound of a patient who had died from tetanus, he took a small fragment of tissue, and placing it under suitable nutrient conditions, *i.e.*, in the specially prepared gelatine at a temperature of little over 30° C., and in an atmosphere of hydrogen, he obtained a very luxuriant growth of anærobic organisms ; amongst these he observed that the drum-stick-shaped organisms developed their spores at a much earlier period than any of the others that were growing in his cultures. As soon as these spores made their appearance he raised the temperature to 80° C., with the result that all those bacteria, in which spores were not already developed, were very rapidly destroyed ; the tetanus *bacilli* were also destroyed (that is, the vegetative forms were destroyed), but the *spores* still retained their vitality, and on being transferred to suitable nutrient media, and placed under other suitable conditions, they " hatched " out into the vegetative form, and a pure cultivation of the tetanus bacillus was obtained. It is a rather curious fact that here, as in the case of diphtheria, the organism seems to be localized at the actual point of inoculation, for although, as we have seen, the bacilli, however numerous in the pus and in the walls of the abscess, and in the infiltrated tissues immediately around the abscess,

are confined to a well-defined and localized area, and the most careful researches, conducted with the help of both cultivation and histological methods, have hitherto failed to enable any observer to demonstrate the presence of these organisms in the internal organs. It would appear, then, that as in the case of septicæmia and diphtheria, the poison is manufactured by the organisms at the site at which they are actually introduced, and that from this point it is absorbed into the body, and is carried to the special tissues on which it acts. Brieger, indeed, was able to separate from the limb of a patient who had died from tetanus an exceedingly virulent basic poison or ptomaine which he speaks of as tetanin, whilst he also found a very poisonous proteid substance, tetano-toxin, which he called a toxalbumen, a substance of which we shall have to speak in a later chapter.

At first sight it appears somewhat extraordinary that the development of the tetanus bacillus should be so extremely localized, and that the localization should be confined to a position so near the surface—*i.e.*, to the surface of a comparatively open wound, to which the oxygen of the atmosphere might at first sight appear to have easy enough access.

When, however, we come to think more carefully of the conditions that prevail in the wound, this does not seem quite so extraordinary. In the first place, there is, covering it, a layer of pus which, as is well known, has little power of holding oxygen in solution, any small quantity that is there being gradually taken up by the leucocytes that escape to the free surface or into the abscess, and utilized by them for their own purposes. Beneath this, especially in the later stages of infiltration, and before the capillary vessels of the granulation tissue have begun to form, or are fully formed, the supply of oxygen must necessarily be comparatively small, and any that is present is promptly taken up by the active wandering and proliferating connective tissue cells. Here then we have the conditions under which the tetanus bacillus is enabled to flourish ; there is a condition of anærobiosis, or oxygen famine. Beyond this, however, where the infiltration is not so great, and where the vessels are considerably dilated, the red blood corpuscles are constantly bringing up their fresh supplies of oxygen to the tissues, and in consequence the fluids at some little distance from the wound contain more oxygen than there is near the surface of the wound, and the conditions

are consequently much less favourable for the continued existence of the tetanus bacillus.

The spores of the tetanus bacillus seem to have a remarkably wide distribution. Originally they were only cultivated from garden soil, but successful inoculations have since been made with such material as the sweepings of a hay-loft, and the dust that had accumulated on the furniture of horses. The specific bacillus has been found on the grime on a man's hand, and on imperfectly cleansed surgical instruments. Tetanus is said to be specially associated with the horse, but the more recent observers insist that this is simply because the horse is susceptible to the action of the bacillus and its poison, and because the germs have such a widespread—in fact, an almost universal—distribution. How universal this distribution is may be gathered from the fact that M. Bossano, who was able to obtain the soil from forty-three different regions in various parts of the globe, got positive results with twenty-seven of them. With the soil from these forty-three places he inoculated a number of animals, introducing a small portion, about the size of a pea, into a little subcutaneous pocket of a white mouse or a guinea-pig, and with the soil obtained from twenty-seven of these places tetanus was produced in from two to four days. He says of the soil obtained from England, that from Bath produced tetanus in two out of three white mice inoculated, both of them dying in about two days. Soil from Portsmouth did not contain tetanus bacilli, whilst that from Plymouth and from Manchester caused the death of some of the animals that were inoculated with it, all the characteristic symptoms of tetanus being developed. Speaking colloquially, a worker at the Brown Institute told a friend that they grew the tetanus bacillus in the garden there. From his experiments Bossano concluded that soils which contain much organic matter, almost invariably contain tetanus bacilli, and that latitude, climate, and special meteorological conditions, have far less influence on its development than defective drainage, imperfect hygienic conditions, and the degree of cultivation of the soil. It appears, however, that our methods of cultivation are not yet perfect, for it is an undoubted fact that failures to produce tetanus with pure cultivations are of very common occurrence, even in the hands of those best fitted to carry on experiments of this kind. So markedly is this the case that

Chantemesse and Widal at one time thought that the presence of other organisms was necessary in order that the tetanus bacillus might act, and it has been suggested that ærobic organisms which have great avidity for free oxygen must also be present in order to allow of the development of the full virulence of the anærobic tetanus bacillus. It is a fact, whatever may be the explanation, that the tetanic organism soon loses its virulence under cultivation, especially when it is grown "pure."

Recently, however, Vaillard and Vincent, as the result of a series of most careful observations, have arrived at the conclusion that it is usually the tetanus poison — which they compare to snake poison—and not the organism itself that gives rise to the tetanic symptoms in animals that are infected experimentally. They also find that until the organism has grown in artificial culture media for some time it has not the power of setting up disease, a fact that was accounted for when they found that no poison was developed until some time after the organism had begun to grow, the production of the poison appearing to go on simultaneously with the formation of a peptonizing enzyme. Even spores, when injected alone, could not set up tetanic symptoms, but when these were injected along with other organisms such as lactic acid bacillus, or even with lactic acid itself, with bacillus prodigiosus, or into a bruised wound, or where they were injected along with a quantity of their own poison, tetanus was invariably set up. The tetanus organisms form their poison slowly, and in healthy tissues they are rapidly destroyed by the tissue cells long before they have time to form sufficient poison to produce the nervous symptoms of tetanus; whilst in the cases above mentioned the tissue cells are so engaged in removing the other foreign matter, or are so paralyzed by the action of the lactic acid, or the small portions of tetanus poison, that they are not able to contend on equal terms with the tetanus bacilli, which being under favourable conditions grow rapidly, give rise to the formation of the special poison, and the patient succumbs. This remarkable poison in doses of 25 millegrammes is quite sufficient to kill a rabbit, or the 25th of a millegramme to kill a mouse.

The tetanus bacillus is a facultative saprophyte, the nature of the wounds through which tetanus is inoculated bearing

out in a most remarkable fashion the experimental proofs that have been already adduced. A horse which, in the stable and in the field, always collects a certain quantity of earth on his skin and in his hoofs, may be easily inoculated ; he, in turn, may readily inoculate a man or another animal by a kick with the sharp iron of his dirty shoe. Gardeners, agricultural labourers, and all who work with horses or in the soil, bear on their hands a virus which only needs a bruise or a cut, but especially the former, to allow of its setting up the characteristic symptoms of tetanus. Soldiers, during a campaign, when their garments and equipments are soiled from contact with the ground during their camping and bivouacking are always more liable to the disease than when they are hurt accidentally during times of peace. It is also pointed out that in warm countries where people are in the habit of sleeping out in the open air and on the ground, tetanus is very frequently met with as the result of comparatively slight wounds, whilst amongst children during the years they crawl on the ground, or play in gardens or in fields, tetanus is always more common than in later life, when the parts that come in contact with the ground are usually protected by shoes and gaiters. Many of these facts are well known to savage tribes, whose powers of observation and opportunities for experimentation are of a very high and extended order, and we find that Dr. Ledantec, in an interesting account of the poisonous arrows used by the inhabitants of Santa Cruz, of the Solomon Isles, and of the New Hebrides, speaking of the deaths from this cause of Bishop Patteson and Commodore Goodenough, with their companions, refers to the fact that they were all attacked by tetanus, or lockjaw. He gives a short description of these poisoned arrows. They are about three feet in length ; the shaft is made of a reed, then comes a middle portion composed of hard wood, and lastly a point which is usually composed of a fragment of human bone, which is carefully sharpened to a very fine point, and is so fixed that it readily snaps off on the slightest shock. With a sticky substance obtained from an incision made in the bark of a tree, the point composed of the fragment of bone is smeared. This fluid, on exposure to the air, becomes thicker, and of a more viscid consistence. Thread is then wound in a spiral direction round and round the sticky point. A quantity of soil from the edge of a mangrove

swamp is taken in a cocoanut shell, or some similar vessel, and into this the arrow-head is plunged. It is then carefully dried in the sun, after which the thread is removed, when a roughened point covered with a film of dry mud and dust is left. In this mud there are probably both septic vibrios and tetanus bacilli, the former, however, are rapidly killed by exposure to the sun, whilst the tetanus bacillus of Nicolaier, which, as we have seen, developes a well-formed spore at one extremity, may remain active for months and even years, although, as the savages well know, the poison gradually becomes more and more attenuated, until old arrows are known to become entirely inoffensive, except as mere mechanical weapons of warfare or hunting.

Stanley, speaking of the hunting appliances and offensive weapons of the pigmies whom he encountered during his African wanderings, makes a similar interesting observation, to the effect that these small specimens of humanity use arrows so poisoned that the slightest scratch with one of them produced one of two things, tetanus (or convulsions), which is evidently due to the action of a poison similar to that above described, or death, accompanied by peculiar gangrenous sloughs at the seat of the wound, which is very probably associated, so far as we can see, with malignant œdema or "black quarter," a condition that was also described by Nicolaier as resulting from the inoculation of soil collected from gardens and forests. These pigmies are described as dwellers in the dense forests of that part of Central Africa through which Stanley travelled.

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CHAPTER XVII.

Diphtheria.

Diphtheria an Infective Disease—The Organism found in the False Membrane in its Deeper Parts—Method of Staining the Bacillus—Characters of the Bacillus—Involution Forms—Cultivation Methods—Appearance of Colonies—Nutrient Media—Results of Inoculation Experiments—Klein's Bacilli differ somewhat from Löffler's—Streptococci found in Diphtheria Poison—Extreme Virulence—Resemblance to snake-bite poison—Toxicity—Predisposing Conditions—Conditions fatal to the Bacillus—Roux and Yersin's Observations—Fraenkel's Observations—Attenuated Diphtheria Virus—Increase of Virulence.

Although it has long been known that diphtheria was an extremely infectious disease, it is only within comparatively recent years that any reliable information as to the nature of the specific infective poison has been forthcoming. Even when the organic nature of other specific infective poisons had been practically proved many difficulties still remained to be overcome ; in most other cases where the disease could be proved to be the result of the vital activity of a micro-organism, such organism could usually be found in a pure condition, or greatly predominating over all others, in the blood, in the internal organs, or in some special fluid in the body. This was found not to be the case in diphtheria. Most careful and elaborate researches were entered upon, but it was found impossible to demonstrate any special organism as occurring in the blood, in the lymph, or in any of the organs of the body. The only position in which any could be found was in the false membranes (composed of fibrinous lymph and altered epithelial cells) that are found in the throat, and it was at once surmised—a surmise that was afterwards found to be correct—that the poison which gives rise to the constitutional symptoms must be formed at the point at which the micro-organisms are found, and that, being of an exceedingly diffusible nature, it is thence absorbed and carried to various

parts of the body, giving rise to heart failure, or to certain forms of paralysis, partly through its action on the nervous system and partly owing to its interference with the nutrition of the various tissues of the body. Here again, however, those who were studying the subject were confronted with another difficulty ; there were so many forms of micro-organisms that found in this false membrane a suitable subsoil on which to grow, it was almost impossible with the methods then at command to separate them and so to obtain pure cultures, in order that the specific and biological characters of each might be investigated.

In 1875 Klebs found in the false membranes a small bacillus with rounded ends, and with, here and there, small clear spaces in its substance, a bacillus that was not readily stained, that grew luxuriantly in broth, and which, inoculated into animals, gave rise to a peculiar dirty fibrinous-looking slough at the seat of inoculation. He found, however, that in certain cases this bacillus was absent, the predominating organism then seeming to be a micrococcus arranged in masses or in short chains. This, when cultivated in broth, gave rise to the formation of chains of considerable length. As a result of these observations he described diphtheria as occurring in two forms, one form resulting from the action of one organism, the second being caused by the other.

These researches were continued by other workers, and Formad, in America, came to the conclusion that the rod-shaped bacillus had little to do with the disease, but that the streptococcus, or chain-forming micrococcus, was the real exciting cause. Matters remained at this stage for some time—in fact, until Löffler took up the subject. After examining a number of cases of diphtheria, he found that, although there are numerous organisms in the false membranes or diphtheritic patches, these were mostly near the surface, and many of them were simply the organisms that were usually found in the mouth now growing under more favourable conditions of nutrition. He found, however, that in the deeper layers, or at the inner margin of the layer of exudation, the Klebs bacillus might almost invariably be found. It was more deeply situated than any of the others, and was always most numerous in the oldest part of the membrane. This was in cases of pure diphtheria. In the so-called diphtheritic sore throats met with in other diseases,

especially in scarlet fever, the streptococcus appeared to be the predominant and characteristic organism.

These rods described by Klebs and Löffler vary much in length, but they average from 3 to 6μ; they are straight or slightly bent, one end or both sometimes being a little swollen. There may be deeply stained or bright glistening points in the protoplasm, though these are not usually met with. Babes indeed describes spores as occurring in the diphtheria bacillus, but, as is mentioned later, these are not real endospores but consist merely of altered protoplasm.

In order to stain the bacillus it is only necessary to remove a small fragment of the false membrane by means of a piece of absorbent cotton wool tied firmly to a pair of forceps or to a pen holder; from this it is transferred to a scrap of blotting-paper, and thence to a cover glass, where it is broken down as finely as possible, heated over a flame in the ordinary fashion, and stained with Löffler's alkaline methylene blue, or by Gram's gentian violet method (washing thoroughly with water before attempting to examine), or by a method adopted by Roux and Yersin, who use a blue, composed of equal parts of aqueous solution of violet dahlia and methyl green, with water added until a clear, but not too deep, blue is obtained. A drop of this is placed on the slide, the cover glass on which the fragments are dried is inverted and lowered on to it, the superfluous fluid is removed with a piece of blotting-paper, and the organism is examined at once.

The vital characteristics of the organism may be used in separating it from false membranes, even where contamination from the organisms of the mouth and pharynx has occurred; and it is recommended that in order, wherever there is any doubt, to be absolutely certain of the diagnosis of diphtheria, cultivations on blood serum should be made.

The specific diphtheria bacilli appear to be stained more readily and more deeply than any of the organisms that usually accompany them. They occur in small groups, as short, straight, or curved rods, with ends sometimes pointed, sometimes curved; they are never absent from cases of true diphtheria in the early stages, and in some cases the membrane consists of an almost pure cultivation of the bacillus. In older cases, however, the organisms do not stain so equally; many pear-shaped and club-shaped bacilli are present, and, in some very old membranes, it is difficult or impossible to distinguish any characteristic bacilli, the accompanying organisms becoming more numerous, especially as the surface becomes fœtid and softened. In such cases the specific organisms can only be found entangled in the deeper fibrinous net-work.

Roux and Yersin hold that microscopic examination gives the most precise

information, even in the case of dried false membranes sent from a distance wrapped in linen or blotting-paper. They also hold that where improvement is taking place, the specific bacilli become less numerous, the other microbes increasing in number, and that this may be followed day by day with a microscope, the course and prognosis of the disease being indicated by the changes that are met with. They also hold that at the beginning of a case of diphtheria it is possible to predict a favourable issue from the presence of a small number only of the specific bacilli, and a large number of other forms, and they also believe that some of the micro-organisms met with under these conditions interfere with the growth and activity of the specific bacillus.

The Klebs-Löffler bacillus cannot be cultivated outside the body on peptone meat gelatine, as it will not grow sufficiently rapidly at the room temperature to outpace the putrefactive organisms which accompany it ; it was, therefore, found impossible to make plate cultivations in order that the different forms might be separated.

By mixing the membrane with boiled distilled water and allowing drops of the mixture to trickle over the surface of solidified blood serum, Löffler succeeded in obtaining pure cultivations. Wyssokowicz, using agar instead of gelatine for making plate cultivations, and incubating at 35° C., was able, on a small percentage of his plates, to obtain pure cultivations of the characteristic diphtheria bacillus ; most of his plates, however, were overgrown with putrefactive and other non-pathogenic organisms.

To obtain a somewhat purer inoculating material, put a scrap of membrane between blotting-paper, or on a cover glass in a test tube, allow it to dry, and then heat to a temperature of 98° C. for a whole hour—a temperature which few ordinary organisms can withstand for even a less time than this—so that, when the dried material from a cover glass so treated is inoculated on solidified blood serum, a very pure cultivation is usually obtained.

Roux and Yersin adopted a simpler method. They used a platinum needle beaten out at the end to form a kind of spatula ; on this they took a particle of the false membrane from a case of diphtheria and then made stroke cultivations on the surface of the solidified blood serum, using the same needle without re-charging for some half-dozen tubes. When these are incubated at from 33° to 35° C. the bacilli rapidly make their appearance ; they are visible at the end of twenty hours and have a characteristic appearance in forty-eight hours. This rapid growth is very characteristic of the diphtheria

bacillus, which appears to be the only organism of all those found in the membrane that can form colonies visible to the naked eye in twenty hours. Such colonies grow as small rounded greyish white points, the centre of each of which is more opaque than the periphery; they spread rapidly, form greyish rounded discs, and continue to develop so quickly that they are very evident before the other organisms have begun to form a colony at all visible to the naked eye. From these points, inoculations on to blood serum may be made.

Probably the best nutrient material for the diphtheria organism is that recommended by Löffler; it is composed of three parts of blood serum, one part of neutralized broth, to which has been added 1 per cent. of peptone, .5 per cent. of common salt, and 1 per cent. of grape sugar. Ordinary blood serum comes next, and then agar-agar jelly. On agar plates the colonies situated in the substance are coarsely granular, dark brown, and somewhat rounded or oval; although where several colonies have run together they may give rise to somewhat irregular outlines. The superficial colonies are much lighter in colour, are not so dense, and have an irregular scalloped border.

These cultivations are found to be made up of bacilli similar to those described by Klebs; they are not quite so long as the tubercle bacillus, but are rather thicker, the extremities, which are more deeply stained than the central portion, are often slightly enlarged. In older cultivations the rods are not uniformly coloured, and there may be seen in the substance of the protoplasm bodies which somewhat resemble spores. It was soon noticed that with this inequality of staining, certain other changes in the organism might be met with; so-called involution forms make their appearance. In these the bacilli are cut up into small rounded masses of protoplasm, some of which have a less diameter than that of the bacillus, whilst others may be considerably larger and are oval in shape. These involution or degenerate forms soon make their appearance where the conditions for the growth and development of the bacillus are unsatisfactory, and it is supposed that many of the failures to find the typical rod-shaped bacilli in old diphtheritic membranes are due, in some measure, at any rate, to the occurrence of these involution forms in the later stages of the disease, where the membrane is invaded by putrefactive and other organisms which interfere with the growth of the specific bacillus. It is also

found that the diphtheria bacillus has a tendency to lose its virulence on cultivation.

It was at first concluded that the bright, strongly refractile particles and deeply stained granules were more or less perfectly developed endospores, and that some of the involution forms might possibly be arthrospores. Although, however, the organisms remain alive and potentially active after being subjected to drying for a considerable time, a moist temperature of 58° C. is quite sufficient to kill them off, which would scarcely be the case were these bodies true spores.

The bacillus, as we have seen, grows freely on meat fluids that have an alkaline reaction, but as it grows it first renders these media slightly acid, but later, if there is free access of air, the fluid again becomes alkaline. The acid reaction is always most marked in those cases in which there is glycerine in the cultivation medium. The organism grows "in vacuo," but more slowly than in air; the exclusion of air, however, interferes with the formation of acid, so that under these conditions the bacillus may retain its vitality for a period of six months, or even longer. Examined in a fluid medium it is found to be quite motionless.

Although Löffler was able to produce some of the symptoms of diphtheria by the inoculation of this bacillus on an excoriated mucous membrane, he was not satisfied that this was the real *causa causans* of the disease, and it was left for Roux and Yersin to demonstrate the intimate causal relation that actually exists between this organism and true diphtheria. They repeated Löffler's experiments of inoculating the bacilli on the damaged mucous membrane of rabbits, guinea-pigs, and pigeons, and in all cases they found that characteristic diphtheritic patches were produced. Injected under the skin, the bacillus causes swelling at the point of inoculation, and the animal dies with symptoms of acute poisoning. In some cases there is found congestion and effusion into the serous cavities; in others there is evidence of fatty degeneration of the liver, similar to, but more acute than that met with in cases of diphtheria in the human subject. A very important point determined by them was, that if death did not take place too rapidly, characteristic diphtheritic paralysis usually supervened. The bacillus in these cases was found only at the point of inoculation, and even in the most congested organs it could not be demonstrated either in the blood channels or in the lymph spaces, whilst it often disappeared

even from the seat of inoculation during the latter stage of the disease ;—another fact that helps to explain Löffler's inability to find the organism in certain of his cases.

From all this it is concluded that the local symptoms of diphtheria are due to the action of a specific bacillus on a weakened mucous membrane or on a wounded surface ; that once having gained a footing it gives rise to an acute inflammatory process, probably by the direct action of the poisonous material that it forms on the cells and on the blood vessels in the immediate neighbourhood ; this caustic action is so intense that the epithelial cells undergo degeneration,—the fibrinous lymph and leucocytes which are exuded also become more or less rapidly degenerated—and give rise to the grey false membranous patches that are so characteristic of true diphtheria. When the growth of the organism is rapid, and where the area of surface attacked is extensive, the amount of poison developed may be very great indeed, and where this latter is greater than can be dealt with in the inflammatory area, owing to the rapidity with which it is produced by a large number of organisms, especially when they are situated deep down in the tissues, there is rapid absorption of the poison, but not of the bacilli, into the system, and the characteristic constitutional symptoms of the disease are set up. We must thus distinguish carefully between the local action of the bacillus and its products, and the toxic constitutional effects of these products.

Additional proof that these products are the active agents in the causation of the disease was found in the fact that from pure cultures of the diphtheria bacillus there may be separated, by means of Chamberland's porcelain filter, a special chemical substance, or series of substances, which, after being proved quite free from bacilli and injected under the skin of animals, gives rise to all the constitutional symptoms and lesions of the disease that follow the inoculation of the bacillus itself, the only feature wanting being the false membrane, which usually does not make its appearance. Animals into which small doses are injected are frequently attacked by diphtheritic paralysis.

Of course it has been objected that the diphtheria produced in animals is not necessarily the same thing, nor is it necessarily due to the same organism, as the diphtheria of

man. Löffler himself has examined the diphtheria of a calf, and he acknowledges that it is not even related to the diphtheria of the human being.

Klein considers that cats are especially susceptible to diphtheria, and that they may act as the intermediate hosts for the development of the diphtheria bacillus between two human patients, whilst he also holds that cows may be inoculated, especially on the udders, with diphtheria bacillus, the disease manifesting itself in the form of small pustules. He considers that milk from such cows may readily become the agent by means of which the disease is spread. Eminent veterinarians, however, are strongly opposed to this view, and it can yet scarcely be maintained that Klein has fully proved his point, although he seems to have obtained strong evidence in support of his position. As he is now working most carefully at the subject, however, it may be well to suspend judgment until he publishes a full report of his observations and experiments.

Klein's bacilli, however, undoubtedly differ in certain essential points from the bacillus described by Löffler ; most important of all in the fact that whilst Löffler never succeeded in obtaining growths of his bacillus at the ordinary temperature of the room, and was not able to grow it in gelatine, those that Klein describes as occurring in the pustules on the ulcerated udder of the cow grow luxuriantly in gelatine at the ordinary temperature of the room.

Klein describes a second bacillus as growing slowly upon gelatine, but this he does not consider to be so important as the more rapidly growing one.

Young rabbits and guinea-pigs are the animals with which most experiments have been made. It has been found that rats and mice enjoy almost perfect immunity from the disease, a fact which has recently been utilized by Behring in connection with the production of an immunity against diphtheria in rabbits and guinea-pigs.

We shall have to wait for further light on these points, but from what we have already seen, young rabbits, guinea-pigs, and young dogs, may undoubtedly contract diphtheria, or something very similar to that disease ; both local and toxic symptoms resulting from the introduction of the poisonous material. It must be borne in mind that there are usually several kinds of bacteria present, that the compli-

cations of the disease are numerous, and that it undoubtedly occurs as a complication in other diseases. But when all this is taken into consideration the fact must still be accepted that careful clinical observation and experimental investigation have been made by many thorough workers, and that these workers have assigned to a special bacillus the power of giving rise to at least one form of diphtheria. It is possible that the streptococcus may play an important part in certain cases of diphtheria, but this has not yet been actually proved ; whilst the evidence in favour of the specific infective Klebs-Löffler bacillus is now almost overwhelming.

As regards the streptococci that are found in this disease, it appears that these organisms so fully described by Klebs, Formad, and Prudden and Northrup, and later by Löffler and Babes, when inoculated give rise to local inflammation, and even to inflammation of joints, &c. ; in no case, however, do they appear to give rise to the formation of a false or a fibrinous membrane. No doubt they play a part in the preparation of the tissues for the diphtheria by weakening them so as to enable them to offer less resistance to the action of the specific organism. These streptococci may actually make their way into distant organs of the body, and it has been found possible to make pure cultivations of them from such organs. These, then, differ very markedly, as regards their distribution in the body, from the diphtheria bacillus, which grows only at the point of inoculation, manufactures all the poison in that position, the poison only being carried into the body ; the bacilli remaining *in situ*, multiply during the advance of the disease, and degenerate as soon as the tissues begin to obtain the upper hand, which they do in all cases where recovery occurs, and even in some cases where the patients afterwards die from the effects of the absorption of the specific poison.

The diphtheria bacillus, having been obtained pure, was naturally seized upon by Löffler, then by Roux and Yersin, and, lastly, by Brieger and Fraenkel for their experiments on toxalbumens ; the way having been paved somewhat by Hankin's discovery of albumoses in certain anthrax cultivations.

The diphtheritic poison is always most active when alkaline ; during the acid period already mentioned, the toxic power is very considerably diminished. Moreover, if an acid be added to a virulent alkaline filtered liquid, its poisonous activity is immediately diminished ; but, curiously enough, this property is immediately regained when the fluid is again neutralized by the addition of a fixed alkali. So virulent is the alkaline liquid that one-fifth of a cc. of the filtered fluid, from a cultivation of the diphtheria bacillus

that has been allowed to grow for forty-two days, proves fatal to a guinea-pig in about thirty hours. Larger doses, from 4 to 20 cc. injected into dogs of various sizes kills them in from fourteen to twenty-six hours. In doses of 2 cc. it proves fatal in four to six days. In all cases the symptoms are those of more or less acute poisoning, resembling septic poisoning in some respects and phosphorus or metallic poisoning in others. A dose of less than 1 cc. of the filtered liquid injected into a dog of middle size causes a temporary paralysis, very similar to the post-diphtheritic paralysis of the human subject. It is a curious feature that when paralysis occurs in a rabbit, death invariably ensues ; but in both the pigeon and the dog, especially in the latter, recovery may frequently follow this condition just as in the case of the human being. Sheep are susceptible to the action of diphtheritic poison, but rats and mice are unaffected by it. The effects on other animals have been already mentioned.

It is always a difficult matter to determine what is the nature of an organic poison. In the first place it is produced in such small quantities that it is difficult to obtain sufficient to determine, even by chemical analysis, its exact nature. Further, it is so unstable that it may become completely altered by the various reagents that have to be used in separating it out from the mixture in which it occurs. A rise of temperature beyond a certain point is fatal to its activity, and it undergoes various oxidations under the least provocation. Roux and Yersin consider that this special poison has certain features in which it resembles the diastases. Thus, when heated in sealed tubes over a water bath to 58° C. for a couple of hours, the toxic activity is diminished at least seven-eighths of its original power ; whilst very large doses of the filtered poison that has been heated to 100° C. may be introduced into the veins of a rabbit, or under the skin, without producing any immediate effect, although symptoms are produced later which can only be due to the action of this material as a modified diphtheritic poison. Like diastase, the diphtheritic poison is rapidly modified by sunlight in presence of air, but if air be excluded the diminution in toxic activity brought about by exposure to sunlight is comparatively slight.

On evaporating a filtered diphtheritic bacillus culture *in*

vacuo over sulphuric acid at a temperature of 25° C., a substance is left which is soluble in water, and possesses marked toxic properties. It is insoluble in strong alcohol, and may be precipitated by it from a watery solution in greyish white flakes. It passes slowly through a dialyzing membrane. The addition of lime water and a solution of phosphoric acid to the filtered culture liquid causes an entangling precipitation of the poison, phosphate of lime appearing to hold it more tenaciously than any other substance. The filtered fluid thus treated loses its toxicity, whilst the gelatinous precipitate inoculated into an animal kills with the utmost certainty, though perhaps on account of the slightly insoluble nature of the substance formed, somewhat more slowly. To form some idea of the virulence of the poison produced by these diphtheritic organisms, Roux and Yersin's calculations that 1 cc. of the active liquid evaporated *in vacuo* leaves 1 centigramme of dried residue, may be accepted. Deducting from this the weight of the ash and the portion soluble in alcohol which has no toxic action, there remain four-tenths of a millegramme of organic material, of which only a small proportion can be diphtheritic poison; even this quantity, however, is sufficient to kill eight guinea-pigs, two rabbits, or one medium-sized dog. If the latter does not succumb to the poison it remains ill for some time. Like snake-bite poison, however (which Waddell has shown to be weakened on being exposed to peptic digestion, a change that is ascribed to the breaking down of the albumose), it may be taken into the stomach in much larger quantities without giving rise to any very serious effect. This poison, when injected into the veins or into the subcutaneous tissue, appears to act specially on the walls of the blood vessels, giving rise to vascular dilatations, minute hæmorrhages, and the small œdematous patches so characteristic of certain forms of the disease. It can scarcely be too strongly insisted on that the activity of a poison formed by a micro-organism is not at all the same thing as virulence, which must be defined, according to Roux and Yersin, as the power that a pathogenic organism possesses of continuing to live and carry on its functions in the tissues of the animal or human host. For example, one might take a young culture of the diphtheria bacillus in which the bacilli are vigorous, but in which the quantity of poison

developed is as yet small; inoculating this into an animal it would be found that death would take place at the end of a certain period, and that, on examination at the point of inoculation, numerous bacilli, evidently the result of vegetative growth of the micro-organism within the tissues at this point, might be demonstrated; whilst on the other hand, if an old cultivation (in which the organisms are weak, and in which many involution forms are present, but in which there is a large quantity of poison which has been developed by the micro-organisms during their period of activity, and which from standing has become diffused into the liquid) be inoculated, death will be produced very rapidly, but no organisms can be found at the seat of inoculation; there is set up, in fact, a true toxic condition. If the residues left on the filter from the above cultures were inoculated, it would be found that in the case of the young culture, death of the animal would take place at very much the same period as when the unfiltered culture was inoculated; whilst in the case of the old culture the period at which death takes place is very much delayed; in the one case, the organisms are active, although deprived of the poison which they form; they can live in the tissues, and can produce fresh poison; whilst the older organisms and involution forms, no longer able to develop in the tissues, and deprived by the filtration of the poison they produced whilst they were active, sometimes do not cause the animal to succumb even at a late date.

This virulence, then, is associated with the power of a microbe to develop in the body of a living animal, a power which may be considerably increased by passage of the organism through a series of suceptible animals, the bacillus acquiring a more and more parasitic habit in each successive host. Researches on micro-organisms are of no value to medicine unless they throw light directly or indirectly on the cause of disease, and so enable the physician to combat its advance. At first sight it would seem that in the case of the diphtheria bacillus there is, on account of the extreme activity of the poison, little hope of rendering the tissues of an animal resistant to its action, as even very minute doses produce marked poisonous effects. On the other hand, however, we have, from the nature and position of the development of the poison, indications as to treatment and also as to prevention of the disease.

The indications as to prevention are of course similar to those for other micro-organismal diseases; if we know the natural history of the diphtheria bacillus, we know at what point it is most vulnerable, the conditions favourable for its

development can be removed, and unfavourable conditions substituted ; whilst, as regards treatment, it is evident that, because of the energetic toxic action of the material formed by the organisms, diphtheria should be attacked as early as possible. If sufficient time be allowed to the bacillus to form a large dose of the poison, it is useless to remove the false membranes, as, though the bacilli may be then destroyed, sufficient poison may have passed into the system to cause the death of the patient, "for in diphtheria, contrary to what occurs in most other infective maladies, the infection is not produced by the invasion of the tissues by a microbe, but by the diffusion through the organism of a toxic substance prepared on the surface of a mucous membrane altogether outside the body, so to speak."

The bearing of recent researches on the prevention of the spread of an outbreak of diphtheria can only be fully understood when some of the facts that they brought to light are enumerated. It was found, for instance, that the presence of the diphtheria bacillus in the mouth is not necessarily followed at once by the appearance of the diphtheritic membrane, and it appears that these bacilli can exert little or no injurious effect where the mucous lining of the throat, larynx, &c., remains sound and unaffected by minor diseases. When once, however, we have such conditions as inflamed tonsils or inflammation and ulceration of the mucous membrane, the diphtheria bacilli find a soil ready prepared for their reception, and typical diphtheritic symptoms are the result. That such ulcerated sore throats, inflammation of the tonsils, and similar conditions usually precede outbreaks of diphtheria, has for long been a well recognized clinical fact ; these experiments give the explanation of it, whilst they also afford indications as to the mode of treatment. Antiseptic throat washes, not merely gargles, plenty of fresh air, and good nourishing food, are what are required. Kill the germs as far as possible by means of the antiseptics, and strengthen the tissue cells by plenty of oxygen, and by promoting the excretion of effete products, by food and exercise, so that the cells shall be able to form their protective products and shall also be able to play their part as phagocytes when called upon to do so. Another important point to be borne in mind is that the disappearance of the bacilli from the mouth is not simultaneous with the removal of the false membrane,

and Roux and Yersin have found that the specific bacterium may persist in the mouth for several days (in one case fourteen days) after all traces of the membrane have disappeared, and they give the good practical advice that diphtheritic patients who are becoming convalescent should not be allowed to associate with their school-fellows, play-mates or families, for at least a fortnight after the membrane has disappeared ; and that it is quite as important to wash out the throat freely three or four times a day with disinfecting lotions as that the clothes and bed linen should be thoroughly disinfected. As regards the tenacity of life exhibited by these bacilli, it is found that at the ordinary temperature of the room these organisms retain their vitality for a period of at least six months, and probably considerably longer. As the temperature rises this period is gradually diminished, for we find that at 33° C. the organisms succumb in about five months ; whilst at 39° C. they are are found to be no longer capable of living and of multiplying when introduced into solidified blood serum or glycerine-agar. When deprived of air and protected from the light, even when kept at the ordinary temperature, they may continue capable of germinating, when introduced on to a suitable soil, for a period of thirteen months. If they are dried and are kept at the temperature of the room, they are killed in four months, at a temperature of 33° C. they are killed in three months, and at a temperature of 45° C. in four days. If a fragment of the false membrane containing bacilli be removed, wrapped in sterilized paper, or linen, and be carefully protected from the action of light, cultivations may be made from it at any time during a period of five months. If, however, instead of keeping it dry and in the dark, fragments of these membranes are exposed to the light and moistened and desiccated alternately, the virus is destroyed much more rapidly. From all this, and from the fact that the bacillus is destroyed by moist heat at 58° C., it is evident that by far the best method of disinfecting clothes, the floor, the walls, and furniture, is by the use of a liberal supply of boiling water ; for although a temperature of 98° C. (dry), continued for an hour is necessary to destroy the vitality of the bacillus, moist heat at a very much lower degree (acting only for a minute or two, according to the temperature) is sufficient to dis-

infect everything on which it is allowed to act. In the case of the cholera bacillus we have already pointed out that under certain conditions it is capable of producing a much more violent form of disease than in others.

Roux and Yersin have been able to demonstrate that the virulence of the bacillus of diphtheria undergoes marked modifications even during the progress of an attack of diphtheria in the same individual, and they believe that the condition of the patient is modified not only by the alterations in the number of the bacilli, but also by their virulence at different stages. They find, for example, that in the early stages of the disease (except in very rare cases) the number of bacilli is comparatively small, but that as the disease advances the number of virulent bacilli present also increases rapidly, this being tested both by microscopic examination and by cultivation and inoculation experiments; whilst, on the other hand, as the case approaches cure the bacilli that can be isolated are not only fewer in number, but those that are cultivated are not nearly so active, for when inoculated into animals they produce neither such marked constitutional symptoms nor such severe local reactions. It is indeed believed that the virulent and non-virulent bacilli represent a difference in degree of virulence only, and not a morphological or specific difference, that the difference is one of degree and not one of kind, and that the pseudo-diphtheritic bacillus described by Löffler and Hoffman is really only the organism undergoing a kind of saprophytic phase which is interpolated in the life of the parasitic bacillus.

Roux and Yersin obtained an imperfectly attenuated virus first by keeping the dried membrane for a considerable time, when they found that cultivations from them of bacilli, in which were present all the typical appearances and morphological characteristics, when inoculated into animals had lost their virulence. They found that they were able to diminish the virulence of the diphtheritic organisms growing in broth by passing currents of air at 39.5° C. through this medium, and that if the process was continued too long the bacilli were completely deprived of life. It would appear that this was simply an interference with the vitality of the organism which was deprived of one function after another until it was killed altogether. They found it impossible, however, by these methods to graduate the attenuation, and although they proved that the virulent bacillus alone could elaborate the toxic material, and then only under favourable conditions, the virulence became modified as the conditions were altered, the activity of the toxine being also modified; these conditions could not be controlled except in a very rough and inadequate fashion.

Recently Fraenkel has found that by heating cultures of the diphtheria bacillus to a temperature of 65° or 70° C. and injecting from 10 to 20 cc. of such cultures into guinea-pigs, after an interval of fourteen days subcutaneous injections of even the most virulent diphtheria poison had no effect. If however, the animals were again injected within the prescribed period of fourteen days they succumbed to the

diphtheria poison. On the other hand, the diphtheritic virus applied to an abraded mucous membrane even after fourteen days was capable of producing typical local symptoms. These points, if confirmed, indicate that the immunity against diphtheria may not be so easily acquired as in some other diseases, or, if acquired, it must be through the application of methods different from those hitherto described.

Another feature that is brought into special prominence by the recent researches on diphtheria is, that the attenuated diphtheria bacillus requires the fulfilment of certain conditions before it can again acquire the virulent form, one of these being that it shall be allowed to grow on the surface of the fauces, or outside the body altogether, in the presence of certain organisms, such as the streptococci found in erysipelas, or those streptococci that occur in the throat affections of scarlet fever, measles, and similar diseases. It is quite possible that other organisms have the same effect, and that the attenuated diphtheria bacillus growing outside the body may become so virulent that it is capable of producing a very grave form of diphtheria, but from what occurs in outbreaks of diphtheria—in which the first cases, as a rule, are mild, successive cases becoming gradually more and more severe until an extremely fatal form of diphtheria attacks susceptible individuals—it would appear that the growth on mucous surfaces of this bacillus, along with these streptococci, is specially favourable to the development of the virulent form of the organism.

These experiments exemplify in a most remarkable manner the use that bacteriological investigations have been to medicine, and Roux and Yersin sum up the practical outcome of their researches as follows :—"The best method of arresting the spread of diphtheria is to recognize the disease as early as possible; consequently a precise diagnosis should be made by microscopic examination of the false membranes, and this should be confirmed by cultivations on blood serum." As the former takes only a few minutes, and as the latter gives results in twenty-four hours, both these methods are available in private practice or where patients can be sent to an observation ward.

"Active diphtheritic virus can remain in the mouth for a long time after the malady is cured. Consequently diphtheritic patients should only be allowed to resume their ordinary mode of life when they are no longer bearers of the bacillus.

"Diphtheritic virus retains its virulence for a long time when kept in a dried state. It is therefore necessary to disinfect in a steam sterilizing apparatus the linen and all articles that have been in contact with diphtheritic patients.

" The attenuated virus of diphtheria is widely distributed and it readily regains its virulence. It is therefore necessary at the very commencement of simple forms of throat disease, and of those associated with measles and scarlatina, to practice careful and frequent swabbing of the throat with antiseptics."

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CHAPTER XVIII.

Hydrophobia.

Pasteur's Experiments—Attempts to Demonstrate Micro-organisms—Hydrophobia does not arise Spontaneously—Disease not Confined to Man or Canine Animals—Pasteur's Early Experiments with Saliva Unsuccessful—Successful Experiments—Symptoms of the Disease—Position in which Virulent Material is Found—Different Animals Differently Affected—Alteration of Virulence—Method of Preparing Inoculation Material—Description of Experiments—Joseph Meister the First Patient Treated—Method of Treatment now Adopted—Treatment of Wolf Bites—The Time Factor in the Disease—Rationale of Inoculation Method—Filtered Virus Non-Virulent—Method of Inoculation—Description of Departments, Apparatus and Methods of Working in the Pasteur Institute.

By some it may be objected that in a work on micro-organisms the subject of hydrophobia can scarcely be legitimately considered. When it is remembered, however, that the methods adopted and the principles involved in the study of the production of this disease are in many respects the same as those concerned in other diseases in which bacteria undoubtedly play the part of causal agents, and when, too, it is remembered that Pasteur's researches on the production of immunity against the attack of this disease were carried on under the full conviction that in hydrophobia the exciting cause is a poison, the most probable source of which is a vegetable organism, one has, I think, ample excuse for the introduction of this subject. When further we take into consideration the extreme interest of the subject, both from a scientific and from a practical point of view, any remaining objection should at once fall to the ground. To treat the subject in a purely historical or chronological order, it would be necessary, in the first instance, to describe Pasteur's experiments; but in order to clear the ground it may be well to give a short *résumé* of the attempts that have been made to find, cultivate, and inoculate a specific organism that might be causally associated with rabies. Pasteur, Chamberland,

and Roux all made most careful search for organisms in the various tissues of animals affected by hydrophobia, and, although they at first imagined that they had been successful, they ultimately came to the conclusion that the small round micrococcus-like bodies that they found were not associated with the disease or had only an indirect relation to it. In 1884 Gibier was able to demonstrate in the medulla of animals suffering from hydrophobia small rounded refractile bodies, which looked like micrococci aggregated into small masses. He demonstrated their presence by mashing down the medulla in sterilized distilled water, and then adding two or three times the quantity of water, and examining some of the opaque fluid thus obtained, under the microscope. Those organisms which remained in fragments of the cerebral substance were perfectly motionless, whilst those that were free in the fluid exhibited a certain motility. No such bodies could be obtained, by a similar method of treatment, from healthy brains. Although these bodies resembled micro-organisms in many respects, they could be only faintly tinged with the aniline colours, and it was never found possible to make cultivations into any nutrient media with which experiments were made. In 1885 Fol brought before the French Academy of Sciences some observations made on sections of the cord taken from rabid animals prepared by Weigert's hæmatoxyline method.[1]

He found in specimens so prepared, small granules resembling micrococci, not only in the lymph spaces of the neuroglia but even between the axis cylinder and its medullary sheath, the cavities in some cases being of very considerable size, and containing these small granules in large masses. Each granule is perfectly distinct and takes on a deep violet stain, they are about .2μ in diameter, are sometimes arranged as diplococci, but seldom or never in chains of any considerable length. Fol believed that he was able to cultivate these organisms, but only directly from the cord itself, and he describes a slight cloudiness of the medium (broth) as making its appearance after the intro-

[1] Weigert's method. Cords hardened in bichromate of potash and then transferred to sulphate or acetate of copper solution. Sections coloured with solution of 1 part hæmatoxyline crystals, 90 of water, and 10 parts of alcohol, then decolorized by a solution of 2.5 of ferrocyanide of potassium, 2 parts of borax, and 100 parts of water.

duction of small fragments of the spinal cord. This cloud is thrown down at the end of the fourth day, and the precipitate when inoculated into a healthy animal produces a modified form of hydrophobia, modified, however, only in that there is a longer period of incubation than in the ordinary disease. He found on treating a thin film of this fluid, dried on a cover glass, as he had treated the sections of the cord, that small groups of micrococci might be demonstrated, and he concluded that he had cultivated the specific organism.

Cornil and Babes appear to doubt the accuracy of these observations; they have never been able to make out any other granules than those which are invariably met with in sections of healthy nerve tissues that have been stained by the Weigert method. Babes, however, claims that he has been able to demonstrate in the brain and in the cord of rabid animals groups of rounded micro-organisms, with a diameter three to four times as great as that of the organism described by Fol; these are stained *in situ* by Löffler's alkaline methylene blue solution, which gives them a peculiar rose colour.

They may be cultivated on blood serum at a temperature of 37° C., on agar-agar, and upon nutrient gelatine made with an extract of the brain of a rabbit. In cultivations the organism grows slowly, appearing as a faint grey spot at the end of several days. The growths spread best in the deeper part of the gelatine. A pure culture of the second or even of the third generation when inoculated into animals occasionally produces hydrophobia, but in most cases the cultures have no pathogenic properties, and it must therefore be concluded that the microbe has either lost its virulence, or that it is not the actual cause of the disease. From what has been learned of the causation of other infective diseases it is quite conceivable that there exists in addition to this micrococcus some hitherto undemonstrable element, which along with the organism is capable of producing the disease. For example, in preparations of the brain and of the cord, another microbe is also occasionally met with. This second special micrococcus can only be coloured by using Gram's staining method, and by leaving the sections for a considerable length of time in the staining reagent. It affects specially the surface of the brain, and is there found in cells which "frequently also contain fatty and proteid granules."

These latter correspond to some of the cocci described by Gibier.

From Babes' description of the larger organism growing in the deeper parts of the gelatine, and also in vacuo, it is evident that the organism is anærobic; other organisms (micrococci) grow on the surface in the form of grey, yellowish, dry streaks, when they are in the form of large cocci arranged in chains, or as curved spindle-shaped bacilli. In all these forms the micrococci or rounded points within the bacilli are stained pinkish with Löffler's methylene blue; whilst the intermediate substance and the bodies of the curved bacilli take on a blue tinge. Similarly, spindle-shaped curved organisms have been found in the cerebral substance of rabbits and guinea-pigs suffering from rabies; whilst curved thick motile bacilli have been found in the blood of rabbits during the stage of fever that precedes the regular nervous symptoms of rabies; the inoculation of such blood, however, seldom produces rabies; in fact, blood from almost any rabid animal produces few or none of the symptoms of ordinary rabies. Ptomaines have been described as present in the nerve centres taken from rabid animals, and it is claimed that with the separated poisons, symptoms analagous to those of true hydrophobia have been produced, but the experiments, although probably accurate, are as yet too few in number to allow of their being very seriously discussed.

It is, however, doubtful whether the real causal agent has yet been observed. It appears to be quite as probable that one of the lower animal protozoa, coccidia, psorosperms or the like, may be the real cause of the disease as that a bacterium stands in this relation to hydrophobia. In the same way it has been suggested that the acute exanthemata from which organisms have not as yet been in most cases successfully cultivated may have a similar *causa causans*.

It was very early determined that hydrophobia or rabies never arises spontaneously; the actual date at which the implantation of the disease took place could easily be traced, as it was found that in the human subject the outbreak of the disease always bore a definite relation to some such injury as the bite of a rabid dog, wolf, or cat, or it might be, to the licking of an abraded surface by an apparently healthy animal, especially a dog which afterwards was known to develop symptoms of hydrophobia.

This disease is not confined to man and the animals mentioned. It has been observed that rabbits, deer, guinea-pigs, and even horses may be similarly affected. Although the disease had been described most carefully from its clinical point of view, and although the saliva of a rabid animal was supposed to contain a specific infective material which was probably the cause of rabies, Hydrophobia or *Rage*, it was not until 1880 that Pasteur set himself to study the virus of this terrible disease. His first experiments were made with

the saliva from a child in whom the disease was developing as the result of the bite of a mad dog. He took a little of the saliva from this child and introduced it into a small pocket under the skin of a healthy rabbit; he found the animal dead at the end of a couple of days. Taking some of the saliva of this animal, he treated another rabbit in a similar manner with a like result, but he also discovered that the blood of the first rabbit when introduced under the skin of another rabbit also produced the disease in a most virulent form, so virulent indeed that the old question of septic *versus* other specific infective organisms again cropped up, and it was suggested that the animals had died of septic poisoning due to the presence of ordinary septic organisms in the saliva, and therefore in the blood of the animal that had first been inoculated with the saliva. The fact, also, that the symptoms of hydrophobia in many cases resemble so markedly those of another form of blood poisoning—tetanus or lockjaw—and the extremely rapid course that it ran in the animals inoculated, made it a matter of extreme difficulty to determine the exact nature of the symptoms, and rendered it impossible for Pasteur to say whether he was dealing with hydrophobia or with some other form of specific infective poisoning. He found, however, that it was specially in the later stages of the disease that hydrophobic symptoms became tetanic in character. After the bite there may be no symptoms at all for a month or six weeks, or even in some cases for twelve months, during which time the poison lies latent in the system, or though active, gives rise to no symptoms. This period is known, technically, as the period of incubation. At the end of this incubation period the wound first of all becomes slightly uncomfortable; there is itching, and the heat becomes almost intolerable, especially as this is usually accompanied by a sharp stinging pain; the patient becomes feverish and very thirsty; the face is pallid and has a peculiar anxious expression, the muscles of the face being drawn and restless, and gradually this expression amounts to one of actual terror or horror. On the second or third day the patient becomes much more excited, is restless in every sense of the word, and a very peculiar feature is that he has a characteristic habit of giving a suspicious side glance as though constantly looking out for some hidden danger; then as the

fever advances a rambling delirium supervenes ; the thirst increases, but along with this there is great difficulty in swallowing—especially fluids—and after making one or two attempts to swallow, the very sight of water suggests such horrors that thirsty as the patient is, he is anxious to avoid it. Then muscular tremors are noted, these become more and more marked and violent spasms are easily stimulated as in tetanus. A sharp sound, a touch, a bright light, or even a breath of air, may give rise to violent muscular convulsions, and eventually the patient is slowly suffocated as in tetanus. One can well imagine that to a man of Pasteur's temperament and mode of thought such a terrible disease as that, the symptoms of which I have just sketched, would just be the subject that would fascinate him. Here was a problem surrounded by difficulty but of such a character that the very difficulties invited success ; whilst if success were attained he would have the satisfaction of feeling that he had done something more to alleviate human suffering—more even than he had already accomplished ; hence the preliminary experiments we have already mentioned. In order to convince himself that the disease which he had produced in the rabbit was really hydrophobia he must obtain the poison in a perfectly pure condition ; as he had already found that Galtier's experiments as regards the blood of rabid animals were incorrect, he determined to repeat the same experiments with fluid taken from the cavities of the brain and spinal cord ; here again he was successful, for although Galtier had been unable to produce the disease with such fluid, Pasteur found that by taking a few drops of the cerebro-spinal fluid and introducing it under the outer membrane of the brain (chloroforming a rabbit and removing a small round disc of bone from the skull cap, then replacing the bone and stitching up the wound, which healed almost immediately) hydrophobic symptoms were rapidly developed. This fluid from the central nervous system of a hydrophobic rabbit contained no septic organisms, and as the disease was rather more slowly developed after the inoculation of such virus (although it could be induced with absolute certainty) than when saliva was used, he discontinued the use of the latter, the inoculations being now made from animal to animal and always with the cerebro-spinal fluid. Fragments of the brain and spinal cord introduced under the

dura mater (the outer skin or covering of the brain) produced similar effects. Nerves were also found to contain the virus, but the saliva, as we have seen, and the salivary glands introduced in the above manner produced a much more virulent and rapid form of the disease than the other tissues and fluids mentioned: quite as virulent a form, in fact, as when the animal was actually bitten by a rabid animal.

Finding that these tissues and fluids taken from a rabid animal varied in their virulence, and knowing that in the case of anthrax virus the virulence may be diminished or increased by inoculating into an animal of another species, he made another series of experiments, as a result of which he found, that although virus taken from similar positions, say, the cerebro-spinal fluid, had always the same action in the same species, when the fluid was taken from an animal of a different species it was weaker or stronger as the case might be. Thus in a dog the virus is of constant strength, and inoculations made from dog to dog kill the animal with the same incubation period, the same symptoms, and practically in the same time. When inoculated from the dog to the monkey, however, the virus becomes less virulent; it is said to be attenuated or weakened, the attenuation becoming more and more marked in successive inoculations from monkey to monkey; the course of the disease becomes longer and longer, until eventually there may come a time at which the virus, when introduced under the skin or into the cranial cavity, is not sufficiently active to cause the death of this species. If this attenuated fluid be now inoculated into a rabbit, a dog, or a guinea-pig, it still remains comparatively weak for a time, through successive inoculations on these animals—*i.e.*, at first it does not kill, then it kills, but only after a considerable time; but gradually the virulence returns, until at length it reaches its original level of malignancy; whilst, if the successive inoculations are made in rabbits with primary fluid from either the dog or the monkey, the virulence may become so exalted that it is considerably greater even than that of the virus taken from the street dog, which at one time was supposed to be the most virulent form except that of hydrophobic wolves, which has always been known to be specially fatal; the virulence is doubled as the inoculation period is reduced to about one half.

Having found, then, that the virus could be intensified or modified, and that different animals were affected in a different degree by the same virus, Pasteur set himself to work out the question whether it was possible so to alter the resistance of an individual that a virulent hydrophobic material would have little or no effect on the tissues. The only way in which this could be done appeared to be by the introduction of an attenuated virus into the animal that was to be rendered immune, as in the case of anthrax, and so to accustom the tissues to the presence of the specific poison, rendering them better able to resist a stronger poison; he thought, in fact, that by successive inoculations of stronger and stronger poisons he would be able gradually to "acclimatize" the tissues to the presence of even the strongest virus, and so enable them to deal with it successfully, probably by converting it into innocuous proteid materials, so rendering it harmless to the delicate and highly organized cells of the nervous system. In his earlier experiments he obtained an attenuated virus by inoculating a monkey, from which he took material from the central nervous system with which to inoculate a series of rabbits; each rabbit supplied a slightly more powerful virus with which to inoculate another one of the series. He thus obtained inoculation material of all degrees of virulence. With material from each rabbit in the series he inoculated twenty dogs, each one receiving a stronger and stronger dose each time it was inoculated. Out of the twenty dogs so treated only about three-quarters were protected against virulent hydrophobia; but this for a first series of experiments was a most extraordinary success, and so satisfied and delighted Pasteur that he was encouraged to continue his research; eventually the results he obtained were even more remarkable.

Having observed that the cords of rabbits that had been dead for some time contained a less virulent poison than the cords of freshly-killed animals—this being especially the case in dry weather—he adopted a method based on this observation, by means of which he was able from the same cord to obtain inoculating materials of very different degrees of virulence, this varying according to the length of time that had intervened between the death of the animal and the use of its cord for protective injection. He proceeded as follows: Having sterilized a glass flask plugged with cotton wool by

dry heat, he filled it to the depth of three-quarters of an inch, or an inch with some hygroscopic material such as solid potassium hydrate ; it was then ready for use.

A rabbit was previously inoculated in the following manner : The skin over the part of the skull covering the brain is carefully saturated with a 5 per cent. solution of carbolic acid. (This serves two purposes : first, it purifies the skin through which the knife is to pass, and, secondly, it renders the skin and the tissues beneath perfectly insensible to pain, so that the rabbit will remain perfectly quiet while the operation goes on ; it does not apparently suffer even discomfort, and I have seen a rabbit going on eating whilst the operation was being performed. I have also seen the operations performed as in the Pasteur Institute, while the animal was under the influence of chloroform ; but I have never yet seen it done without either a local or a general anæsthetic being administered). An incision is made through the insensitive tissues so as to give either a cruciform incision or a semi-circular flap, the soft tissues are dissected from the bone, and then with a small tube with teeth at the end, and a sliding pin in the centre (a trephine), a little circular disc of bone is removed, as far as possible without injuring the external covering membrane of the brain.

With a subcutaneous injection syringe, carefully purified by means of some chemical germicidal reagent, or heat, a very minute quantity of the cerebro-spinal fluid, taken from an animal that has succumbed to the disease, is then injected under the dura-mater (the membrane above mentioned), which is immediately beneath the bone of the skull. The disc of bone is then replaced ; the wound is closed by means of a couple of stitches ; a pad of cotton wadding, carefully purified by heat, is used to dry the skin as much as possible ; after which a little of the same cotton wadding is used as a dressing ; this dressing is kept in position by a free application of flexile collodion, the two together forming an air proof shield, through which no organisms from the external air can make their way to the wound, which, as a rule, heals up most perfectly in less than a couple of days. The operation does not appear to affect the animal so treated in the slightest, and until the seventh day it appears to be as lively as any of its companions ; it then gradually begins to lose the power of the muscles, first

in the hind legs, then gradually in the muscles throughout the body ; other nervous symptoms appear, the animal becomes unconscious and comatose, and about the tenth day after inoculation it dies. The cord is taken out as soon after death as possible, and great care is exercised to prevent organisms, septic, putrefactive, or any others, from finding their way to the surface of the cord, which as soon as removed is suspended by a sterilized silk thread in the flask, the air of which, having been rendered extremely dry by the potash, now absorbs a very large proportion of moisture from the cord, and prevents it from undergoing putrefactive change. Originally the cord was left in this flask from twenty-four hours to fifteen days, the material from the cord that had been left for fifteen days having almost or completely lost its virulence, the one day cord remaining nearly as virulent as a cord that had undergone no desiccation.

On the 26th of October, 1885, Pasteur described this method to the French Academy of Sciences. He showed that by inoculating animals on ten successive days with fragments of different cords, each beaten up with twice its volume of sterilized bouillon, commencing with the weakest virus, and continuing until he had used an emulsion from the cord that had been exposed only two or three days to the dried air, and kept pretty constantly at a temperature of 17° or 18° C., they were protected against hydrophobia, even when extremely virulent virus was afterwards injected subcutaneously, or into the membranes of the brain. Of fifty dogs so treated (no two exactly in the same way), every one was refractory to the disease in proportion to the theoretical degree of protection that had been given ; such protection lasting apparently for at least two years, and probably more.

Having obtained such success with dogs the next step was to protect patients who had already been bitten by mad dogs or wolves. The first human being so inoculated against hydrophobia was a little boy, Joseph Meister, aged nine years, who, on the 4th of July, 1885, was bitten so severely on the arms and legs by a mad dog, that it was with difficulty the poor child could walk. He was attended to by a doctor who cauterized the worst of the wounds with carbolic acid, but not until twelve hours after the child had been bitten. As the dog was undoubtedly mad, and as there was little chance of the survival of the

child in the ordinary course of events, Pasteur resolved, after consulting with Professors Vulpian and Grancher, who agreed to share the responsibility, to treat the boy as he had treated the dogs that he had already been successful in protecting. During the following ten days he made thirteen injections :—

2 on the 1st day with emulsions made from cords that had been exposed to the air in the flask for 14 and 10 days respectively :

2 on the 2nd day	,,	,,	11 and 9 days respectively
1 ,, 3rd day	,,	,,	8 days
1 ,, 4th day	,,	,,	7 days

and so on until the 10th day, when he inoculated with the cord of a rabbit that had died on the same day, *i.e.*, the cord in which the rabic virus still retained its full virulence.

For every injection that was made into the child, a corresponding one was made into a test rabbit, and it was found that the five rabbits inoculated with the first five injecting materials, had no hydrophobia, whilst the other eight succumbed to the disease ; the period at which the animals succumbed being gradually shortened as the cords exposed to the dry air for the shorter times were inoculated. It was remarkable that although the later vaccines proved fatal to rabbits, in the patient, prepared by the previous inoculations, they did not produce the slightest discomfort, he never had the faintest symptom of hydrophobia, and now, five years later, we are told that the boy is still alive and well. Since that time an enormous number of patients have been inoculated, and it certainly appears from statistics, given monthly in the "Annales de l'Institut Pasteur," that the percentage of deaths after inoculation has been much lower than in those patients left without the anti-rabic treatment.

Babes, in order to make the virus as constant as possible, finds that it is advisable to make a mixture of cords from three or four different days (*i.e.*, cords that have been exposed to the drying process for different periods), and to inoculate at least twice a day, or more frequently in serious cases, such as those in which there are wounds about the face, or where the wounds are inflicted by a mad wolf ; the period of treatment now also is longer, in addition to which much

larger quantities of the protective material are used, especially in severe cases.

Take as an example of this method of treatment, one given by Cornil and Babes in their work on bacteria, that of a child severely bitten about the face, which only came under treatment four days after it had been bitten.

On the 1st day it received injections of 2 grammes of emulsion, made up of cords that had been exposed 13, 12, 11, and 10 days respectively.

On the	2nd day	2 grammes	,, ,,	10, 9, 8, and 7 days ,,
,,	3rd day	,,	,, ,,	7, 6, 5, 4, days ,,
,,	4th day	1½ grammes	,, ,,	4 and 3 days ,,
,,	5th day	,,	,, ,,	3 and 2 days ,,
,,	6th day	2 grammes	,, ,,	8 and 7 days ,,
,,	7th day	,,	,, ,,	7 and 6 days ,,
,,	8th day	,,	,, ,,	6 and 5 days ,,
,,	9th day	,,	,, ,,	5 and 4 days ,,
,,	10th day	1½ grammes	,, ,,	4 and 3 days ,,
,,	12th day	2 grammes	,, ,,	8 and 7 days ,,
,,	13th day	,,	,, ,,	7 and 6 days ,
,,	14th day	,,	,, ,,	6 and 5 days ,,
,,	15th day	,,	,, ,,	5 and 4 days ,,
,,	16th day	1½ grammes	,, ,,	4 and 3 days ,,
,,	18th day	2 grammes	,, ,,	8 days
,,	19th day	,,	,, ,,	7 days
,,	20th day	,,	,, ,,	6 days
,,	21st, 22nd days.	2	,, ,,	5 days
,,	23rd, 24th days.	,,	,, ,,	4 days
,,	25th, 26th days.	,,	,, ,,	3 days

On the 11th and 17th days there was no treatment.

At Bucharest, where wolf bites are of frequent occurrence, the treatment may last for more than a month, four and five injections being made on the first few days, and as much as 12 or 13 grammes of the emulsion being injected per diem; the whole series of attenuations being gone through in mixtures of three each in three days. Then the same process is gone through with mixtures of two attenuations, and lastly, the emulsions from single cords throughout the whole series are injected. For instance, on the 1st day there are injected 4 grammes of an emulsion, made from the cords of 13, 12, and 11 days; then 4 grammes from the 12, 11, and 10 days; 3 from the 11, 10, and 9 days; then 2 from the 10, 9, and 8 days; the last injection on the 3rd day consisting of 1 gramme of the 3, 2, 1 days, or the strongest virus; then on the 4th day similar injections are made of the most virulent virus; on the 5th day 16 grammes of the weaker virus from the 12th to 9th days are injected; and on the 30th day 4 grammes of the strong virus (2 days) are injected. This treatment is modified for special cases, but this is an example of the treatment for very severe bites.

Bujwid adopted a somewhat different process, he injected 2 grammes daily of the mixture of 12 and 10 day cords on the first day; 8 and 7 day cords on the second day; 6 and 5 cords on the third; 4 and 3 on the

fourth, and then again 12 and 10 cords on the fifth, and so on through a third series, the whole treatment lasting 14 or 15 days.

Cornil and Babes have collected some most interesting statistics, which we may here quote. In the Institut Pasteur in 1886, out of 2,682 cases treated, there was a mortality of 13.4 per 1,000; in 1887, in 1,778 cases, a mortality of 11.2 per 1,000; in 1888 7.7 per. 1,000; and in 1889 5.4 per 1,000. In St. Petersburg, in 484 patients treated, the mortality was 26.8 per 1,000; in Odessa, in 1,135 treated, 17.1 per 1,000; in Moscow, out of 107 treated, 34 per 1,000; but out of 500 inoculated with stronger virus, and for a longer period, the mortality was only 13 per 1,000. At Warsaw, of 297 people treated by the milder method, 80 per 1,000 succumbed; whilst of 370 inoculated by the intensive method, none succumbed to hydrophobia. At Charkow, of 233 treated, there was a mortality of 38 per 1,000; at Turin, of 502 treated in cases where the dogs were undoubtedly rabid, there was a mortality of 25 per 1,000; at Bucharest, out of 310 patients, a mortality of only 2.9 per 1,000; at Naples, of several hundreds treated, the mortality was only 15 per 1,000; and at Havannah, where there were 170 patients, only 6 succumbed.

The factor of time between the bite and the commencement of the treatment plays a most important part, and it has been found that there is always the greatest success obtained in the cases of those patients who submit themselves for treatment within two or three days of being bitten. One of the most convincing proofs of the efficacy of this inoculation that has been given, is that recorded by Babes. Thirteen men and thirty animals, cattle, horses, pigs, and dogs, he states, were attacked by rabid wolves; of the thirteen men so attacked, twelve came to Bucharest for treatment, and all of them recovered except one whose head was fearfully torn and lacerated by the fangs of a wolf; the thirteenth man, who would not present himself for treatment, died of hydrophobia. A very significant fact was that every one of the thirty animals succumbed to typical hydrophobia. The patient who died succumbed ten days after the completion of a very intense and prolonged treatment (thirty-two days). The other patients were then subjected to a further treatment during six days of mixtures of two cords from the twelve days down to the one day; and as we have seen they all recovered.

It is interesting to note in connection with Galtier's early observations, which were at first supposed to be disproved by Pasteur, that they were confirmed by Nocard and Roux, and by Vestea and Zagari, who have proved that the injection into the blood of rabic virus does not always produce hydrophobia, and that in certain ruminants,

such as the sheep and the goat, the intravenous injection of rabic virus, although it does not produce hydrophobia, protects these animals against rabic infection. It is now generally acknowledged that when properly performed, the inoculation of even very large quantities of virus may be safely carried out, and as it is found that greater success is obtained where these larger quantities are injected we may look forward to still greater improvement in the treatment of this disease, and to even further diminished mortality.

This process of inoculation in hydrophobia brings up a phase of the vaccination question that has not yet been fully developed but one that appears to be destined to cast an important light on some of the questions relating to immunity. Pasteur's explanation of the results he obtained does not appear to be entirely satisfactory. I am inclined to think that the explanation advanced by Wood and myself, that the treatment consists essentially in causing the tissues to acquire a tolerance before the microbe has had time to develop, is more in accordance with facts. The tissue cells are acted upon by increasingly active virus, each step of which acclimatizes the cells for the next stronger virus, until at length when the virus formed by the micro-organisms introduced at the time of the bite comes to exert its action, the tissues have been so far altered or acclimatized that they can continue their work undisturbed in its presence ; and treating the micro-organisms themselves as foreign bodies, destroy them. When the cells are *suddenly* attacked by a *strong dose* of the poison of this virus they are so paralyzed that the micro-organisms can continue to carry on their poison-manufacturing process without let or hindrance, but when the cells are gradually, though rapidly, accustomed to the presence of the poison by the exhibition of constantly increasing doses they can carry on their scavenging work even in its presence, and the micro-organisms are destroyed, possibly even before they can exert their full poison manufacturing powers. Some such explanation as this would account for the interference with the course of the disease even after the patient has been bitten. The micro-organism is localized, it takes some time to form its poisonous products, and whilst this is going on the whole of the nervous and other tissues are being gradually acclimatized by the direct application of small quantities of the poison artificially introduced.

It is a most remarkable fact that although no micro-organisms can be found in the virus, filtration through the Pasteur filter keeps back the effective part of the virus, whilst heating to 100° C., or even a prolonged heating to 80° C., destroys the activity of the virus; further, an alcoholic extract of the emulsion does not contain any substance that will confer immunity, nor will it, when inoculated, produce any symptoms of rabies.

That the patients themselves have great faith in the treatment, we have ample evidence in the fact that so many present themselves for treatment. It was an extremely interesting sight to see the members of the polyglot crowd in the waiting-room brought in, one by one, for inoculation in the Pasteur Institute. As the patient's name was called out, one assistant standing at a table on which was arranged a row of little conical glasses, each covered with a paper cap, and containing an emulsion of a cord of a certain length of exposure, filled or partly filled a Pravaz syringe with emulsion from one of these glasses, according to the stage at which the treatment had arrived (determined by reference to the case book). He handed the syringe to the operating doctor, who, taking a fold of the skin (which had been previously washed and purified with carbolic acid, or some such antiseptic reagent), just above the crest of the ilium, a part very easily exposed from the arrangement of the attire of both males and females, inserted the point of the needle, injected about half a syringe full, withdrew the needle, diffused the fluid as far as possible by pressure, and the patient passed on. The syringe was handed back to the assistant, who carefully sterilized it by plunging it into hot water. The whole operation seemed to take only a few seconds. A continual stream kept passing from one room to another, each patient, as he went through, undergoing the same process of inoculation. Everything was done in the most orderly fashion, and one could not but feel that Pasteur, who was standing in the room whilst this was going on, had every right to feel proud, not only of the splendid Institute in which he was working, but of the excellent work that was being carried on, both in the field of investigation and in the application for the benefit of suffering humanity of the results of the investigations.

In France they manage some things better than we do,

and, although France should not get all the credit of work towards which most European nations have contributed, through representatives of all classes, the grants from the Imperial treasury formed a basis on which to found the now celebrated Pasteur Institute, and to France and Pasteur much of the honour is due. The Pasteur Institute is a building set apart for the study of micro-biology, the most perfect of its kind in the world, and a short account of it cannot fail to prove interesting. For many of the details I have referred to the January number of "Annales de l'Institut Pasteur," 1889, as unfortunately, when I visited the laboratories, the place was not in full working order, but I saw enough to convince me that the spirit which animates the founder of the Institute pervades also those of his disciples who carry on the work he has begun. It covers an area of eleven thousand square metres in the Rue Dutot, and consists of two large blocks running parallel, one behind the other, united by a long corridor which runs from the main entrance in the front, connecting the two buildings, and through to the back, with a number of separate outbuildings scattered over the gardens behind. In the large block facing the street on the right are M. Pasteur's apartments ; on the left his laboratory occupies the basement. In this laboratory he continues his researches, but during the time that patients bitten by mad dogs are being treated he may frequently be found watching with great interest the treatment, and chatting with those who have come to see the place and the method of work. In this block also is collected everything connected with the preparation of, and despatch of, the various vaccines—rouget de porc (swine fever), anthrax vaccine, etc. On the first floor is a large hall, which serves the double purpose of library and council room. This room is well lighted by four large windows, and is provided with the current scientific literature, both periodicals and books, all of which may be consulted by the workers in the laboratories, who, however, are not allowed to remove them from the room. The storey above this is occupied by the servants and attendants of the institution. The large corridors, four metres and a half broad (nearly five yards), well lighted, run between the corresponding storeys from this block of buildings to the larger one behind, which is entirely devoted to laboratories.

This block is divided into two wings, each about twenty-five metres long and fifteen metres from back to front; in the right wing, on the ground floor, are the rooms set apart for the examination of patients who have been bitten by mad dogs, and for their treatment by the preventive inoculation method. The patients who enter the grounds at the right are directed by an intelligent porter through a door at the right of the building to a waiting-room heated and well lighted, surrounded with benches on which they may rest while waiting for their turn to come; they then pass into a room where their names, addresses, and particulars of their cases are carefully recorded. After being duly registered, they are passed on to the inoculation apartment, where they are treated in the manner already described. Should any patients show symptoms of faintness, they are assisted into a small room in which is a sofa, on which they may recline; the others pass out into a central passage, on the opposite side of which is a room where severe bites and lacerations are attended to surgically. Here also are an operation room, archive room, and others. Next to the room in which we left the faint patients, is the laboratory in which the preparation of the cords that are used for the manufacture of the virus is carried on. It is maintained at a constant temperature of 23° C. by means of a special regulator. Around this is a regular array of bottles, arranged on shelves fixed to the wall, and grouped according to the time that they have been exposed to the dry air. A screen within the door prevents draughts, and helps to maintain a constant temperature, even when the door is frequently opened.

In the left wing, on the ground floor, is a lecture-room for biological chemistry, which will accommodate about fifty auditors; it is separated from a laboratory by a wide doorway, which, when open, permits of those in the lecture-room seeing what is going on in the laboratory. This opening may be closed altogether by a large black screen, or by a ground-glass plate, on which lantern projections may be thrown. M. Duclaux, professor of biological chemistry in the Sorbonne, now delivers his course here, and M. Roux lectures on Practical Micro-biology. From time to time those who are working in the laboratory, or others, also give demonstrations and explanations of their work and of any recent discoveries in this

theatre. Close to the lecture-theatre is a photographic department, specially designed and fitted up by M. Roux, for the reproduction of bacteria and other microscopic objects. At the end of the block, at the right and left of the central corridor, are two rooms with well-fitted aquaria, which are specially fitted up for carrying on researches, under the supervision of M. Metschnikoff, on aquatic animals. The two rooms on the ground floor, specially set apart for experiments upon large animals, are provided with a good wide door opening to the outside. The concrete pavement, sloping towards a green, permits of this being readily cleansed. The remainder of this floor is occupied by a kind of store-room and a laboratory set apart for general use, in which are stored and prepared the bouillon and other nutrient media. Here also is carried on the glass-blowing ; a skilled artizan supplying workers with flasks, pipettes, tubes, and other vessels they may require. A large staircase situated at the end of the central gallery puts the laboratories of the basement in easy communication with those of the storeys above. The first storey is divided into duplicate halves, one on the right, the other on the left of a passage. That on the left is set apart for M. Duclaux's department of general micro-biology ; that on the right to practical biology, over which M. Roux presides. The central corridor leads to a large concreted workroom, nearly twelve metres square, which is well lighted with nine large windows. Around the room are seven work tables, each of which is covered with a thick sheet of volcanic lava, the surface of which is enamelled so that it has the appearance of an immense sheet of porcelain. Each is fitted for two workers. Every worker has before him a window, from which light is obtained for the microscope on his right or left side, according to his position. At the table is a gas connection, from which gas may be conveyed to any desired point ; here also is water, which is received into a sink, also of enamelled lava. A small sliding board, which may be drawn out and used as a desk, is placed on the other side of the table, away from the central projection which carries the sink ; this allows the worker to make for himself a little retreat, in which he sits surrounded by all that he requires. When work is over for the day everything except the microscope is put away in a couple of small cupboards which are placed at the disposal of the worker. This is absolutely necessary in order that the room and the

tables may be kept clean. Two tables placed parallel to one another in the centre of the room are used for chemical operations which cannot be carried on at the smaller special tables. Then there are large evaporating chambers, in which are placed the sterilizing and incubating apparatus. In addition to the incubating apparatus used in the common laboratory, there is a large common room, which is really made up of three rooms :—An entrance chamber, in which the temperature varies somewhat from time to time ; it contains the heating apparatus, and is specially designed as an air cushion insulator. The second chamber—nine feet ten inches long, eight feet broad, and six feet high—is heated by means of hot water, by which it is kept at a constant temperature. Above this, and heated in connection with it, is a third chamber, in which the temperature is intermediate between those of the two preceding ones. This group of incubating chambers has very little cooling surface, and is separated from the outer wall by a room in which is collected and washed all the dirty glass used in the laboratory. In these constant-temperature chambers are shelves and tables, on which may be seen all kinds of flasks, test tubes, and sterilized moist chambers, in which, growing on various media, and under different conditions, are all kinds of micro-organisms that are being experimented with in the various departments of the Institute. The laboratory "preparateur" has a room next to the laboratory, with which it communicates directly, so allowing ready access thereto and constant superintendence. From this small laboratory is the only entrance to the museum, for the care of which the preparateur is directly responsible. A special laboratory for the performance of experiments in chemical biology, and a lavatory, are also added to the suite on this floor. The laboratory and the room of the director of the department are placed in each wing. These are entered from the passage which leads to the common laboratory. In the storey above this there are no teaching laboratories ; we have simply two series of rooms, divided by a passage, each room designed to become a research laboratory, and fitted up to meet the requirements of the *savant* who occupies it. Investigators have every facility for carrying on their work, and may, if they desire it, have the advice and guidance of any of the directors of departments, each of whom is responsible for the

management and work of the whole wing that is placed under his supervision. In the left wing M. Chamberland superintends the department of applied or practical bacteriology; in the right wing M. Gamaleia presides over the subject of comparative micro-biology or bacteriology. In each of these departments, in addition to the special incubating chambers required by the workers, is a general incubating chamber, constructed like that in the first storey, but on a larger scale, and also a general laboratory, in which may be carried on all the sterilizing, preparation of gelatine, broths, &c., for which special apparatus is required.

This is the extent of the laboratory proper, but in the garden which surrounds the main building are found scattered other structures, apparently less important, but all of them required for the carrying on of the work of this immense establishment.

First comes a building, parallel to the main block, in which is accommodation for animals, which are kept in elevated cages constructed of iron bars on every side; they are so raised from the sloping asphalt floor, that they can readily be flushed. At the two ends of the building three small rooms have been set apart for operation on small animals that come to be treated in the Institute. In a special house, which is very well arranged for the reception of dogs, each animal has its special cage, where it can be properly looked after, watched, and fed, without it being necessary for the attendant or observer to come actually in contact with it. Near this is another series of animal houses, also with asphalt floors, which are used for stock, or for animals that being experimented upon require special isolation. The rabbits that are inoculated for the preparation of the specific fluid, during the incubation period of their attack of hydrophobia occupy a special house, which is also kept at a constant temperature in order that the periods of incubation may be kept as uniform as possible. A special arrangement of the cages in which these animals are enclosed allows of their bedding being changed and of their being kept clean without its being necessary to open the cage. A gutter, with walls of glass placed below the cages, which can be well flushed by a stream of water, serves to carry off the urine which makes its way through the floors of the cages. Then come a run for hens, and smaller hen-coops, an aviary, and

stabling for large animals, all of which are so constructed that they can be kept not only in a state of perfect cleanliness, but can be thoroughly washed out with boiling water. They present no porous surfaces, and there is no material used into which germs can penetrate and become inaccessible to the usual microbicidal agents. Lastly, in one corner of the grounds are two cremating furnaces, in one of which gas is used, and in the other ordinary fuel. In these every particle of infected matter and all diseased tissues not kept for microscopic examination, are carefully calcined in order that nothing of an infective nature may be carried beyond the walls of the establishment. The arrangements generally are such that absolute cleanliness may be maintained with the slightest possible amount of labour, so that not only the workers, but also the people residing in the neighbourhood, may be perfectly at ease as regards any danger of infection. Even in the old laboratory, situated in the gardens and in contact with the Normal School in the Rue Vauquelin in the immediate vicinity of hotels, experiments with the most virulently infective diseases have for ten years been carried on by Pasteur and his pupils without the least accident having happened. The new Institute will accommodate about fifty workers—about fourteen in the laboratories on the ground floor, and the remainder in the research laboratories. The staff of directors and assistants consists of about fifteen persons, all of whom are studying some branch or other of micro-biology. The only passport required for working in the laboratory is a capacity for doing good work. If there is a place at liberty a good worker is always sure of it, and Frenchmen and foreigners alike are admitted to the use of these tables in one or other of the principal departments, of which there are six, associated with the names of such men as Straus, Grancher, Duclaux, Gamaleia, Chantemesse, Widal, Charrin, and others. These departments are named according to the special kind of work carried on. Thus, one is devoted to the study and treatment of hydrophobia, another to general micro-biology, a third to practical bacteriology, a fourth to micro-biology applied to hygiene, a fifth to morphological and comparative micro-biology, a sixth to biological chemistry. Most extensive and elaborate arrangements are made for the instruction of pupils in both general and special methods of bacteriological

investigation, and the pupil, after being instructed in the first, will, in the laboratory of general micro-biology, follow out special methods, each one having his own subject and pursuing his research by appropriate methods. M. Roux takes them first in small classes, and in five or six weeks he gives all the general principles of work, and indicates all the details of technique necessary to ensure the competence of these pupils in ordinary matters relating to the study of micro-biology. In the course is included regular practical laboratory work ; but the main work is the carrying on of original investigation, and the results of the researches made by the distinguished staff and their pupils are now known throughout the world, whilst there still comes a stream of brilliant papers, such as may well fill us with admiring envy, and, let us hope, provoke in us a generous rivalry at some not far distant date.

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CHAPTER XIX.

Bacteria of the Mouth.

The Mouth a Good Forcing Ground for Bacteria—Food Material—Kinds of Bacteria found in the Mouth by Miller and Vignal—Bacteria in the Teeth in Caries, in Milk Teeth, and in Abscesses—Combination of Lactic Acid with Lime Salts—Organisms Attack Decalcified Base Substance—Artificial Decay—Mode of Invasion of Teeth by Bacteria—Poisonous Saliva—Micrococcus of Sputum Septicæmia—Pneumococcus—Other Organisms found in the Mouth—Septicæmia following Slight Operations in the Mouth.

It will be remembered that many of Leeuwenhoek's observations as to the morphological appearances of the minute structures which he examined with his wonderful lenses, were made on bacteria which were found adhering to the teeth, and certainly some of his most interesting observations were on bacteria derived from this source. From what we now know of the biology of these organisms we can readily understand that the mouth should form a kind of hothouse or forcing-ground for their cultivation. Here is a moist cavity kept at a comparatively high temperature, covered with an epithelium which is constantly being partially or completely shed, to which there is ready access from the outside air, and through which food material is constantly being passed, particles of which, despite the exercise of the greatest care and the utmost cleanliness, always remain in small crevices between the teeth, or perhaps, more important still, between the gums and the teeth. It is also said that the fact that starch is constantly being converted into sugar by the ptyalin is in favour of the growth of bacteria. Then we have the dead epithelium, which is readily attacked by organisms of various kinds, supplying proteid or other nitrogenous materials; as a matter of fact, there are often found in these dead epithelial cells a number of micrococci, which appear to be gradually disintegrating their substance and utilizing the materials of which they are composed. In

a similar way the various constituents of the teeth are made to serve as nutrient elements for these bacteria if once the protecting enamel is removed.

One author, writing of the micro-organisms that may be found in the mouth, points out that almost every organism that has been described as occurring in any position has also been described as growing in this cavity; but it is now

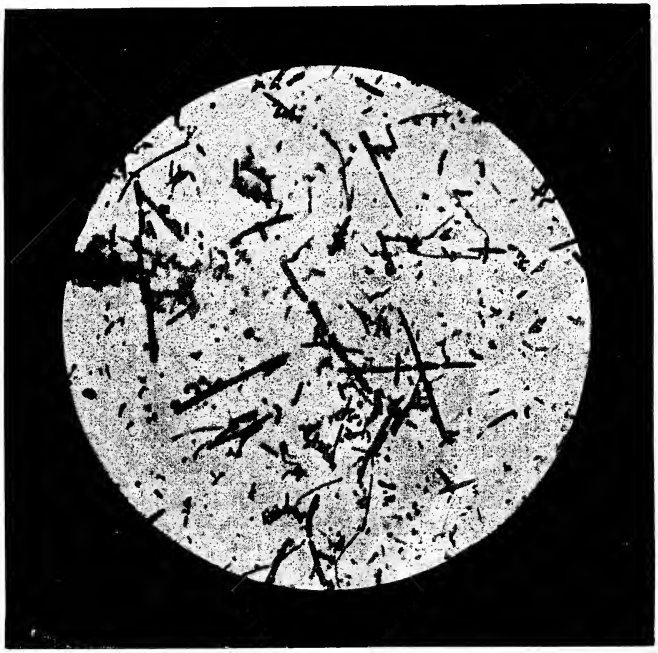

Photo micrograph of scraping from the teeth in which different kinds of micro-organisms are seen, × 1000. Spirilla very imperfectly stained.

generally accepted that about eight or ten are almost constantly met with, and that six may be said to be invariably present. Of these we give a short description, as they may be examined by any one who wishes to see what these organisms really are, though we warn any who may attempt to cultivate them that they will almost inevitably fail, as these special mouth organisms seem to require

special conditions, as yet not at all understood, for their existence, and although one or other of them may be found in enormous numbers in one case, and may be comparatively few in another, there is usually some explanation to be offered for such preponderance.

(1) The *Leptothrix innominata* (Miller) is found, usually, on the soft white material that is deposited on teeth, it may probably be but a generic term for more than one form, as the constituent elements of which it is said to be made up vary very much both in length and in thickness; they are usually .5 to .8μ thick, and though there may be shorter cells or rods, the organisms usually consist of tortuous non-motile threads. These rods and threads as a rule contain no spores.

(2) The second form, *Bacillus buccalis maximus*, occurs as isolated bacilli or threads, generally arranged in bundles, which may interweave with one another. These bundles are sometimes of very considerable length, but each thread is divided into short rods, from 1 to 1.2μ broad, and from 2 to 10μ long. This bacillus is, as a rule, not found within the dentine tubules. In addition to this bacillary form there is a leptothrix form, in which the filaments are considerably longer, but otherwise the organism is much like that already described, although it is scarcely to be looked upon as related to the leptothrix innominata.

(3) The next organism is one called by Miller the *Iodococcus vaginatus*, which is found especially in mouths that are not properly cleansed; it appears to be made up of cocci arranged as diplococci or tetrads, which are usually arranged and held together in a kind of sheath, though now and again a single coccus or diplococcus may be seen, around which no sheath can be demonstrated. When this organism is stained with iodine a marked reaction is obtained, the sheath being stained yellow, whilst the cocci are stained dark blue.

One of the most interesting forms met with in the mouth is: (4) the *Spirillum sputigenum* (Miller), which always occurs in certain numbers, but is found in especially large quantities in those cases in which the presence of tartar has set up slight inflammation of the gums around the teeth; it usually occurs in the œdematous gum just at its junction with the teeth. This organism has more than usual interest from the fact that it was for long supposed, by Lewis and Klein, to be identical with the cholera bacillus. Morphologically it certainly resembles this latter organism in many respects. The simplest form is that of a small curved bacillus; sometimes two or three of these may be joined together to form an S-shaped organism, whilst in other cases it may be found as long spiral threads. This organism, as we have said, is almost invariably met with in the mouth, but it is curious that it is very seldom found between the teeth, or in carious cavities; it thrives apparently upon the exudation from the slightly inflamed gums, and so necessary is this food for its existence that it has never yet, so far as is known, been cultivated in any of the artificial nutrient media outside the body, although (5) a somewhat thicker, curved, comma-shaped bacillus, identical in almost every respect (except as regards size), can be cultivated in gelatine quite readily at the ordinary temperature of the room. This differs from the first-mentioned in that it has the true comma-shape, tapering towards one end.

(6) A third curved or spiral organism is also described—somewhat longer than the others; it occurs in longer or shorter, straight or spiral filaments, which may break up into rods and even into chains of cocci; this organism when cultivated in gelatine does not give rise to any liquefaction, differing in this respect from either the Koch's cholera or comma bacillus or the Finckler-Prior bacillus, the latter of which has also been described by Miller as occurring in the mouth. Miller maintains indeed that some of these curved organisms may pass into the lower parts of the alimentary canal, but that even when he took them from this position he never succeeded in making pure cultures of them; they will not grow on any of the nutrient media that have been as yet devised.

(7) Then, of these more important forms, is the *Spirochæte dentium* or *Spirochæte denticula*, which is usually found associated with the (8) Spirillum sputigenum, and under similar conditions; it consists of a long spiral thread from 8 to 25μ in length, of which the spirals are irregular and of unequal thickness. Very little is known of this organism, and it is quite possible that the thicker spirals may represent some stage of development of the preceding organism, especially as in this case it is also impossible to obtain any artificial cultures. As already stated, other organisms are very frequently, though not invariably, met with.

Vignal describes a number of bacteria and bacilli as occurring in the mouth and amongst these certain common forms that are generally recognized, such as Bacterium termo (?) Bacillus ulna, the potato bacillus, Bacillus alvæ, and a number of others, whilst amongst the cocci are found the Staphylococcus pyogenes albus, and S. pyogenes aureus, &c. Hueppe describes organisms that give rise to the lactic acid fermentation, whilst others, especially those giving rise to pigment and pathogenic bacteria of various kinds, most of them well recognized, have a peptonizing action, and a few appearing to secrete a true diastatic ferment may also be met with in the mouth.

Miller gives some interesting statistics as to the action of the bacteria found in the mouth upon carbohydrates; he finds that of twenty-two kinds of mouth bacteria which he mentions, "sixteen brought about an acid reaction when cultivated in beef extract peptone solution, four produced an alkaline reaction under the same conditions, whilst in the case of two only the reaction remained neutral." Many of these organisms also exert an enzyme function in a somewhat marked degree; most of them have the power of peptonizing coagulated albumens, during which they give rise to the usual ultimate products—sulphuretted hydrogen, ammonia, carbonic acid gas or combinations of these, peptones of course also being formed.

In thin sections of decalcified teeth, stained with fuchsin and vesuvin, it is seen that bacteria are scattered, though somewhat irregularly, throughout the dentine (the hard substance of which the tooth is principally composed) that is undergoing decay or softening. This is always most marked near the surface, but it must be noticed that as the bacteria travel along the dentine tubules (little canals that run vertically through the dentine), there is usually a small part of the softened area in which micro-organisms are not seen, as it seems that these cannot make their way readily along the fine transverse branches that connect the vertical tubules, and, as Miller puts it, although the organisms "keep up with the softening in the direction toward the pulp, they fall considerably behind in the lateral directions, so that the invasion, particularly in the lateral direction, is usually much less extensive than the softening;" and the tubules near the surface always contain more bacteria than those deeper down in the softened area. It is important to remember that bacteria may be found in apparently normal dentine canals, whilst a similar invasion seems to go on in the roots of "milk" teeth, and also where abscesses have occurred in the roots of "permanent" teeth. It is now held by most of those who have given attention to the subject, that the bacteria which are found in decaying teeth are only playing a secondary part, though a very important one, in the process of caries. In the first instance there appears to be a softening of the various parts of the tooth by acids, commencing with the enamel; in the case of people who take perchloride of iron or nitro-hydrochloric acid this softening may go on exceedingly rapidly. As we have already seen, lactic acid is constantly present in the mouth, though often in very small quantities; if left to act on the lime it may give rise eventually to softening at the margins of the gums and to caries, acids preparing the way for the invasion of various bacteria, by combining with the lime and softening the tooth. Where once the lime salts have been removed, bacteria can attack the basic substance most easily; they are now in a position to make their way along the dentine tubules, and by the intertubular spaces, and once in this position they attack the surrounding tissue with very great vigour; they use it as a food material, absorbing and digesting it until they have made their way into the greater

part of the softened area. It was at one time stated that the "leptothrix buccalis," as it was called, was the organism that was always found in these cases, but this is now known not to be the case : micrococci, leptothrix threads, bacilli, and spiral forms may all be met with even in the same decayed tooth, and in tubules lying close together ; the softening and absorption going on indiscriminately, whichever of the organisms may be attacking the basic substance. It is owing to the peptonizing power of these organisms that they are able to carry on the disintegrating process, and we thus see that although the bacilli may not be actually present in all parts of a decaying area, their products, such as lactic acid and the peptonizing enzyme, are really carrying on the work that ends in the decay of the tooth, perhaps considerably in advance of the bacteria themselves. In experiments carried on with the object of proving that decay might take place in teeth removed from the mouth, Miller placed a number of perfectly sound teeth in a mixture of saliva and bread ; this mixture was renewed from time to time, whenever the slightest trace of alkalinity appeared, and the pulpy mass in which the teeth were embedded was kept at a temperature of 37° C. during a period of three months, with the result that the dentine became softened, and there was what he describes as a condition of "white decay" ; he found that where the enamel was perfect, even acids had no power to attack the dentine beneath, but in those cases where the enamel was soft or imperfectly developed the dentine had become softened by any acid that was present, and the canaliculi were filled with bacteria ; this gave rise to irregular erosion of these canals which thus appeared to be unequally distended. Near the surface of the tooth the organisms are not strictly confined to the tubules, but they invade the basic substance from the surface, softening it as they advance, but filling up the microscopic cavities as they are formed.

The organisms described by Galippe and Vignal that were cultivated on gelatine were six in number, all occurring in decaying teeth.

1. A short thick bacillus 1.5μ in length, and nearly as broad as long ; it grows somewhat rapidly on gelatine, giving rise to an opaque white growth along the track of the needle ; it liquefies the gelatine about the third or fourth day, rendering it somewhat opaque, from which we should gather that the organism is motile ; on plates it forms colonies which are usually

2 or 3mm. in diameter before they begin to liquefy; after th they extend and liquefy very rapidly.

2. A bacillus slightly constricted in the centre about 3μ in length, and about one-half as thick as long; in cultures it is similar to No. 1, except that it has a larger surface growth before it gives rise to any liquefaction.

3. A bacillus very similar to No. 2, but with no constriction in the centre; it has square ends and frequently grows in long chains, especially in liquid media; it causes only slight softening of the gelatine.

4. Is a small thin bacillus so short that it might even be mistaken for a micrococcus; it gives rise to a white growth along the needle track in gelatine which it turns yellow, and then causes to liquefy.

5. Was not found in all cases (in eight out of eighteen decayed teeth). It is a bacillus with rounded ends, which grows almost exactly like No. 1.

6. Found five times only, is a large coccus; it was found only in advanced stages of decay, where the canals had been opened up by other organisms as it was so large that it could not make its way along the ordinary dentine canals; it forms a white line along the track of the needle in gelatine, of which it causes no liquefaction.

The very favourable incubating chamber of the mouth is, however, not monopolized by the organisms that so far have been mentioned, and during epidemics, or when people come in contact with persons suffering from various diseases, the organisms associated with such diseases are, as might be anticipated, frequently taken into the mouth, where they accumulate, multiply, and eventually may set up any of the various diseases with which they are associated in the hitherto healthy person.

In the very act of developing in the mouth they are supposed to give rise to ptomaines and other poisonous products which may render the human saliva toxic when introduced by a bite or a wound into the individual himself, or into another individual; in fact, septic poisoning from injection of the saliva from mad dogs was one of the great difficulties with which Pasteur had to contend in his early experiments on hydrophobia. Quite recently Sternberg, Fraenkel, Klein, and others have shown that, though the pure saliva as it runs into the mouth is non-pathogenic, it acquires toxic properties as soon as it becomes mixed with the organisms which are usually found in the mouth, this being especially the case in patients suffering from certain infective diseases. One observer has found that his own saliva is permanently so toxic that it invariably causes the death of small animals into which it is inoculated; it is almost as fatal as hydrophobic saliva. Some of these pathogenic organisms, like those we have already mentioned, cannot be cultivated artificially, for it

is sometimes found that sputum inoculated into mice or rabbits causes their death in a comparatively short period, bacteria being found in their blood ; this blood inoculated into another animal produces a similar disease, such as acute or chronic abscess formation, which may be carried on from generation to generation by simply inoculating a healthy animal with the contents of the abscess. In these cases, however, the organisms that are cultivated from the blood or the pus fail to produce any symptoms at all, and it must be concluded (until further evidence is obtained) that the pathogenic organisms cannot live on the artificial culture media, those that survive on these media being non-pathogenic.[1] There are, however, certain pathogenic organisms found in the mouth which can be readily enough cultivated, the first, and one of the most important of which is the micrococcus of sputum septicæmia, which may be grown on blood serum or agar-agar at the temperature of the body ; it grows as a transparent greyish-white gelatinous coating on the surface of the nutrient medium, and looks almost like a dewdrop. It is encapsuled like the pneumococcus described by Friedländer, and usually occurs in sputum in the form of single or paired cocci ; it is found almost invariably in patients suffering from pneumonia, but it also occurs frequently in the mouths of healthy persons. When injected into animals, either in the sputum or as a pure cultivation, death usually occurs in from twenty-four to thirty-six hours ; numerous capsuled cocci are found in the blood, the spleen is enlarged and contains a number of organisms ; the symptoms, in fact, are those of an acute septicæmia. It has been observed, however, that pigeons and dogs are unaffected by this disease, whilst rabbits and mice are almost invariably killed by its inoculation. It would appear that when it makes its way from the mouth to the healthy lung this organism has little or no power of attacking the tissues, but that if there be slight congestion or inflammation, just as in the case of inflammation of the gums around the teeth, this organism, finding its way from the mouth (where it may have existed for some time without giving any evidence of its presence) into the air vesicles, is enabled to grow on the exuded fluid constituents of the blood, and to set up at once

[1] Another explanation of this will be found in the chapter on Leprosy.

those intense inflammatory changes characteristic of croupous pneumonia, or acute inflammation of the lungs, and in some cases, where the organisms appear to be more virulent, septic pneumonia and gangrene of the lung. It may be, however, that this gangrenous pneumonia is the result of the invasion and action of another organism. It has already been mentioned that the Streptococcus aureus and S. albus sometimes occur in the mouth, but it would appear that these forms are more frequently met with in the posterior nares and in the cavities of the nose. To their action is supposed to be due the suppuration or festering that almost invariably follows small operations of the mucous membrane of the nostrils, unless the mucous surface is previously prepared by careful antiseptic washing out of the cavities and by frequent application of antiseptics after the operation has been performed. The micrococcus tetragonus is also found in the mouth, whence, in cases of tuberculosis, it makes its way into the lungs, and is there found, especially in suppurating cavities; this organism, which is fatal to white mice and guinea-pigs, usually occurs in little packets of four, each coccus being about 1μ in diameter. On gelatine, according to Eisenberg, it grows as small white colonies, which when magnified appear to have a peculiar ground glass appearance; it does not give rise to any liquefaction of the gelatine. Various other septic forms have been isolated from sputum. It will thus be seen that in certain cases injury of the mouth, of the periosteum of the jaw, or the soft tissues of the pharynx, may lead to infections of very different kinds, but it may be laid down as a general rule that septic infection is frequently the result of invasion from these regions, and very numerous are the cases recorded in which death has resulted even from the most trifling operations in the mouth and naso-pharynx. I have seen several cases where death has ensued, with all the symptoms of most acute septicæmia, or with symptoms of more chronic poisoning, as in pyæmia, from the extraction of a tooth or the lancing of the gums in patients with imperfectly cleansed mouths, or in persons who have been engaged in attendance on patients suffering from certain infectious diseases; the organisms in such cases finding their way from positions in which they were comparatively harmless, into the wounds that were unavoidably made, whence they invaded the lymphatics or passed

directly into the blood stream and set up septic or other mischief.

It is scarcely necessary here to enter into the different forms of septic tooth disease or to consider the points at which the different kinds of poison may enter, but it should be mentioned in the interests both of antiseptic purity and suffering humanity that a good stout tooth brush, plenty of water and some antiseptic dentrifice applied morning and night afford a greater safeguard against many diseases than most people are aware.

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CHAPTER XX.

The Bacteria of Colour and Phosphorescence.

Colour-Forming Bacteria—Micrococcus Prodigiosus—Magenta Micrococcus—Beggiotoa Roseopersicina—Bacillus of Blue Milk—Sulphur Pigments—Iron Pigments—Bacillus Fluorescens Putidus—Phosphorescent Bacteria—Six Species—Method of Cultivation—Conditions under which they produce Light.

If an organic medium, such as boiled potato, bread soaked in broth, or nutrient gelatine, be exposed for some time to the air of an ordinary room, it will be found that at the end of a few days, in addition to the moulds of various colours that develop on it, there appear minute yellow, pink, or brown points, which on examination are found to consist of yeasts, of sarcina, of micrococci, and of bacteria, of which there are numerous species that give rise to these coloured masses present in soil, air, and water. It is a well-recognised fact that most putrefactive and decomposition changes are associated with changes in colour of the putrefying media; these colours being due to the activity of some of the above colour-forming organisms which, in the exercise of their full assimilating and colour-forming powers, decompose nitrogenous substances, the elements of which are converted into the protoplasm of the organism, into coloured material, and into the other excretory products to which these organisms give rise. In some cases the colour is actually contained within the substance of the organisms; usually, however, it is accumulated in the sheath, as in the case of the Micrococcus prodigiosus, the organism to which the bright red colour of "bleeding bread" and "bloody sweat" is due. This pigment is quite insoluble in water, but by means of alcohol it may be extracted and obtained in solution; this and other pigment-forming organisms, such as the magenta micrococcus, mentioned by Greenfield as

occurring in the water of the Tweed, contain a colouring matter which has been described as resembling in a most remarkable manner the aniline dyes; in fact, in the case of the pigment of the magenta micrococcus, the resemblance is carried so far that in old cultures even the peculiar metallic lustre of the aniline dyes is reproduced. Other pigments are soluble in water, but not in alcohol, whilst the bacterio-purpurin formed by Beggiatoa Roseopersicina is like chlorophyll, insoluble in both alcohol and water. The name bacterio-purpurin, however, has been given by Engelmann to the pigment produced or possessed by a whole group of organisms. He concludes that it has, in these lower organisms, the function of the chlorophyll of the higher plants. If exposed on the microscope stage to the light of a sub-stage spectroscope apparatus, these bacterio-purpurin bacteria invariably tend to collect on that part of the slide that is over the ultra-red bands of the spectrum, the portion of the spectrum where the absorption of light by the bacterio-purpurin occurred. He found that the analogy with plants and chlorophyll became still closer from the fact that wherever this occurred, oxygen was set free, and that light in fact was necessary for the continued existence of these bacteria and for the development of their characteristic colour-producing power. In the case of this organism, according to Ray Lankester, the colour is actually contained within the protoplasm of the organism, where it appears in some cases to be combined with sulphur, to form dark granules. In some cases the colour, in place of remaining within the organism, becomes diffused into the surrounding media. As an example of this may be cited the bacillus of "blue milk," which, growing along the track of the needle in a gelatine culture, sends out a peculiar iridescent green coloration into the surrounding gelatine; as time goes on this green is replaced by a smoky brown colour. Similarly the Bacillus fluorescens putidus imparts to the gelatine, or any other material on which it is growing, a peculiar fluorescent green, and at the same time gives off an odour of herring brine.

That the decomposition of the sulphates in the presence of iron and organic matter plays a most important part in the production of these pigments has now been fully recognized. If it be borne in mind that sulphides of the various metals appear as beautiful precipitates when thrown down from

solution it is quite evident that very minute traces of iron, say, acted upon by the sulphuretted hydrogen set free by the decomposition of the sulphates, can easily account for the production of certain pigments. Cladothrix dichotoma, for example, growing in water, appears able to separate iron from the surrounding substances ; this becomes accummulated in the form of an oxide in the sheath. In the Beggiatoa the power of reducing the sulphates is especially well marked, sulphur, which appears in regular granules in the substance of the organisms, being stored up in the protoplasm to be utilized as it may be required. The sulphuretted hydrogen that is formed, acts on the iron and gives rise to the formation of sulphide of iron. In this way may be explained the presence of the pigment that is formed in muds where these putrefactive organisms are present, as, for example, in the mud of a tidal river. It should be remembered, however, that iron is not the only metal that may be present, and that there is always a tendency for the sulphuretted hydrogen to be set free from the sulphide and to give way to the formation of oxides, especially in the presence of air and moisture.

Only by the application of some such explanation as this is it possible to account for certain of the beautiful brown colorations that make their appearance in gelatine ; for example, that which surrounds the track of a needle inoculation of some of those organisms, which, though colourless themselves, give rise to most beautiful coloration of the surrounding gelatine. So important is the presence of iron in these cases, that Miller holds that to the action of organisms on it is due most of the discoloration that occurs in decaying teeth.

He points out that the colours characteristic of decaying dentine only make their appearance some time after the process has commenced, the depth of the colour being in direct proportion to the length of time that the decay has been going on, and he considers that this coloration is due to the formation of sulphide of iron in the decomposing enamel, dentine, and pulp. He performed an experiment which may be repeated by any individual who is unfortunate enough to be compelled to have a tooth of his own drawn for decay, or who is fortunate enough to obtain one from some other source. He says :—

" A tooth was cracked in a porcelain mortar, so as to thoroughly expose the pulp, and then placed in a mixture of dilute hydrochloric acid, to which was added a small proportion of a ten per cent. solution of ferrocyanide of potassium. The hydrochloric acid, as well as the water used for diluting it, must be free from iron ; neither must any iron implement be brought in contact with the freshly broken surfaces of the tooth. Those parts of the

tooth containing iron, even in minute quantities, will, after an exposure from one to sixty minutes, assume a blue colour—Prussian blue being formed. One source of error is introduced in the necessary use of an iron instrument in extracting the teeth, but this will only affect those points on the external surface of the tooth with which the forceps come in contact, and therefore may be easily eliminated." He found by these experiments that there were minute traces of iron in Nasmyth's membrane, in the dental pulp (though not constantly), in carious dentine and in enamel, and he considers that it is quite possible that the sulphide of iron that may be formed during putrefaction of the pulp—a process that is set up by micro-organisms—may have something to do with the discoloration, though it is quite possible that much of the discoloration is due to the iron that is taken into the mouth along with the food, the putrefactive processes set up in the mouth liberating the sulphuretted hydrogen, which, combining with the iron brought from outside, gives the discoloration already referred to.

In addition to this coloration, the result of the formation of inorganic salts, we have those violet, magenta, green and yellow colours that appear to be distinctly organic in character, and to be the result of the metamorphoses of albuminoid substances. As above stated, these may be related to the aniline colours, though this has certainly not yet been proved to be the case. It may be well to bear in mind, however, in this connection what takes place in the process of colour formation set up by the Bacillus fluorescens putidus, in which we have not only a colour resembling an aniline colour, but we have a distinct odour of trimethylamine, a substance nearly allied to the cyanogen compounds from which, as we know, the most beautiful red, blue, and yellow products are readily obtained when combining with iron in certain definite ways. Of course this is only given as an example of what might take place, and not as representing any accurate work that has been done, for it appears that up to the present very little definite knowledge has been obtained as to the nature of the pigments contained within the protoplasm of micro-organisms or of those diffused from it into the surrounding tissues. What we do know is, that a large number of the saprophytic decomposition-producing bacteria give rise, when grown under certain conditions, to most exquisite colour products, that by altering the conditions, as in the case of the Micrococcus prodigiosus—subjecting it to a higher temperature, for example—the power of forming these colours may remain in abeyance, the energy of the protoplasm being diverted into the formation of some other substance—in this instance, lactic acid. It has, however,

been objected that such lactic acid formation can only take place in the presence of sugar.[1]

In place of colour a certain amount of the energy of the organism may be diverted to the production of light. Although phosphorescent micro-organisms have for some little time been known to exist, and special organisms have been described as giving rise to phosphorescence in different regions, they have not been very carefully studied until comparatively recently, when Forster, Tilanus, B. Fischer, Kunz, Beyerinck, Lehmann, and Tollhausen have added very considerably to our knowledge, not only of the morphology, but of the biology of these special bacteria. In certain seas, and especially on clear dark nights at the mouths of rivers, any one who has rowed over them or steamed through them may have observed a beautiful phosphorescence or fluorescent glow at the bows or at the stern of the boat. As the oars dip into and leave the water, they seem to shine with a pale phosphorescent light. All kinds of explanations have been given of this beautiful phenomenon, but it is now known to be due in part or entirely to the presence of certain low forms of life amongst which the bacteria take an important place. First of all there was described *the* phosphorescent bacillus, then another was added, after this a third, and now there are described no fewer than six of these light-producing bacteria, arranged in three groups of two each. The biological characters of these groups have been very carefully studied by Beyerinck, who gave the result of his observations in a most admirable paper presented to the Royal Academy of Sciences, Amsterdam, 1890.

We may mention briefly some of the characteristic forms and features of these light bacilli. *Photo-bacterium phosphorescens*, which is 1.3 to 1.9μ long, and 1.5 to 1.7μ broad is motile and is surrounded by a gelatinous membrane; it is readily cultivated on fish broth containing a small quantity of peptone, or in sea-water; it also grows (though slowly) on ordinary nutrient gelatine or on nutrient gelatine to which herring brine or 8 per cent. common salt has been added; it brings about the fermentation of glucose and maltose, its power of producing light being apparently closely associated with these fermentations, as when oxygen is cut off both the light and

[1] A number of the more important colour-producing organisms will be found described in the Appendix.

the ferment-forming powers of the organism are at once interfered with, although growth and multiplication appear to go on much as usual. The process of light production is evidently somewhat of the nature of an oxidation of the food elements within the protoplasm under certain definite conditions, the most important of these conditions being the presence of oxygen and a temperature ranging between 3° and 35° C. The Photo-bacterium phosphorescens grows entirely as a surface colony, and although a ferment action is set up, there is no peptonizing power exerted, the gelatine remaining quite solid.

In a tube culture the organism grows down below the surface along the track of the needle, but the phosphorescence is developed only on the surface, where the organism can obtain a plentiful supply of oxygen. In the neighbourhood of the colonies, after a time, the gelatine takes on a yellowish-brown tinge. On all surface growths, whether on agar, gelatine or potato, the growth increases in thickness rather than in surface area. It grows best at from 15° to 25° C.

A rather pretty story is related in connection with this power of the organism to develop light. A lady, Madame Salomonsen, the wife of Professor Salomonsen of Copenhagen, was able to obtain photographs of the light bacillus made on gelatine plates and so cultivated them as to form the letters of a complimentary message to M. Pasteur ("Hommage à M. Pasteur"); the photographs came out very distinctly, and conveyed in a most delicate and striking manner the message which the lady wished to send.

Photo-bacterium Fluggeri is the most phosphorescent of all the light bacilli; it grows in nutrient gelatine as longer and thinner threads than the preceding form. It differs from it also in that, although it exercises its characteristic light function when supplied with peptone and glucose, maltose cannot take the place of glucose. Both organisms in setting up their fermentation processes bring about the evolution of CO_2 and hydrogen.

If a number of fresh cod or herring, the surfaces of which have not been allowed to dry, be placed between a couple of plates and kept at a temperature of about 15° C. or upwards, there may be made out at the end of about twenty-four hours a number of small phosphorescent points, and at the end of a couple of days the whole of the fishes are covered with a phosphorescent glow; but as putrefaction sets in this glow is gradually lost. There may be separated from these patches an organism 1·5

to 1.9μ long and 1.3 to 1.7μ broad; these rods have rounded ends and appear to divide exceedingly rapidly, in consequence of which the cells are usually almost round, and are then very like large micrococci, in fact they are sometimes compared to the Bacillus prodigiosus which was for long spoken of as a micrococcus. Sometimes a few organisms may be held together in a short chain; the bacterium is motionless, and no spores have as yet been observed. On plates prepared with peptone gelatine, to which a small quantity of glucose, and from two to three per cent. of common salt have been added, the organism develops luxuriantly, giving rise to small white mother-of-pearl-like colonies, about the size of a pin's head, with no surrounding zone of liquefied gelatine. Under the microscope these are seen as small, round, yellowish-white, granular drops, with sharp but irregular margins.

Another organism, the *Photo-bacterium Fischeri*, found in the waters of the Baltic, peptonizes gelatine, causing it to liquefy very rapidly. It can exist in a medium to which a small quantity of raw sugar has been added, this addition of sugar increasing in a most remarkable manner the intensity of the light given off, although a large quantity of the same material (three to five per cent.) interferes with, or altogether stops, the phosphorescent activity of the organism. This organism is motile; it occurs in short chains and grows on gelatine and agar, the former of which is liquefied by its action. Grown on plates, the colonies after making their appearance emit a kind of bluish-white light, and the organisms themselves as they lie at the bottom of the fluid gelatine have also a somewhat bluish tinge. It grows best at a low temperature, from 15° down to 0° C., or even lower.

Photo-bacterium Balticum also liquefies gelatine, but more slowly than the above. It is not, however, dependent upon glycerine for its growth, and is not nearly so sensitive to the presence of a considerable quanity of sugar, as it can live in a medium containing from three to five per cent. of that substance. These four forms are all what may be called peptone carbon bacteria, as they cannot develop their functions to their highest point without the presence of some substance from which carbon may be readily obtained such as sugar, glycerine, glucose, &c., as well as peptone, but the Photo-bacterium Fischeri and Photo-bacterium Balticum do not set up any ferment action as do the first two mentioned.

Beyerinck states that all four are best cultivated in fish broth made with sea-water, to which are added one per cent.

of glycerine, one-quarter per cent. of asparagin, and eight per cent. of gelatine.

The *Photo-bacterium Indicum* of the West Indies and the *Photo-bacterium luminosum* of the North Sea, both liquefy gelatine very rapidly, and appear biologically to be much more like ordinary putrefactive micro-organisms, though the most favourable conditions as regards temperature for the performance of their functions differ somewhat; that from the West Indies giving off most light at from 30° to 35° C., that from the North Sea being most active in this respect at about 15° C. Both of them may multiply and give off light in peptone gelatine without requiring the presence of any sugar; in fact, they are both extremely sensitive to the presence of this substance, one per cent. preventing the phosphorescence, and three to five per cent. interfering with the liquefaction of the gelatine and eventually killing the bacteria, though, if a small amount of asparagin also be added to the medium in which the West Indian phosphorescent bacillus is growing, light may continue to be given off for some time. The Photo-bacterium Indicum is a motile rod of medium size which grows very readily on all the ordinary nutrient media; the light is best seen in this case by taking a fish that has been boiled and inoculating with a fragment of the artificial culture; there then appears in a very short time a soft glistening point, which is found to be covered with bacteria. On darkening the room a beautiful bluish-white light can be seen at the point of inoculation. The light of Photo-bacterium phosphorescens is yellowish.

Beyerinck gives a number of experiments to show that the formation of light bears no direct relation either to the respiration or to the growth of the organisms, but he finds that certain food substances are necessary for this light to make its appearance, although the growth of the colonies may go on perfectly well without oxygen, even if the formation of light be completely stopped. As soon as the organism is grown anærobically, certain food substances also become necessary.

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CHAPTER XXI.

Poisonous Alkaloids and Albuminoids.

Early Observations—Burrows, Kerner, Panum, &c.—Ptomaines—Leucomaines—Brieger's Work—The Alkaloids, Poisonous and Non-Poisonous—General and Local Reactions—Structure and Composition—Sketch of Chemistry—Cholera Poisons and other Members of this Group—Mussel Poisons: two Classes, viz., those Without Oxygen and those Containing Oxygen—Ptomaines the Result of the Activity of Micro-Organisms—Löffler's Experiments on Diphtheria Poison, an Enzyme—Roux and Yersin's Diastase—Hankin, Albumose—Brieger and Fraenkel, Toxalbumen—Method of Preparation of Albumoses and of Toxalbumens—Near Relationship of Proteid Poisons and the Ptomaines—Martin's Observations.

It has long been known that the products of putrefaction, especially those formed during the putrefaction of fish, are extremely poisonous. In 1814 Burrows, in this country, described such a poisonous substance in putrefying fish; in 1820 Kerner described a poisonous alkaloid which resulted apparently from the decomposition of albumen; it resembled in its physiological action a substance that he had found in poisonous sausages, and which he compared with atropine as regards its toxic reaction. In 1856 Panum was able to obtain a substance from decomposing animal matter which appeared to him to be derived from albuminoid substances through the action upon them of micro-organisms. He looked upon this as a purely chemical material. He was able to isolate it by dissolving it in water or alcohol; in quantities of about six centigrammes it proved fatal to dogs, and this substance, to which the name sepsin was given, came to be looked upon as the cause of putrid infection or intoxication. Twelve years later Bergmann and Schmiedeberg obtained what they took to be a similar substance; it contained nitrogen, could be crystallized out and separated, and was also evidently the result of a putrefactive process.

The first mention that is made of the probable chemical composition of such organic poisonous substances is found in a paper by Zuelzer and Sonnenschein, who describe as an alkaloid a substance that they were able to obtain from decomposing animal matter, which they said closely resembled atropine in its physiological actions; it caused dilatation of the pupil, paralysis of the inhibitory fibres of the vagus, so allowing the heart's action to become accelerated, and paralysed the non-striped muscular fibres of the intestine. This alkaloid was supposed to resemble the vegetable alkaloids of which a considerable number had then been described: more recent observations have shown that these alkaloids are very nearly related, from the fact that they all appear to have as a common basis or ground structure a substance named pyridine, of which more immediately. In 1872 Armand Gautier described as the products of albuminous decomposition a number of alkaloids; and Selmi, between 1871 and 1880, described what he called cadaveric alkaloids or ptomaines, and he was able to obtain two new alkaloids from pure albumen that had undergone putrefactive changes. Pouchet in 1880 described an alkaloid in urine, and in 1882 Bouchard also described alkaloids in the human urine, which, he considered, were the result of the decomposition of proteid matters in the alimentary canal, and which were excreted from the body by the intestines and by the urine, through the kidneys. He concluded that these alkaloids are usually found in health in certain definite quantities, whilst in certain diseases—typhoid fever, for instance—they may be enormously increased in quantity, and can then be separated from the urine in very considerable quantities.

The first ptomaine separated pure was obtained by Nencki, then Brieger obtained several of these alkaloids from pure cultivations of micro-organisms, but as early as 1880, Pasteur, after failing with the sterilized products of Anthrax, was successful in producing the symptoms of Fowl Cholera with the sterilized products of that organism, *i.e.*, with the poisonous alkaloids or proteids. Of those in the pathogenic group Brieger first described the substances that he was able to obtain from pure cultures of the typhoid bacillus and of the tetanus bacillus. From the former he obtained typhotoxine; from tetanus cultivations tetanine, which produces characteristic tetanic symptoms in animals, tetanotoxin

which also produces some of the symptoms of that condition ; and two other alkaloids both of which give rise to certain definite symptoms, acting physiologically somewhat like strychnine. The importance of the presence of these substances in pure cultivations of pathogenic organisms can scarcely be overestimated. In the vegetable kingdom there are recognized whole series of substances that have an alkaline reaction, combine with acids to form salts, are evidently formed by the protoplasm of the plants in which they are found, and which, injected into the tissues of an animal or taken by the stomach, exert a most energetic poisonous action either upon the end organs in muscles or upon the muscles themselves. Of these we may take such well-known examples as strychnine, atropine, nicotine, cinchonine, thebaïne, morphine, brucine, and others. They are all of them built up by vegetable cells and all exert a specific action on animals. Similarly it is found that bacteria —minute vegetable organisms—can build up substances, as they grow in dead or living animal tissues in which they are living as saprophytes or parasites, which substances exert a most deadly influence on the nerve centres or the parts above mentioned of animals in which they are formed or into which they may be injected, but in addition have an extremely injurious local "caustic" influence, giving rise in many cases to the death of the tissues with which they may come in contact at the points where the poison is formed, or at the seat of inoculation. Thus tetanine is a substance that appears to act through the nervous system much as does the alkaloid strychnine, whilst in sepsine, material formed by those bacteria that are found in a local abscess, we have a powerful acrid substance which by its caustic action causes the death of the cells with which it is allowed to come immediately in contact. Some of the most deadly of the poisons formed by micro-organisms, however, are not of the nature of alkaloids, but are said to belong rather to the classes of globulins and albumoses. A number of them, however, give some of the reactions of the alkaloids, but they must not on that account be looked upon as belonging to that group. Brieger includes under the term ptomaine all nitrogenous bases that are formed by the action of bacteria, such of those as are poisonous being spoken of as toxines. It thus happens that certain ptomaines that are formed

during putrefactive processes are non-poisonous, whilst others formed during the same process may be extremely toxic ; a considerable number of the non-poisonous forms especially have been manufactured synthetically or by analysis ; trimethylamine and dimethylamine, for example, both of which as well as Pentamethyline diamine (Cadaverine) may be obtained as putrefactive products and may also be prepared synthetically by the chemist, the artificial Cadaverine in sufficiently large doses giving rise to all the symptoms and post-mortem appearances of an attack of cholera. Of the poisonous kinds a substance known as betaine, which is closely related to nicotine and glycocol, has been found in both vegetable alkaloids, and in the human urine, in the latter being apparently the result of decomposition changes going on in the alimentary canal under the action of bacteria. It is one of the substances formed during the processes of decomposition of albuminoid bodies ; it also has been prepared in the laboratory.

The formulæ of a number of these substances is exceedingly complicated, but they all appear to be allied to or even to be derived from what is known as the pyridine base, a non-saturated alkaloid, derived from the products of dry distillation of bone or wood, from the ammoniacal liquor of coal distillates, and from the action of heat or strong alkalies on the vegetable alkaloids. Its composition will be best understood by reference to the diagram given by Pictet (Fig. 1). It is very nearly related to benzol ; the only difference being that one of the CH groups is replaced by N. Thus benzol has a formula (Fig. 2). Neither of these substances has all the carbon bonds satisfied, so that each C and the N having a bond free (those drawn within the hexagon) to combine with other atoms, or groups of atoms, there may be enormous numbers of derivative or addition and substitution products formed ; for instance, all the bonds free in pyridine may be saturated by the addition of a single atom of hydrogen to each, when we have what is known as the piperidine alkaloid (Fig. 3), and by adding one, two or three ethyl or methyl groups to the free bonds in place of one, two, or three of the H's we may obtain methyl piperidine,

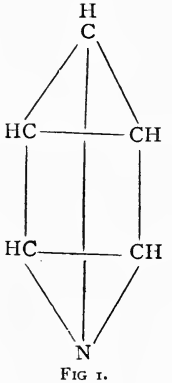

FIG. 1.

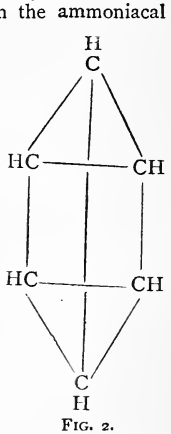

FIG. 2.

dimethyl piperidine, and so on throughout the whole group. In fact, where there are so many bonds to be acted upon the number of organic compounds is almost endless, and it can be readily seen how substances varying much in chemical composition can still be arranged in groups under a common head; the members of each series having very much in common not only as regards their chemical character, but, as has recently been pointed out, as regards their physiological action. As a considerable number of the benzol series have been manufactured by the chemist, it comes quite within the range of possibility that a number of the ptomaines (especially those of the pyridine series) may also be built up synthetically.

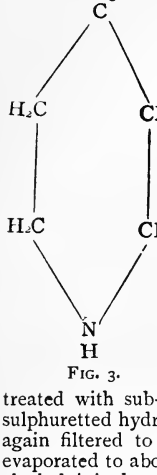

FIG. 3.

The most important of the methods used for the separation of the ptomaines is that used by Brieger, who is perhaps the greatest authority on this subject. He goes to work with the salts of the heavy metals, and with picric acid. When he wishes to separate an alkaloid from any putrefying mass, this mass is first boiled with water, and then filtered; the filtrate is then treated with sub-acetate of lead; from this the lead is precipitated by sulphuretted hydrogen which is passed through the filtrate, and the fluid is again filtered to keep back the lead sulphide. This second filtrate is evaporated to about one-third of its original bulk, and is mixed with amyl-alcohol, it is then thoroughly washed with water, and again reduced in bulk by evaporation, and sulphuric acid and ether are added; the ether is evaporated, after which the remaining liquid is concentrated by careful evaporation to one-third of, or one-fourth of, its bulk; the evaporation driving off most of the volatile fatty acids present, after which the fluid neutralized by the addition of baryta, is again filtered, carbonic acid gas is passed through it, by which baryta carbonate is thrown down which is separated by filtration. After careful heating over a water bath, the fluid is cooled, and bichloride of mercury is added, when a somewhat dense precipitate is formed. This precipitate is carefully washed and decomposed by sulphuretted hydrogen, when sulphide of mercury is thrown down; the fluid is again filtered and the filtrate is evaporated to obtain as great concentration as possible. From the liquid so obtained all inorganic substances crystallize out first; these are removed, and then in the fluid that remains "organic" acicular crystals are thrown down. These may be dissolved in water, but they are insoluble in absolute alcohol, ether, benzine, or chloroform. It is found that the substances so given, the ptomaines, may be precipitated by the salts (especially the chlorides) of the heavy metals. These precipitates or crystals differ, however, very considerably as to their solubility; hydrochloride of putrescine obtained by the above method separates out in acicular crystals, and on the addition of chloride of gold gives very insoluble crystals of an octahedral form, whilst on the addition of chloride of platinum, octahedral crystals which are much more soluble, are also formed. Phospho-molybdic and phospho-wolframic acid added to this substance give respectively a yellow and a white crystalline precipitate. Iodide of mercury dissolved in iodide of potassium also gives rise to the formation of prisms;

with ferrocyanide of potassium there is a yellowish amorphous precipitate; with picric acid a yellow precipitate composed of delicate needle-shaped crystals; and with a watery solution of bichloride of mercury an exceedingly insoluble acicular crystalline precipitate is thrown down. This substance and the reactions obtained with it may be taken as typical of the whole group, although there are certain differences; for instance, cadaverine treated with chloride of gold gives a very soluble substance, whilst with chloride of platinum there are thrown down well-formed very insoluble crystals. Mydaleine is exceedingly soluble in most of its combinations, and it is at present almost impossible to separate it from the mother liquid; in fact, its salts have not yet been separated, and in consequence it has been found impossible to determine its exact chemical nature. These, along with saprine, were obtained by Brieger from flesh that was being decomposed by the action of putrefactive micro-organisms.

Cadaverine and putrescine are both poisonous, giving rise to local death of the tissues along the course of the intestine; they have, however, not nearly such marked general toxic activity as have some of the other alkaloids. They are all somewhat volatile, and cadaverine as we have already seen is very readily formed where the cholera bacillus is allowed to grow and act on proteid matter such as egg albumen. Of a more poisonous nature is neurine, a substance that is also formed in connection with the putrefaction of flesh; it has been separated by Brieger and is readily obtained in crystalline form by the addition of chloride of platinum. Choline, which was supposed at one time to be identical with neurine, and is by Brieger said to be the same substance, is really hydrated neurine. These two substances are exceedingly toxic and appear to have an action very similar to the vegetable poison, curare. A very similar substance, muscarin, has been described by Schmiedeberg and his pupils, as occurring in a kind of poisonous mushroom, and was found to have a composition very like that of choline and neurine. It is an extremely toxic substance and is specially interesting from the fact that we have it formed as a vegetable alkaloid in the mushroom, whilst it has also been found in putrid fish, thus giving us another link between those so-called animal alkaloids found in decomposing albuminoid matter and the vegetable alkaloids. This substance is a most powerful muscle poison, its action being somewhat like that of eserine.

It will be remembered that not long ago there was a sad case of poisoning in Dublin, in which the wife and family of a well-known journalist died after partaking of mussels.

The poison in this case was probably an alkaloid substance that was separated from decomposing mussels by Brieger, who gave to it the name of mytilotoxine. Dr. Vaughan in America separated from cheese that had undergone putrefactive changes a substance that he called tyrotoxine, and he was able to separate from some ice cream that gave rise to most acute poisoning a very similar substance.

Jacquemart, giving an account of these ptomaines, divides them into two groups—those that are fluid and are volatile, that have a peculiar characteristic smell, and that contain no oxygen, being in the first group; those in the second are solid, non-volatile and contain oxygen. Those of the first group are soluble in ether and also slightly in amyl-alcohol and chloroform. The members of the second group are usually crystalline, are soluble in water, but are insoluble in alcohol, benzine, and chloroform. Although they are extremely unstable they unite with acids, in excess of which, however, they are soluble, when we have first a red colour and then a brown deposit of acicular crystals. An excess of chloride of platinum or strong light usually causes their disintegration. It should be remembered that corrosive sublimate does not precipitate some of these alkaloids, although there is undoubtedly a salt formed which can be obtained in white crystals by evaporating from watery solutions. The only substance that gives invariable reactions with all these ptomaines is phosphomolybdic acid.

The following ptomaines contain no oxygen: Parvoline, described in 1881 by Gautier and Étard, who obtained this substance from putrefying mackerel and horse-flesh; its formula is $C_9H_{15}N$ (also given as $C_9H_{13}N$). It is a light yellow substance readily soluble in water, alcohol, ether, and chloroform, it turns brown on contact with the air; with chloride of platinum it forms a somewhat insoluble crystalline flesh-coloured substance, which rapidly becomes rose-coloured on exposure to the air. These authors also described a substance Hydrocollidine (formula $C_8H_{15}N$, sometimes given as having two atoms less hydrogen). It is a colourless, oily fluid, becoming brownish on exposure to the air; when treated with carbonic acid gas it becomes sticky. It forms a double salt with chloride of platinum, a pale yellow crystalline insoluble substance, though it dissolves on heating without undergoing any disintegration. Collidine, with the formula $C_8H_{11}N$, was first obtained from decomposing pancreas and gelatine. It is a yellowish, mobile fluid with an extremely offensive odour, very soluble in water, but much more soluble in methyl and ethyl alcohol and in ether. Neuridine, $C_5H_{14}N_2$, Cadaverine, $C_5H_{16}N_2$ (sometimes given as isormeric with neuridine). Putrescine, $C_4H_{12}N_2$, Saprine, $C_5H_{14}N_2$, and Mydaleine, all belonging to this group, have been already mentioned. The ptomaines that contain oxygen hold a kind of intermediate position between the above group and the Leucomaines or physiological alkaloids.

Neurine, $C_5H_{12}N$ (OH), is a strong base exceedingly soluble in water. Choline, $C_5H_{15}NO_2$, Muscarine, $C_5H_{15}NO_2$ (or $C_5H_{13}NO_2$, or Choline from which H_2 has been removed by nitric acid), and two other ptomaines described by Pouchet, and having the formulæ $C_7H_{13}N_2O_6$ and $C_5H_{12}N_2O_4$, make up the solid ptomaines of the second group; whilst Gadinine $C_7H_{17}NO_2$ is not solid, although in other repects it resembles the members

of this second group. To this group also belong Mytilotoxine, $C_6H_{15}NO_2$, Typhotoxine, $C_7H_{17}NO_2$, and Tetanine $C_{13}H_{22}N_2O_4$, which appears to be really a double pyridine molecule, and is therefore probably a mixture, as are also a number of the others above mentioned.

Most of these substances are found to be associated with the decomposition of dead material by micro-organisms, but it has long been known that substances similar in many respects (some of them of an exceedingly poisonous nature) are formed in the body of the living subject, resulting from the purely physiological nutritive changes in the protoplasm of the various organs and tissues in the body; they are in fact excretory products which must be got rid of, and which if retained interfere, in some cases very materially, with the vitality of the protoplasm. These were named leucomaines by Gautier to distinguish them from the ptomaines; they are fully described in physiological text-books with the uric acid and creatinine groups of substances, to one or other of which they belong as far, at all events, as their chemical composition is concerned. Some of these leucomaines are, as we have said, exceedingly poisonous, and when retained may give rise to very serious toxic symptoms. Brieger and others, however, deny that any such bodies are formed or at any rate have yet been found in the tissues of the living body or that they owe their existence to the tissues. They consider that they are simply absorbed from the intestinal canal where they are formed by bacteria.

In 1887 Löffler, when examining the products of a pure culture of the diphtheria bacillus that he had obtained, found that the fluid from which all the organisms had been removed by filtration through a porous porcelain cylinder, when injected into a guinea-pig gave both the local reaction and the paralytic symptoms that were obtained when the organism itself was introduced into the subcutaneous tissue. In order more readily to determine the nature of this material, he added to a pure culture of the organism a quantity of glycerine; this when filtered and dropped into absolute alcohol gave a floculent precipitate which could be freely washed with alcohol without passing into solution, but on the addition of water it was again dissolved. It could be again precipitated by alcohol, and after the passing of carbonic acid gas through the precipitate, it retained a certain toxic

property,—still setting up distinct local reactions. Löffler eventually concluded that he was dealing with an enzyme.

I have already mentioned Roux and Yersin's experiments, from which they also concluded that they had obtained a substance similar in many respects both to a disastase and to an enzyme. Hankin, working from the fact that a poisonous albumose had been discovered in snake poison, set about the task of isolating an albumose from anthrax cultures. He made a cultivation of anthrax bacilli, then precipitated it by large quantities of absolute alcohol and washed the precipitate thoroughly to dissolve out any ptomaines that might be present; this precipitate was filtered and dried, then re-dissolved in water and the solution passed through a Chamberland filter. This albumose is very similar in its characters to the albumoses ordinarily described, which really consist of albumen that has been altered by hydration either by super-heating by steam under pressure, or during the process of natural digestion, in which albumens are converted stage by stage into peptones. These albumoses are intermediate non-coagulable hydrated albumens. Some forms are soluble in water, others are insoluble. That described by Hankin as formed by the anthrax bacillus is a soluble form. Brieger and Fraenkel obtained what were apparently similar substances from pure cultivations of cholera bacillus, typhoid bacillus, tetanus bacillus, from staphylococcus aureus, and diphtheria bacillus, with all of which they were able to produce toxic effects, some at least of which were similar to those met with as the result of inoculation with the bacteria themselves. These observers, however, did not separate from the albumoses that were formed any enzymes that might be present, consequently they were working with a mixture of substances. The products that they obtained gave most of the reactions of albumoses; they were certainly toxic, but they probably contained both enzymes and albumoses.

As these albumoses, described though not recognized under that name by Wooldridge, are destined apparently to play a most important part in the production of immunity against disease, it may be well here to give a short description of the methods adopted by Hankin to obtain his albumose from the anthrax cultures, and by Brieger and Fraenkel and Babes from cultures of other organisms (Sidney Martin has been able to

separate from anthrax cultures, in addition to a poisonous alkaloid, two albumoses, which apparently represent slightly different stages in the transformation of albumen into peptone) : it may be well also to give the characteristic reactions that are obtained with these albumoses.

Hankin's method is as follows : A 1 per 1,000 pure solution of Liebig's extract of meat is carefully sterilized by being heated in a small sterilizer for two or three hours on two or three successive days ; to the fluid so sterilized a quantity of pure fibrin is added and the whole is again sterilized "by repeated heating to boiling point for a short time only on each occasion" ; if this is heated for a longer period a considerable quantity of the fibrin is digested and converted into peptone, a substance that would interfere very considerably with the after-examination, in addition to which the anthrax bacillus would have little material left on which to exert its "peptonizing" function. This is inoculated with blood from an animal that has died from anthrax and is "kept at the ordinary temperature." The cultivation is allowed to go on for a week, at the end of which time the albumose is extracted. If the flask be kept at the temperature of the body, 37° C, the transformation of the albumose into the peptone goes on much more rapidly. To separate the albumose the culture fluid is first acidulated with acetic acid, and then thoroughly saturated with ammonium sulphate, when there is thrown down a bulky precipitate of albumose. In order to concentrate the solution, instead of using Brieger's method of evaporation *in vacuo* or under pressure at a low temperature he resorted to the method of diffusion or dialysis. A quantity of thymol, to prevent putrefaction, is added to a watery solution of the albumose, and the whole is placed in a parchment sausage skin which is immersed in a foot glass full of methylated spirit. The spirit can be changed after some hours if it is necessary to prolong the process, but this is not usually necessary. "In this way," says Hankin, "I have been able to bring 400 cubic centimetres of albumose solution down to 100 c.cm. in the course of a single night, at the ordinary temperature without risk to the albumose or trouble to myself. The concentrated solution is then poured into absolute alcohol, which precipitates the albumose and removes any impurities that might be derived from the methylated spirit. This prolonged treatment with alcohol will tend to remove any free ptomaines or other substances soluble in alcohol." In order to remove any ferments that are capable of acting along with the albumose, Hankin, following Roux and Yersin, adds a quantity of lime water to his solution, so that, on the addition of a solution of phosphoric acid, a gelatinous precipitate of calcium phosphate is produced, in the formation of which ferments are usually entangled and carried down, and on filtration a purer solution of albumose is obtained.

Brieger and Fraenkel have adopted Hankin's method in the preparation of their toxalbumens, but instead of dialyzing, they evaporate down *in vacuo* at a low temperature until the liquid has been reduced to less than a fourth of its original quantity. They again wash in alcohol and filter.

Brieger and Fraenkel found Millon's reagent gave a white precipitate, which on heating became brick red in colour [1] this indicating its proteid nature ; it is precipitated by magnesium sulphate in saturated solution ; it is therefore not an ordinary albumen ; whilst on the addition of a drop of dilute sulphate of copper solution and a slight excess of potash solution (the so-called biuret reaction) a rose red and not a violet colour is given, indicating that this material belongs to the albumose rather than to the globulin group. There are a number of other tests which it is not here necessary to describe.

It must be remembered, however, that these proteid poisons and the ptomaines are very closely bound up with one another. Martin indeed holds, that as some of the albumoses are less toxic than the alkaloids with which they occur and as they also have a marked alkaline reaction, the alkaloid may really be bound up in a nascent condition in the albumose molecule. This may undoubtedly be the case with certain vegetable alkaloids and albumoses, but in the cases of diphtheria and tetanus, it would appear that some of the so-called poisonous alkaloids owe their specific properties to the presence of minute traces of an enzyme, or proteid poison that is present along with them. It has even been suggested that the alkaloid may be the poisonous agent formed by bacteria, whilst the albumose is to be looked upon as the protective agent. This, however, can scarcely be maintained except in certain cases, as in the case of tetanus the poisonous agent is certainly not of an alkaloidal nature. Still, these points should be borne in mind by any one going to work at the question. It seems to be undoubtedly the case, that in accordance with the well-known fact that certain products of micro-organisms, such as those of acid or alcoholic fermentation, act deleteriously on the bacteria that produce them, especially as they accumulate in large quantities. On the other hand, it may be that these, or similar products in a more or less dilute form, may be necessary

[1] Millon's reagent is prepared by adding one part of mercury to two parts of strong nitric acid, gently warming until the mercury is thoroughly dissolved : to one part of this mixture two parts of water are added ; a precipitate is formed ; the supernatent fluid only is used. A few drops of this solution give the above characteristic reaction with all proteid materials held in solution except in the presence of common salt.

in order to allow of the growth of bacteria as parasitic organisms in animal tissues.

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CHAPTER XXII.

Vaccination.

Natural Immunity—Ingrafting of Small-pox—Jenner's Discovery—Klebs—Pasteur—Chauveau—Grawitz's Theory—Buchner's Theory—Metschnikoff's Work—Greenfield's Observations on Anthrax—Toussaint's Vaccinal Fluid—Pasteur's Classical Experiments—Other Methods of Diminishing Virulence—Chauveau's Soluble Poison Experiments—Protection by Alkaloids, Albumoses and Toxalbumens—Saprophytic "Anthrax"—Hueppe and Wood—Antagonism by Blue-pus Products—Immunity not the same as Antagonism—Summative Action of Bacteria.

It has long been recognized that in a number of infective diseases, one attack confers a certain immunity against that special disease in the future. This protective influence may last for a very considerable period, or there may be merely a temporary immunity. It was early noticed, however, that the severity of the primary attack of the disease appeared to bear no very definite relation to the degree of immunity acquired by the patients, although immunity of an animal usually bears a very direct relation to the strength of the vaccine which it has withstood. In fact every degree of immunity may be produced, though this does not always correspond with the severity of the symptoms. Immunity appears to be governed by no very regular laws, for it is found that even in the same species, individuals may differ very considerably as regards susceptibility to first or subsequent attacks of some of the infective diseases. It is known, for example, that whilst certain people have never more than a single attack of measles, others may have several, and that similarly, certain individuals are specially liable to the recurrence of scarlatina, suffering no fewer than three, four, or even more attacks. When differences of species are taken into account these differences of susceptibility are even more marked ; thus sheep and cattle are protected against subsequent attacks of anthrax by

a first, whilst in the human being and in the horse, anthrax may occur again and again, though in all probability a temporary immunity is conferred by an attack even in these extreme cases.

Cholera and typhoid fever both belong to the group of diseases of which one attack usually confer a certain degree of immunity against a second, the period during which this may last, however, varying in different cases ; in some it extends over a few years only, whilst in others it appears to be almost permanent. The acute exanthemata, of which we may take small-pox as an example, belongs to this latter class.

Amongst the Turks the ingrafting or inoculating of small-pox matter was very early resorted to as a routine practice in order to produce a milder but protective attack of the disease. We have a record of this in one of Lady Mary Wortley Montague's letters, dated 23rd March, 1718, and written from Belgrade, in which she describes the inoculation of her little boy three years old. She says : " The boy was ingrafted last Tuesday, and is at this time singing and playing, very impatient for his supper ; I pray God that my next may give as good an account of him. I cannot ingraft the girl ; her nurse has not had the small-pox." The after progress of the patient in this case was satisfactory, and, as is well known, Lady Mary was afterwards instrumental in drawing attention to the matter in this country.

Then came Jenner's remarkable discovery that similar immunity against small-pox might be conferred by the inoculation of lymph from animals that were suffering from what was known as cow-pox ; an attack of the one disease in this case conferring an immunity against an attack of the other.

There has for long been the utmost anxiety on the part of physicians and others to obtain some explanation of these remarkable facts. Klebs and Pasteur explained them on the assumption that during the course of a first attack of a disease some material that was essential for the nutrition of the pathogenic organisms that had by this time been found to be associated with some of these diseases, had been used up, and the supply being cut off, the organisms were no longer able to exist in the body and exhibit the characteristic evidence of their presence. This substance must have been present in exceedingly small amount, as no

alterations in the composition of the blood or other fluids of the body could be determined by any methods of chemical analysis that could then be applied. Then came the theory advanced by Chauveau and others : that just as micro-organisms when growing in artificial media produced excretory products, the presence of which was inconsistent with the continued life of the organism ; so in the body, bacteria during the course of the disease gave rise to some material which might act deleteriously on their own protoplasm, and which, remaining in the body for a considerable length of time, interfered with the growth of any similar organisms that might in future be introduced. Here, again, these special chemical products could not be detected in the blood, and must have been present in such infinitesimally small quantities that it is difficult to see how they could exert any very marked influence on the activity of the bacteria ; whilst, as Flügge points out, our knowledge of the action of the tissues on foreign bodies of various kinds would lead us to the conclusion that any such material would be very rapidly eliminated. Then Grawitz suggested that in any battle between the cells and the bacilli that may occur in the body during the course of a disease, if the cells can but manage to obtain the upper hand and to destroy the bacteria, they should become hardier, as it were, through the training of the contest, their vital energy and assimilating power should be increased, and they should thus become able to deal in a more summary manner with any organisms with which they might afterwards be brought into contact. Then came Buchner's theory of the inflammatory cause of immunity, which offered another explanation, or modification of Grawitz's explanation. He argued that bacteria made their way into the body at certain special points, these points or seats of election differing in different diseases, and that in consequence of the development of the bacteria, there was a reactionary alteration, inflammatory in its nature, in the tissues, which fitted them for the future to resist the special organism that had previously made the attack ; this minute alteration in the function of the special cells at the seat of invasion enabling them to resist the further action and invasion of the same organism even at a considerably later period. Again based on the same principles as Grawitz's theory came the now celebrated Metschnikoff theory. Metschnikoff holds that the protection against the

attacks of micro-organisms on the body is entirely due to the action of the amœboid cells of the body, that these cells are living pieces of protoplasm, that they are constantly taking into their own substance all foreign particles which find their way into the body, that wherever there is an extra demand on their energies, a large number are attracted to the point at which the work is to be done, and that these cells acting on the micro-organisms just as they do on foreign bodies, take them up into their substance, digest and convert them partly to their own uses, and gradually throw into the circulating fluids of the body small quantities of effete substances which are removed by the ordinary physiological channels. Some observers, however, hold that the process is not so simple as it would appear ; certain bacteria secrete substances which appear to exert a paralysing effect on the cells, and may so alter them that they are unable to perform their proper functions ; whilst, on the other hand, the cells secrete in the performance of their work a material which has an unfavourable influence on the activity of the bacteria. This at first sight is an extremely feasible explanation, but when we come to consider more carefully the conditions under which immunity against diseases is conferred, we find that, although in certain cases an attack of one disease protects against an attack of a more serious and deadly malady, this occurs only within certain definite and well-defined groups of diseases ; there appears, therefore, to be something more than a mere general protective influence generated within the body. We must have specific powers of resistance developed in or by the cells in order that they may be able to resist specific bacterial activities, and the effects of specific bacterial poisonous products. I have in previous chapters spoken of the effects of the bacteria and of their products in protecting against the various diseases to which they themselves give rise ; it may now be well to give a concrete example of the theories that have been advanced as to the nature of this protective inoculation ; let us take the development of the methods that have been devised for protecting animals against anthrax.

It was found that on devitalizing the anthrax organism by one of several methods it might be introduced into the subcutaneous tissue of a sheep without giving rise to any very serious symptoms. The first note that the virulence

of the anthrax could be modified was communicated in this country by Greenfield, on June 17, 1880, to the Royal Society of London. This observer found that by cultivating anthrax bacilli through several successive generations, in fluid taken from the front part of the eye of the ox, he was able to obtain a virus so modified that when injected into animals susceptible of being affected by ordinary anthrax, the animals did not die, whilst in animals injected with aqueous humour cultures of the twelfth generation no symptoms whatever were developed ; earlier cultures giving rise to a modified form of anthrax only.

In the following month Toussaint intimated to the French Academy that he had been able to obtain a protective anthrax vaccinal fluid, *i.e.*, one that might be inoculated into an animal without causing death, and which conferred on animals so inoculated protection against a second attack of the disease.

His original method of procedure was as follows :—The blood of an animal dead of anthrax was carefully defibrinated by whipping and straining through linen and then through ten or twelve thicknesses of blotting-paper. Some animals were undoubtedly protected by the inoculation of this prepared blood, but the method was very uncertain ; and in some cases the vaccine itself caused the death of the animal. Instead, therefore, of filtering the defibrinated blood, he heated it for ten minutes to a temperature of 55° C. With material so prepared he was able, by inoculation, to render an animal immune to the action of the more virulent anthrax bacillus.

Pasteur, who concluded that the vaccination depended not on the bacterial products but on an alteration in the virulence of the bacillus which might be the result of the altered temperature, subjected the bacilli to a temperature of from 42° to 43° C. ; these were found to have lost all their vitality at the end of about six weeks, this loss of vitality during the six weeks going on progressively in proportion to the rise of temperature. It is stated that at the outset the pure culture had all the virulence of anthrax blood ; whilst only half of the sheep inoculated with the culture, that had been heated for twelve days succumbed to anthrax. On the twenty-fourth day of heating, the culture, when inoculated, although giving rise to mild febrile disturbance, did not cause the death of a single animal. It was found, too, that if now, twelve days after the first inoculation, these surviving animals were inoculated with a culture from the

twelfth day, which before had killed half the animals, there was still only a slight febrile disturbance, and none of the inoculated animals died. Virulent anthrax blood, might, after a further interval of twelve days, be introduced into animals that had been subjected to the double inoculation, without giving rise to anything more than a slight febrile condition similar to that noticed as resulting from the inoculation of the modified virus. If, however, virulent anthrax blood was introduced into animals in which only the first protective inoculation had been made (*i.e.*, with material that had been cultivated for twenty-four days), a large proportion of the animals died. It was evident, therefore, that it was absolutely necessary to use both a first and a second vaccine if the protection was to be complete. This attenuation was not confined to the generations of bacilli that were directly acted upon. If the temperature were lowered to about 35° C., vegetative activity was immediately set up, rods in enormous numbers were formed, and eventually spores might be observed in these rods. Now comes the interesting fact: the attenuated properties of the original bacilli were handed on to the spores; these spores might be kept in a latent condition for a considerable length of time, and on being introduced into media suitable for their growth they sprouted out, *not into virulent anthrax bacilli, but into modified anthrax bacilli*, so that the conservation of the vaccine (on silk threads) became a comparatively simple matter.

Pasteur attributed the diminution of the virulence of the anthrax bacillus to the action of heat in the presence of oxygen, but Chauveau, working on Toussaint's plan, found that heat alone continued for a very short period was quite sufficient to modify the virulence of the bacillus. Blood is taken from a guinea-pig about thirty-six or forty-eight hours after inoculation with an active virus; it is carefully defibrinated and is run into small glass pipettes of about 1 mm. in diameter. One end is carefully sealed by heat so far from the blood that the heat cannot injure the organisms; the sealed end containing the blood is plunged into water at a temperature of 50° C., and is kept in this for about a quarter of an hour; in this way is prepared what is known as the primary vaccine, corresponding to that obtained by Pasteur, by heating for twenty-four days. The second vaccine is heated for only nine or ten minutes, and corresponds to that obtained by heat at the lower temperature for twelve days. These vaccines are injected in the same way as Pasteur's at intervals of from ten to fifteen days and they are found to protect very fully against the most active virus. Where large quantities of the vaccine have to be made by this rapid method, Chauveau used sterilized broth which is inoculated with anthrax blood from a newly killed animal; the flasks are then kept at a temperature of 43° C. for about

twenty hours and the temperature is then raised to 47° C. for a period of three hours. This is the second vaccine. The first vaccine is a culture made from one that has been heated for three hours at 47° C.; this is incubated for from five to seven days at 35° to 37° C., and then for one hour at 80°. Of these vaccines two drops are used for inoculating a sheep and four for cattle, the animals being injected, cattle on the outer aspect of the ear, sheep inside the thigh. The great drawback associated with the use of vaccine so prepared is that it cannot be preserved for any length of time, as under cultivation the original virulence is regained at once.

Pasteur's classical experiments made in May 1881 gave abundant evidence of the utility of this method of treatment. On the 5th of May, twenty-four sheep, one goat, and six cows were inoculated with a protective vaccine; twelve days afterwards they were again inoculated with a somewhat stronger vaccine than that at first used, and on the 31st of May these animals that had already been vaccinated, and twenty-four sheep, one goat, and four cattle that had not previously been inoculated with the protective virus, were injected with material from a virulent anthrax culture.

On the 2nd of June all the animals that had been protected were found in apparent health; of the others, twenty-one sheep and the goat were dead, two other sheep were dying, and the other was attacked later in the day. The non-vaccinated cows were not dead, but they had all marked local symptoms. Next day one of the vaccinated sheep died, but its death was said not to be due to anthrax. The experiment was repeated in a modified form by injecting a quantity of blood and spleen pulp from a sheep that had died of anthrax into sixteen non-vaccinated animals, and into nineteen protected animals, with the result that on the third day all the unprotected animals but one had succumbed, whilst the others remained apparently healthy.

Equally good results were not always obtained by other experimenters, but in some cases, at any rate, the experiments appear to have failed through want of attention to detail rather than from any defect in the method itself, and from the failure to recognize that the initial virus is not always of the same strength, that different animals have very different degrees of susceptibility and natural immunity, and that the quantity of the virus injected very materially alters the conditions of the experiments. No tissues can be expected to cope equally well with large and with small doses.

A number of other methods of preparing a less virulent (or vaccine) material have been described by different observers. Thus Toussaint

found that if anthrax were treated with a one half per cent. solution of carbolic acid, it became distinctly attenuated. Chamberland and Roux found that fresh cultures started from one that had been subjected for twelve days to .16 per cent. solution of carbolic acid were fatal to guinea-pigs and rabbits, whilst if the time during which the bacillus was exposed to this solution of the acid was extended to twenty-nine days and a cultivation then made, such cultivation was no longer capable of killing a rabbit. They were thus able to produce a virus of any degree of attenuation and to preserve it for some time, as the cultures made from their attenuated bacilli inherited the same degree of attenuation that had been developed by the bacilli that had actually been treated with the acid. They found that bichromate of potash and other antiseptics exerted a similar attenuating influence. It has also been shown that the passage of the anthrax bacillus through a series of animals of a certain species will render the anthrax bacillus more or less virulent, according to the species that is used. Thus Klein found that blood taken from a white mouse which had died of anthrax was a protective vaccine for sheep, whilst Sanderson and Duguid observed that the virus obtained from a guinea-pig dead of anthrax was modified so far as cattle were concerned. Roy made a series of similar observations. It must be borne in mind, however, that cattle very frequently recover from anthrax under ordinary treatment, so that these latter observations can, as yet, scarcely be accepted as fully proved.

Up to this time it had not been recognized that the immunity was really conferred by the action of the soluble products of the organism. Pasteur had indeed shown that the general symptoms of fowl cholera could be induced by the inoculation of the sterilized products of the fowl cholera germ, and Chauveau had suggested that an acquired immunity was due to the action of the soluble products of the microbe. He argued from an observation that, although in pregnant sheep, anthrax bacilli with which they had been inoculated were unable to pass into the fœtus, the lambs exhibited an extraordinary immunity against attacks of anthrax, this immunity, he considered, being necessarily the result of the action of the soluble products that had been able to pass over from the maternal to the fœtal circulation. We have now a whole series of diseases from which immunity may be conferred by the inoculation or introduction into the tissues of an animal of the soluble products of pure cultures of micro-organisms. In America, hog cholera has been vaccinated against, the vaccinator using the sterilized cultures of the hog cholera organism as his protective virus. Wooldridge, who was the first to adopt this principle in connection with anthrax, was followed by Pasteur and Perdrix, and by Hankin, whose

researches on the albumoses formed by the anthrax organism have opened up a new field for the chemistry of bacteriology. Fowl cholera, certain forms of septicæmia, and a number of other diseases, amongst which may be mentioned hydrophobia, in which, however, the facts do not belong to quite the same order, all were brought within the same zone, when it was found that the introduction of the sterilized products of a specific organism, first in minimal doses and then in gradually increasing doses, could confer a protection against the subsequent action of even the most virulent organism that under ordinary circumstances gives rise to the same products as those injected. Gradual "acclimatization" is the ideal method though in most cases the results were obtained by a single injection. It was for long supposed that the products through which this immunity was conferred were of an alkaloidal nature, and there can be little doubt that in some cases, at any rate, these alkaloids may, if given for a sufficiently long period of time, and in gradually increasing doses, have some effect in "acclimatizing" the tissues to the action of the poison. As pointed out by Sewall, who carefully studied the substances contained in snake poison, the albumose contained in such poison, given in very minute doses to pigeons, confers upon them the power of withstanding seven times the ordinary deadly dose of snake poison, even three months after the inoculation has been made. A single dose of the ordinary hemi-albumose of proteid digestion confers a similar immunity against the action of this same albumose for a period of twelve hours. Hankin, working on this analogy, concluded that the albumose that he was able to separate from anthrax bacilli was really the substance that conferred the immunity against attacks of the bacillus itself, and he found that, although he could kill rabbits with doses of the five millionth of the body weight, a dose of one-ten millionth of the body weight rendered the animal immune to the action of virulent anthrax. He found that he had obtained an instrument of such delicacy, although of so great power, that he was able to protect even mice against anthrax, which had only been done once before—by Hueppe and Wood. It would appear, however, that it was necessary to allow a certain interval to come between the inoculation with the albumose and the injection of the virulent anthrax organism. This is

an exceedingly interesting fact, for from it we gather that the protective material acts in much the same way as does the poison of the anthrax bacillus itself, showing that the poisonous and the protective agents may be one and the same, in certain diseases at any rate. We have, in fact, if we introduce it at the same time as the living organism, a cumulative action during the earlier stages, the albumose helping the anthrax bacillus (by additional albumose being formed) to do its work. Where, however, there is an interval allowed between the introduction of the albumose and the inoculation of the virulent material, there is time allowed for the tissues of the body to become acclimatized, as it were, to the action of this special material, so that when the stronger poison is introduced the cells are more ready to deal with it. A very interesting fact in this connection is, as pointed out by Hueppe and Wood, that a certain putrefactive organism, the earth bacillus, which in all morphological characters resembles the anthrax bacillus, and differs only in the fact that it does not give rise to any fatal disease, even in mice, was able when inoculated into mice and rabbits, to afford protection against anthrax that otherwise proved fatal to these animals. These observers concluded from this fact that the saprophytic organism must be closely related to the anthrax organism, and that it formed much smaller quantities of the *same* specific poison as the disease organisms, so that by its previous introduction these cells were prepared for the attack of the specific anthrax poison, and the disease was checked or modified. They indicate that the relation of the saprophyte to the parasite is merely a quantitative one, having an analogy in the relation that one of our cultivated flowers bears to its wild progenitor. On the other hand Wood and I have observed in a series of experiments that we carried on with the products of the blue pus bacillus that we had a kind of antagonistic influence exerted by the blue pus products on the action of the anthrax bacillus. That the favourable influence exerted by the blue pus products in the course of an attack of anthrax was not due merely to an antiseptic action was proved by the fact that the anthrax bacillus could actually grow in the blue pus products, although, under these conditions, it was undoubtedly somewhat weakened; and we came to the conclusion that we had to deal with a kind of biological antagonism acting indirectly

through the cells. We observed that animals treated by inoculation of the blue pus products and then with anthrax bacillus not only passed through the disease at the time, but they were protected against anthrax, even of a virulent order, when inoculated later. This we looked upon as most interesting, as in other experiments that had been carried on with the blue pus bacillus itself as a protective agent against anthrax, it was found that although an attack of anthrax was cut short in the presence of the blue pus bacillus in a rabbit, it could still be so inoculated at a later date that the animal died with symptoms of true anthrax. The inference we drew was, that the action of the products of the blue pus bacillus and the action of the anthrax albumoses on the cells are essentially different but that the one may interfere with the action of the other. Thus, an animal that has been rendered immune to blue pus is none the less susceptible to anthrax. In our experiments the products of the blue pus bacillus were injected only at intervals, and during part of the time between these intervals there was little of the substance in the fluids of the body ; during these intervals the albumoses of the anthrax had the opportunity of acting on the tissue cells and of so acclimatizing them to its presence that immunity was conferred. Where, however, the blue pus bacillus was in the body, forming its products continuously and acting antagonistically to the anthrax bacillus, the tissues had never any opportunity of becoming acclimatized to the action of the albumoses and no immunity was conferred. Immunity produced by the attack of a specific disease must then be looked upon as an acquired tolerance or adaptation of the cells of our body to the specific poison of the special bacterium of that disease, and the process of recovery from an attack of anthrax, for example, is really the development of such immunity, which gradually passes into the more perfect form during the course of the disease and remains after the patient has recovered. The antagonism of the products of one organism on another which occurs in mixed infection can never in all probability act directly in the body, but through the agency of the cells in the body such action may come to play a most important part in holding in check the active poisons until an immunity can be acquired. On the other hand we may look forward to a period when it will be possible to obtain, by the action of

one organism in the body neutralizing the action of another or by means of antagonizing drugs, a method of treating and ameliorating disease, and at the same time allowing of an acquisition of a condition of immunity by the patient. It is a well-known fact that certain substances which are in themselves non-poisonous or are only slightly "depressant" in their action may so restrain the powers of the tissue cells that organisms that are otherwise incapable of growing in the body are enabled to give rise to septic and putrefactive changes even during the life of the animal. Thus papain and the soluble poison of the Jequirity bean when introduced into the circulation both so alter the conditions in the body that ordinary putrefactive organisms can make their appearance in enormous numbers in the blood of the animal injected.

It appears probable that both the antagonistic action and this summative action are due to the bringing into play, or the depressing, of certain specific functions of the protoplasms of the cells by the products of different microorganisms. It is not necessary that these functions should always be manifesting themselves; after being once evoked and exercised they may remain latent for a considerable period and only be again called into action under the regular specific stimulus. It is a case of writing on the looking-glass with ink and with French chalk; the ink is always in evidence, and we might say that it corresponds to the enzyme or the peptonizing functions exerted by certain cells, animal and vegetable, whilst the French chalk, though always there, is only brought out when the glass is breathed upon. This may be said to exemplify very roughly the specific power that the cell has acquired of resisting the anthrax or other special poison. It only comes into play when the specific poison is present; it is actually present though in a latent condition the whole time.

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CHAPTER XXIII.

Bacteria in Air, Earth, and Water.

Spores in the Air in Hospitals—Effects of Currents and Altitude—Direction of Wind—Nature of Country over which it passes—Frankland's, Carnelley's, Haldane's and Petri's Experiments—Few Bacteria in the Air of Sewers—Tyndall's Glycerine Chamber—Examination of Air—Koch's Method—Miquel's Method—Hesse's Apparatus—Modified Hesse's Apparatus—Miquel's Sugar Method—Bacteria in Water—Effect of Standing—Sluggish Movement—Oxidation of Organic Matter in Water—Organisms carried by Sewage—Number of Organisms in Water—Method of Procedure in Analysing Water—Pfuhl's Flasks—Petri's Dishes—Petruschky's Flask—Plate Cultivations—Cooling Apparatus—Von Esmarch's Tube Cultures—Effect of Rains, Frosts and Thaws—Relation of Bacteria to Ammonia—Basis on which to determine whether water is fit for Drinking or not—Filtration—Method of Examination of Soil.

As will have already been gathered from what has been stated in connection with the distribution of the cholera bacillus, tubercle bacillus, anthrax bacillus, and similar organisms, there are marked differences in the facility with which organisms may be carried even by currents of air. Those organisms that do not produce spores, and that are easily killed by drying, are very seldom contained in the air, at any rate in a condition capable of growing, whilst it is found that those which resist drying, and especially those which form resting spores, may be carried about from place to place, either alone or adhering to dust or other particles.

In connection with this question of micro-organisms in air, there are certain general rules that should always be borne in mind. Their presence must, we are afraid, be looked upon as inevitable in those hospital wards in which patients suffering from infective diseases are gathered together for treatment; tubercle bacilli, for example, are found in the air of wards where phthisical patients are collected,

whilst the special bacteria associated with other diseases have also been demonstrated in the air of wards specially set apart for the treatment of these diseases. Then, again, it must be remembered that there are always more micro-organisms in the air in those regions where decomposing organic matter of any kind is allowed to accumulate, this being especially the case in positions in which the air cannot be continuously renewed by currents setting in from other and purer regions. Consequently, it is found that in valleys and in low-lying country generally, especially where there is any accumulation of decayed vegetable matter, or where people are massed together in towns or villages, many bacteria are usually present in the air. Some of the organic growths obtained from such air belong to the mould fungi, the spores of which, as is well known, have considerable resisting power, and may be readily carried from point to point by gentle currents of air. As the low-lying lands are left, and the hill country is reached, micro-organisms are comparatively few in number, and at certain elevations, especially where the temperature is low, the number of these germs of moulds and bacteria may sink almost to zero. Again, if the air brought by breezes coming from the sea on the one hand and from the land on the other, be examined, it will be found that in the former case it is impossible to demonstrate the presence of organic life of any kind ; whilst in the latter, collected it may be at the same point, an enormous number of micro-organisms of different kinds may be met with. It is, however, impossible to give any general rule as to the number of organisms that should be found under these various conditions, though we may take it as a result of Aitken's experiments on the presence of solid particles in the air and their relation to fogs, that the more solid particles there are in the air, the more micro-organisms of various kinds are to be found. Some idea of the number of organisms present at different seasons of the year may be gathered from P. F. Frankland's investigations, the results of which were presented to the Royal Society in 1886. He determined the number of colonies found in two gallons of air (examined by Hesse's method), collected on the roofs of the Science Schools at South Kensington. He found fewest present during the month of

January, on an average of 4 per two gallons (ten litres); the numbers gradually rose until August, when there was an average of 105, and then gradually fell, though he records no observations made during November and December. He had previously confirmed the general results of Miquel, Hare, and others, that as we leave the ground the number of micro-organisms in the air rapidly diminishes. On Norwich cathedral spire, at a height of about 300 feet he found in ten litres of air only seven micro-organisms on one occasion, and on the tower, at a height of 180 feet, he found nine, whilst at the base of the cathedral (in the close) eighteen were found. In another series of experiments made at St. Paul's cathedral a similar volume of air taken from the golden gallery yielded eleven, that from the stone gallery thirty-four, and that in the churchyard seventy micro-organisms. He gives a number of other most interesting experiments, for which, however, the reader must refer to the original paper. Carnelley, Haldane, and Petri have been able to show that the number of micro-organisms in any air depends to a very great extent on the moisture or dryness of the atmosphere, for they found that in the air of sewers, which is necessarily very damp, the number of micro-organisms present is extremely small, unless rapid fermentation is going on, or there is splashing from irregularities in the course of the drain, or from falling in of sewage from a height, the bacteria always tending to settle and to adhere to the moist walls of the drain, so that unless a considerable number of the organisms are carried into the air by the escape of bubbles and gas, or by other agencies, the tendency of these organisms to gravitate allows of their removal from the air. Here, however, it appears to be the moisture on the walls that prevents the escape into the air, rather than any moisture in the air itself. Tyndall demonstrated that exactly the same thing occurred in his chamber coated with glycerine; bacteria, or other solid particles to which these bacteria were adherent, fell to the floor, or were carried on to the walls, where they were held fast by the glycerine, the air in the chamber thus becoming practically sterile, or deprived of its micro-organisms; there might still be an enormous number of organisms attached to the floor and walls, but the air itself remained absolutely free, and flasks opened in this chamber would remain sterile for a very considerable, or even an indefinite, period. Griffiths,

gives the result of a number of similar observations, in which the above statements are in the main confirmed.

Of the methods of examination of the air for micro-organisms the simplest and most convenient, but perhaps the least reliable, is that of allowing the germs to settle on plates of sterilized gelatine or potatoes, that are left uncovered for a definite length of time. Currents of air may completely destroy the accuracy of these results, but in rooms that have been left undisturbed for some time, in which the doors are closed, and in which no currents are set up by heat coming through the windows or from fireplaces, moderately accurate average "countings" may be obtained. In place of these glass plates covered with gelatine, shallow glass trays with glass lids soon came to be used to contain the gelatine. Those used by Koch for this purpose are shallow glass capsules about half an inch deep and a couple of inches in diameter, in which the sterilized gelatine is placed. These are placed inside tall glass cylinders about five or six inches high, the mouths of which are closed with large cotton wadding plugs. The glass capsule is lowered into the cylinder and again removed from it by means of a piece of soft metal bent at right angles. After the whole has been sterilized the cotton wadding plug is removed, the gelatine is left exposed, say, for ten minutes, the plug is re-inserted, and organisms that have settled on it are allowed to develop at the temperature of the room. These soon make their appearance as small, white, yellow, or pink points, according to the nature of the germs that are present in the air. In addition to these, however, a number of fluffy white, green, or black, forms make their appearance. The former consist of bacteria, sarcina, or yeasts; the latter of penicillia, mucors, and aspergilli.

Another method of examining the dust and micro-organisms contained in the air is one in which an apparatus somewhat like a chemical "wash bottle" with the bottom knocked out is used. In the neck of the bottle is an india-rubber cork with two holes; through one of these holes the long limb of a tube bent into a U shape, with a long limb and a short limb is passed; the long limb, which is drawn out into a pretty fine point, projects about two-thirds down into the bottle; in the other hole of the stopper is a short glass tube, to which is attached a piece of india-rubber tubing; the bottom of the bottle carefully ground, is luted with vaseline on to a glass plate on which has been placed a microscope slide, so supported as to rest with its upper surface immediately under the drawn out opening of the longer tube. This slide has previously been coated (as recommended by Miquel) with a mixture of one part of grape sugar and two parts of glycerine. A given quantity of air is then drawn into the bottle by means of an aspirating apparatus, and all particles of dust, spores, or moulds, and organic and inorganic fragments of all descriptions are made to impinge on the sticky surface, where they are retained and may afterwards be examined under the microscope, or, if necessary, the glycerine and sugar mixture may be washed into a nutrient fluid such as peptonized meat gelatine, and a regular biological examination of the organisms may be made. A modification of this æroscope used by Miquel is a flask containing a small quantity of fluid, into which the air is drawn. This apparatus is somewhat complicated; it is like a Pasteur flask, with three openings, through one of which the air enters, the neck of the flask being continued as a kind of tube down into the fluid in which the organisms are to be

cultivated. Hesse's apparatus, for the estimation of the number and nature of bacteria, &c., in the air, consists of a glass tube of about 18 to 25 inches long, and 1½ inches in diameter, slightly funnel-shaped at each end ; in one end an indiarubber bung is fixed. This has a central opening through which passes a short glass tube about 5 or 6 inches in length, and one third of an inch in diameter. In this short tube is inserted a plug of cotton wadding. At the other end of the larger tube are two membranes of india-rubber tied on separately ; the inner one has a perforation of about one-third of an inch in diameter, the outer one fits over this and so closes the opening. The tube, bung, and indiarubber membranes are then sterilized with a one per cent. solution of bichloride of mercury and rinsed with boiled distilled water. The inner cap is firmly tied in position with good stout thread, the tube is half filled with water, and with a pair of scissors a hole is clipped in the centre of the indiarubber membrane. The second cap is then firmly tied on, water is again poured into the tube, the bung is replaced, and the whole apparatus is thoroughly boiled for a quarter of an hour in the steam sterilizer. After allowing time for the glass to cool, the bung is removed, the water is carefully poured out and liquid nutrient gelatine is poured in to replace it ; the bung is again fixed in position and the apparatus is boiled for ten minutes at 100° C, after which the tube is put in a cool place in a horizontal position, so that the gelatine may consolidate in a thin layer along the whole length of the tube. Just as the gelatine is beginning to "set," the tube may be gently rocked from side to side so as to obtain a rather larger cultivating gelatine surface. This tube, so prepared, is placed in a V-shaped support resting on a tripod, where it is held in position by a couple of elastic bands. To this tripod an aspirating apparatus is fixed. This consists of two bottles, each fitted with corks and tubes like those of a wash bottle. These bottles are placed at different levels and are connected by an indiarubber band, in the middle of which is a pinch cock. The indiarubber tube connects the longer tubes in the flasks. A litre of water is then measured into the upper flask, and, by applying suction to the orifice of the lower flask that is now left free, water commences to flow from the upper into the lower flask. If now the second tube in the upper flask be attached to the little tube projecting from the bung of the tube containing the gelatine, the aspirating apparatus will communicate with the chamber in which the gelatine is contained ; the outer layer of indiarubber membrane which covers the orifice in the inner membrane is then removed, and by setting the syphon system in operation water is slowly and regularly run from the one flask into the other, and a corresponding volume of air is drawn in at the small opening in the india-rubber membrane. As soon as one litre of air has been drawn through, all that is necessary is to close the pinch cock, reverse the positions of the bottles, and repeat the whole process. This may be done until a volume of from 1 to 5 litres of air has been drawn into and through the tube containing the gelatine, the micro-organisms, drawn in along with this air, settling on the nutrient medium in the tube. If the estimation is to be made in the open air, it may be necessary to draw through the tube 20 or 30 litres. It is, however, usually necessary to expose a number of tubes, drawing different quantities of air through each ; then by taking an average of those in which the organisms are most readily counted, a fairly accurate result is obtained. The flow of water, and consequently the rate of flow of air, may be regulated by the introduction of pieces of glass tubing of known

calibre into the indiarubber tube that connects the two flasks. When the operation is complete the tube with the contained organisms is disconnected, the imperforate indiarubber membrane is again tied in position, and the whole is set aside until the organisms begin to develop; they may then be counted *in situ*, or with a long platinum needle special points may be removed for microscopic examination, or for the purpose of making pure cultures.

In place of Hesse's tubes I have, with Mr. Coghill's assistance, devised a flat glass bottle with a large central opening at the top and two side openings just at the level of the gelatine surface. These orifices are placed at the opposite sides of the jar. They are used just as are Hesse's tubes, but possess several very obvious advantages over them.

Another method used in estimating the number of bacteria in the air was that of filtering the air through tubes containing sterilized glass wool, asbestos, or sand; these are now seldom used, as they all interfere more or less with the transparency of the gelatine. Indeed, it is often a very difficult matter to distinguish grains of sand from young colonies of organisms. In place of these substances Miquel recommends that sterilized cane sugar should be used. Here the method of procedure is as follows: Loaf sugar is carefully ground in a mortar and passed through a couple of metal sieves, the latter of which allows grains of not more than half a millimetre in diameter to pass; this sifted sugar is packed into a glass tube about eight inches long and the sixth of an inch in diameter. Near one end of this tube is a slight constriction, on each side of which is placed a little sterilized cotton wadding or glass wool, one of which serves to prevent the sugar from escaping at the lower end, the other acting as a sterilized plug. At the other end of the tube is a glass or indiarubber cap (see description of Pasteur-Chamberland flask in Appendix) carefully sterilized and plugged with cotton wadding. The tube is sterilized for an hour at 150° C., and allowed to cool; the cap is then removed and the sugar, which has been previously well dried, is poured into the tube, which should be filled to a depth of about 4 to 4½ inches. The whole is again sterilized at a temperature of 150° C. When the filter is to be used the sugar is packed against the plug by tapping the tube gently, and the tube is held vertically whilst the air is being drawn through the filter; this may be done slowly for twenty-four hours, or very rapid aspiration may be carried on where it is required to ascertain the number of micro-organisms present in the air at any definite period of the twenty-four hours. When the process is completed, the plug nearer the aspirator is carefully removed with forceps and placed in a sterile glass box, which should be got ready beforehand, the inner plug is then removed and is put into a test tube containing a small quantity of liquefied nutrient gelatine; this test tube is carefully plugged and laid aside, the outer plug is immediately replaced, the cap is removed and the sugar is poured out into a flask filled with sterilized water. A short indiarubber tube is then slipped over the plugged end of the glass, the upper end, from which the sugar has been emptied, being placed in a test tube containing sterilized water; this water is alternately sucked up into the filter tube and then expelled, until the whole of the sugar with its contained germs has been dissolved and driven into the test tube. This water is added to that of the flask and well shaken until the whole of the sugar is dissolved. The number and character of germs so obtained from the air are determined by making plate cultures. One portion should be utilized for the demon-

stration of the different ærobic organisms present, another for the anærobic species, and others may be used to determine the mere numbers of these bacteria that are present, there being sufficient material for a dozen or even twenty analyses. If a large number of organisms be present it is necessary to dissolve the sugar in a large quantity of water so as to obtain a sufficiently dilute "solution," whilst if a small number only are expected to be present a smaller quantity of water should be used.

Water is one of the most convenient vehicles for the distribution of micro-organisms. It has been noted that a shower of rain diminishes the number of bacteria suspended in the atmosphere in a most remarkable manner; these organisms being afterwards found in the puddles in the road, in the pools in the sidewalks. In stagnant water and in surface water of all kinds bacteria may be found in enormous numbers, though the numbers and varieties in which they are present may be very considerably modified by the amount of organic material contained in the soil through which such surface water comes, by the depth at which the water is taken from the surface, and by the facilities for oxidation or aeration that may be present. In the sluggish streams of valleys, where there is a constant drainage from the surface land, and where the water is so little disturbed that the air of the atmosphere cannot obtain free access to the organic matter suspended in the water, the number of micro-organisms will, as a rule, be very considerable, whilst the rapidly-flowing shallow streams that make their way over shingle or gravelly beds will be found to lose their micro-organisms very rapidly indeed; if the organic matter has not already been left behind in the sluggish reaches of the stream it is very rapidly converted by oxidation into the ultimate products of decomposition, and ordinary putrefactive micro-organisms at any rate are no longer able to obtain any subsistence, although, as Bolton maintains, the "water" bacteria can still flourish. They can even grow in distilled water. In the water that comes from springs there may be scarcely a single bacterium as the water rises to the surface, but if such spring water be allowed to stand exposed to dust and contamination of various kinds it very quickly swarms with micro-organisms just as do the waters from other sources. A very small quantity of sewage, which really consists of water in which is suspended an enormous quantity of organic matter, finding its way into a water supply may contaminate it for a whole neighbourhood, such

contamination being only too plainly indicated by some of the epidemics of typhoid fever and cholera that have from time to time devastated our badly drained villages both at home and in new countries. In view of all these facts, the biological analysis of water has come to be a subject of importance of the first rank, and too much stress can scarcely be laid on the necessity for such analysis in order to determine the suitability of any water supply for the purposes to which it is to be put ; fortunately such examination is comparatively easily carried out. In addition to this, however, regular examination is absolutely necessary both to provide an indication that no contamination is creeping in during the process of distribution and to test how filter beds where such are used are performing their work. Water should always be examined for bacteria immediately after the sample is drawn from its source, for although the amount of nutriment for micro-organisms may be comparatively small, it is all in solution, and in such a form that it can be easily utilized by the organisms which in the fluid make their way with the utmost rapidity from one point to another. In consequence of these extremely favourable conditions their rate of multiplication is most remarkable. If a sample of even the purest water (containing, say, 200 germs per cc.) be left to stand in a room, in which the temperature is comparatively high and therefore suited for rapid growth of these organisms, it may be found that in place of 200 germs per cc. there may be present on the second day 5,000, on the third day 20,000, whilst on the fourth, as pointed out by Carl Fraenkel, they are almost innumerable. This multiplication may go on for some time, but at length there comes a period at which the small quantity of food contained in the water is used up, the bacteria begin to die, and the number of living cultivatable organisms gradually falls until eventually it may become extremely small.

Water for analysis is collected in a sterilized Ehrlenmeyer flask, well plugged with sterilized cotton wadding ; in place of this a wide-mouthed stoppered bottle may be used. In either case the stopper or the wadding should be covered with an indiarubber cap previously carefully sterilized in corrosive sublimate solution and boiled distilled water. If the water is to be collected from a tap it should be allowed to run for several minutes before any is taken ; water from the surface of a pond or a river should be collected by means of large sterilized pipettes ; whereas if it is to be taken from a depth or from a well a stoppered and weighted flask is

lowered, then, when it has reached the required depth, the stopper is removed, the bottle is allowed to fill, after which it is then drawn to the surface, the water so obtained being at once transferred to a number of smaller bottles prepared as above.

Quite recently an exceedingly convenient apparatus for the collection of water has been described by Dr. Pfuhl. It consists of a tall glass vessel with a flat bottom 2.5 centimetres in diameter and 10 centimetres long, with a glass tube 6 or 8 centimetres long, which can be easily closed, leading from this vessel. To prepare this it is only necessary to sterilize by heating in a flame, and while the air is rarefied to seal up the point of the tube by heat. When this has to be filled it is plunged under water or in the running stream from a tap or pump, the tip of the tube is broken off with a pair of sterilized forceps, water rushes in and about half fills the vessel. The outer surface of the tube is then carefully dried with blotting-paper, gently warmed to drive away the moisture from the glass near the opening, and finally sealed as before in a spirit lamp flame. After it is thoroughly cooled the flask is well shaken, and should there be any leak this is made good. For transport these flasks are packed in zinc cases with cotton wadding and ice. To remove the water the tube is nicked with a file, broken off, and a sterilized pipette is introduced. In all cases, however, there is the difficulty of transport, and it is a great matter if plate cultivations can be made at the time that the water is drawn. Petri gets over this difficulty by making his cultivations in double glass dishes, which are kept in position by india-rubber bands; and various other pieces of apparatus have been devised, perhaps the best of these being Petruschky's flask, which can be used for the cultivation of either ærobic or anærobic organisms. This consists of a thin flask flattened on two sides with an indentation at the neck to prevent the flowing out of the softened gelatine. It may be used simply with a plug of cotton wool. The mixture of gelatine and water is poured in and then allowed to settle on one of the flat sides. For anærobic cultures, however, a couple of glass tubes similar to those used in a wash bottle are introduced through openings in an indiarubber cork. Hydrogen is driven through the bottle to displace the air, and the ends of these tubes are carefully sealed, the flask is laid on its side, and the gelatine is allowed to cool. These flasks are so constructed that a microscopical examination may be made through the thin glass walls, especially if care be taken to obtain a layer of gelatine or agar sufficiently thin and of equal thickness throughout. Measure the size of the drop delivered by a pipette in the following manner. Weigh a filter paper on a fine balance, then put a gramme weight into the opposite scale, and drop by drop deliver exactly one gramme of water on to the filter paper, counting the drops as this is done; then mark the pipette with the number of drops that it delivers per gramme, after which it may be used for measuring the water in making gelatine plate cultivations. It is now sterilized in the hot air chamber at 150° C., or by being thoroughly washed out with bichloride of mercury, then with distilled water that has been boiled and allowed to cool, and then with absolute alcohol, the last traces of alcohol being driven out by the heat of a spirit lamp. If a very large number of organisms are present a single small drop of the water, corresponding to about the fiftieth part of a gramme or cc. will be sufficient, whilst a larger drop, the twentieth part of a gramme, or even six or eight of these drops, may have to be used in order to obtain a sufficient number of organisms in a plate cultivation. Then prepare a number of glass plates in the following

manner :—Thin plate glass is cut into squares of four and a half inches, the sharp edges are removed with a file, and the glass is carefully cleaned. It may then be sterilized in one of the following ways : Wrap each plate separately in a sheet of hard tissue paper, and place in the hot air chamber, leaving it for about an hour subject to a temperature of 150° C. It may then be taken out and kept in a dry place until required for use. If the plate is required at once, leave it slightly damp after cleaning, then, laying hold of it with a pair of strong forceps, heat it carefully over a Bunsen burner or spirit lamp until the whole moisture disappears, great care being taken that one side at any rate is sterilized by the action of the flame, wrap up in a piece of sterilized paper and leave it until the other materials are ready. Or the plate may be tilted against a clean wooden block or iron upright with the more carefully heated surface downwards. Bell jars and glass benches have previously been prepared by being thoroughly washed with soap and water, and then with a 1 per 1,000 solution of bichloride of mercury ; a piece of absorbent filter paper thoroughly saturated with the bichloride solution is placed in the bottom of one of the jars ; on to this all germs that are suspended in the air within the jar gradually fall and are destroyed. On a surface made as level as possible by means of a tripod levelling apparatus and a small round spirit level, (if these can be obtained), a plate of metal resting on three metal feet is placed in a mixture of ice, salt, and water; on this the sterilized plate of glass, with the more carefully prepared side uppermost, is laid, and the whole is covered with a bell jar that has been previously sterilized by means of heat or of bichloride of mercury. A test tube, with a large overhanging cotton wadding stopper, containing gelatine or a mixture of agar and gelatine is taken, the stopper is removed for a second or two, and the lip of the glass tube is carefully heated in a flame, the cotton wadding stopper is also thoroughly singed and then replaced. The gelatine is melted by placing the tube in water at a temperature of about 35°–39° C. for gelatine, and a much higher one for agar gelatine. As soon as the medium is thoroughly melted, the quantity of water that is to be used is dropped from the sterilized pipette into the tube, then with a rolling motion the water is thoroughly incorporated with the nutrient medium, great care being taken that as few air bubbles as possible shall find their way into the viscid fluid. The plate by this time being thoroughly cooled, the gelatine is poured out so as to form an equal and regular layer ; it is spread gradually from the centre of the plate to near the margins, over which, however, it is not allowed to run ; it is then allowed to solidify, after which the plate is transferred to the bell jar prepared for its reception. By means of slips of glass, or of glass, porcelain, or zinc benches, carefully sterilized, three plates may be placed in the same bell jar ; they are then allowed to incubate at the temperature of the room, and at the end of two or three days colonies of bacteria make their appearance (each one from a single seed if the mixing has been perfect), and may be isolated and described. Where the number of organisms is unknown the method described for the isolation of cholera organisms (see under Cholera, p. 155) should be utilized. It is sometimes necessary to make a whole series of plate cultures to obtain a single pure growth, especially where zoogloea masses are formed. This method is also used for the separation of different species of micro-organisms, and it can be easily understood how micro-organisms may be more or less isolated by being shaken in a fluid medium, and how when once they are isolated they

are prevented from running together again, for a time at any rate, by the solid gelatinized medium. Where it is necessary to isolate and obtain pure cultures of any special organisms, the method described as used in separating any colony of organisms should be used (see page 155). Salomonsen recommends a cooling apparatus made of an ordinary plate on which a shallow glass dish with a ground rim rests. This is filled up to the surface, but not to overflowing, with water and lumps of ice, a plate of ground glass is fitted on to this, and then a sterilized glass cover or bell jar is used to cover the glass plate; the further procedure is then much as above. The organisms may be counted as a whole, but it is more convenient to use a plate of glass marked into squares, which is supported over the gelatine plate, a low power lens being used to define the smaller colonies. The colonies in a number of squares in different positions are counted, then the number of squares that cover the gelatine plates is determined, and an average is obtained from which the whole number of organisms may be reckoned. It must be remembered, however, that a small proportion of the gelatine still remains in each tube, therefore it is necessary when making the plate cultivation to spread the remaining quantity of gelatine over the walls of the tube by keeping the tube rotating in water until the gelatine is fixed in position; the organisms left in the tube also give rise to colonies. These should be counted and added to those found on the plates. In place of plates von Esmarch recommends the use of test tubes. The inoculation is made, exactly as above, into wide test tubes containing from eight to ten cc. of nutrient gelatine, a second tube may be inoculated from the first, and a third from the second by means of the sterilized pipettes. The plug is then thrust well home after being singed, and a tight-fitting indiarubber cap is placed over the mouth of the test tube, or melted paraffin is run in to protect the wadding. The mixture is then effected by rolling the tube rapidly between the hands, keeping it in a vertical position. When the fluids are sufficiently mixed the tube is placed into ice-cold water and, in a horizontal position, is kept rotating on its longitudinal axis until the gelatine is "set" in a thin layer all over the walls of the tube; the tubes are then put aside and kept under observation. Should the presence of a large quantity of oxygen be necessary, the indiarubber cap may be removed and the plug may be pierced at one or two points with needles that have been carefully heated, these needles passing through both paraffin and gelatine. These tube cultivations may be made at once and on the spot, are readily carried about, and serve as control experiments even when plate cultivations are made in the ordinary manner. In place of glass plates and large moist chambers, glass capsules are sometimes used for making plate cultivations, and Petri has devised a shallow glass tray with a lid which answers the purpose admirably. The method of procedure is much the same as in the above cases, but is much simplified from the fact that the gelatine is allowed to cool on the floor of the capsule, the space above serving as a moist chamber. Before sterilization these boxes are wrapped in a sheet of the hard tissue paper, they are then subjected to dry heat and are ready for use at any time. In working with gelatine there is the disadvantage that certain organisms peptonizing it cause it to liquefy exceedingly rapidly; it has the advantage that it is exceedingly clear, is readily melted, and solidifies rapidly. Agar-agar on the other hand is not liquefied by the peptonizing organisms, but it is not nearly so clear as the gelatine, and requires a much higher tempera-

ture (at least 90° C.) to melt it, although, as should be borne in mind, once melted it will remain fluid at 40° C., at which temperature any ordinary inoculation may be made A mixture of agar and gelatine prepared by adding 5 per cent. of gelatine and .75 per cent. of agar to the peptone beef broth, or other nutrient fluid combines the advantage of both gelatine and agar-agar media. It is almost as clear as gelatine, is readily manipulated, and melts at a considerably lower temperature, although it will remain solid between at 30° and 40° C., and it is not nearly so difficult to prepare as the pure agar. The number of organisms in water is always calculated per cubic centimetre, but having obtained the number of organisms in the fraction of a cc., it is easy enough to convert the figures to the desired standard. In every case a sample of water should be allowed to stand in conical glasses, so that any sediment may be obtained for microscopic and biological examination. This is especially important where the presence of pathogenic micro-organisms such as those of typhoid is suspected. These organisms are usually brought in along with solid particles of sewage matter, and to these they usually adhere so that they may all be deposited along with such particles.

The conditions under which micro-organisms are most numerous in water have been already referred to in general terms. It is of course found that where great rain storms, or thaws after the action of frost, break up the earth's surface, many organisms are set free and are carried away into the water supplies, the number usually varying to a certain extent with the amount of solid matter that is suspended in the water ; as a general but by no means invariable rule it may be stated that most bacteria are found where there is most ammonia. It is sometimes said that if water does not contain more than one thousand organisms per cc. that it may be used with safety for drinking purposes, but it must be borne in mind that this thousand organisms may contain a larger number of pathogenic organisms, whilst on the other hand five thousand organisms in the same quantity might not include a single pathogenic germ. It has been found indeed that no general biological examination will give us absolutely accurate indications as to the nature of bacteria in water ; to obtain such information a rigid examination of every species present must be carried out. The number of liquefying organisms has indeed been taken as giving an indication of the quality of water, and this is undoubtedly a safer plan than to take into consideration the mere number. A safer rule still, however, is to take the number of different species of organisms in a drinking water as indicating its purity or impurity for drinking purposes, for it follows that if any water contains a

considerable number of species, there must be several centres from which these must be derived, each additional source, of course, bringing in an additional element of danger. After examining 400 springs, wells, and streams, W. Migula concluded that when there are more than ten species of bacteria in any sample of water, especially when these are species not ordinarily met with, the water should not be used for drinking purposes. In only 59 out of 400 was such a number of species obtained, whilst 169 contained more than 1,000 individuals per cc., 66 of these having over 10,000, and 40 over 50,000. He found in all 28 species, and observed that the number of colonies does not by any means correspond with the number of species, though in some cases it undoubtedly does. Ordinary putrefactive bacteria are almost invariably absent from spring water, but they are usually found where the number of colonies is between 1,000 and 10,000 per cc., but they also occur where the number of germs is below 50 cc., but very seldom where the number is over 10,000. Of course the only perfect method is to examine each separate species by itself and to examine carefully any organisms that bear the slightest resemblance to any of the pathogenic species. It is a good rule to observe that all water taken from near the surface, or spring water that has been allowed to come to the surface and remain there for some time before use, should be filtered through sand or through porcelain filters. If in the process of filtration pure air can be mixed with the water so much the better; the best of all filters for this purpose being the Pasteur-Chamberland, which may be readily applied to every household water supply. In large water-purification works gravel and sand are by far the best filters, especially if these are frequently renewed, the old filtering medium being burned before being again used.

For the examination of soil the first method used was simply to sprinkle a little of the earth on a plate of nutrient gelatine, and then to examine the organisms that grew on it. This, of course, was an exceedingly imperfect method. The next step was to mix a small quantity of the earth with sterilized nutrient gelatine in a test tube, and then to make a plate cultivation either on a tube or on a flat surface. Now, however, that it has been found that there are so many organisms on the surface of the earth, the mass of earth to be examined has been diluted by adding a considerable quantity of sterilized distilled water. This is allowed to stand for a considerable time in order that the two may be thoroughly incorporated into a thin brown liquid with as little sediment as possible, and from it plate cultivations

are made as described in the case of water or cholera organisms. Perhaps, however, the most certain way of obtaining all the organisms that are in any sample of earth is to break it down in liquid gelatine as above described, and then make an Esmarch tube cultivation. It is of course an easy enough matter to take a sample of earth from near the surface, but it is much more difficult to take samples from the deeper layers, which can only be done by means of special boring rods, unless access can be gained to a clean cut surface such as those met with in the making of a drain or other such cutting. As most organisms, however, such as the bacilli of anthrax, malignant œdema and tetanus, and the ærobic putrefactive bacteria are usually found near the surface, and as the deeper layers are so frequently almost entirely free from micro-organisms, just as is the ground water that we find in these deeper layers, this is as a rule a matter of comparatively little importance.

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APPENDIX.

THE short outline of bacteria and their products contained in the preceding pages will not have had the effect desired if it does not induce in a certain number of readers an ambition to undertake some experiments in bacteriology, in at least some of its branches. As it is often extremely difficult to obtain the most elementary knowledge of the technique of a subject without being compelled to dive into elaborate and erudite articles, it has been thought advisable to supplement the descriptions of methods already given by a short *résumé* of some of the simpler methods of experimental investigation (the apparatus required for which is extremely simple, and can be obtained at comparatively trifling cost), and to give a short description of some of the commoner forms of bacteria that they may be readily identified.

HOT AIR STERILIZING APPARATUS.

C. Salomonsen recommends a very inexpensive form of sterilizing oven in which glass utensils, metal apparatus, wadding, paper, &c., may be heated to about 150° C. It consists of an ordinary large-sized biscuit box, such as may be obtained from any grocer. A round hole is cut in the middle of the lid, and into this a cork is fitted to carry a thermometer registering to 200° C. A small hole is punched in each of the four sides near the top, and similar holes are made close to the bottom. On the bottom of the box, inside, is fitted a piece of strong sheet iron, with the ends bent at right angles to raise it about two-thirds of an inch, so that materials to be sterilized do not touch the metal which is in immediate contact with the flame. The lid is covered with a piece of felt, in the middle of which is a hole for the thermometer. Another piece of felt, the breadth of which is three-quarters the height of the box, and long enough to go round it outside, is fastened round the chamber, just below the upper, and above the lower, air holes, and is kept in position with stout copper wire. The box so prepared is supported on an ordinary tripod, between which and the box rests a loose piece of thin sheet iron or tin (this latter prevents the burning of the thin tin bottom of the box). Such a chamber may be heated by gas or by a small oil stove. An ordinary cooking oven may also be used as a hot air sterilizer. A more complicated apparatus is a double-walled sheet iron box of about the same size as the above, or a little larger, with a hole in the top to receive a thermometer, a door in front, and a movable bottom, which can be easily renewed from time to time as it is burned through. All objects placed in this sterilizing chamber must be protected against dust on their removal. This is done in the case of flasks and test tubes with their cotton wadding plugs by covering them with a layer or two of strong, hard,

thin "preserve" or "type-writing" paper; other objects, such as watch glasses, slides, cover glasses, cultivation plates, &c., may be enclosed in small iron or copper boxes, or they may be carefully wrapped in cotton wadding, or in a couple of thicknesses of the above paper, before they are put into the oven to be sterilized. One must always be careful that the paper or wadding does not come into actual contact with the bottom or sides of the oven, the hot metal of which may cause singeing. Even hot air at 150° C. will "brown" cotton wadding or paper slightly, so that such a temperature in the chamber may be at once recognized, even when no thermometer can be obtained, by the appearance of a slight brown coloration of these materials.

Steam Sterilizer.

For sterilizing by means of steam, an ordinary fish kettle or potato steamer may be used; either of these placed on a fire will, for private work, usually serve the purposes of the more complicated Koch steam sterilizer. In this steaming apparatus the heat penetrates rapidly and does its work thoroughly, moist heat being much more effective as a sterilizing agent than dry heat. If it is thought necessary to obtain a piece of special apparatus for the purpose, the best is a tin or zinc cylinder about eighteen inches high and six inches in diameter, to which a small water gauge is added. The bottom of this should be made of block tin; the top is closed by a conical lid in which is a hole for a thermometer. The lid and sides are usually covered with felt, though this is not essential, unless economy of heat is advisable. The felt should not in any case extend quite to the bottom, or it is readily singed by the heating flame. Inside the cylinder are a couple of shelves, one about one inch, and the other about ten inches, above the level of the water; on each rests a perforated tin plate on which tin vessels about four or five inches in diameter and with perforated bottoms are supported. The objects to be sterilized are placed in these vessels, the cylinder is filled to a depth of three or four inches with water, the pails are put into their places, and the lid placed in position. After the water has commenced to boil briskly, the steaming is continued for about twenty minutes, at the end of which time most of the pieces of apparatus are thoroughly sterilized. This sterilizer may be lengthened by the addition of a tin cylinder with a ring or collar near the base, which fits into the top of the sterilizing cylinder, the lid being placed at the top of the additional cylinder. This lengthening portion is especially useful for sterilizing Hesse's air analysis tubes.

Incubating Apparatus.

Most of the ordinary micro-organisms may be cultivated at the temperature of the room, but many of the pathogenic organisms, such as the tubercle bacillus, will only grow at a temperature approximating that of the human body, and for the cultivation of these some sort of incubating apparatus is necessary. The simplest apparatus, however, if supplied with a good body of water or a wrapping of felt and cotton wadding, will in most cases serve our purposes. Any one with a little ingenuity will be able to devise an incubator for his own use, if he has a greenhouse or any warm room at his disposal, an ordinary oil lamp being sufficient, if properly trimmed and regulated, to keep a double-walled chamber covered with felt at a fairly constant temperature, varying only three or four degrees for months together. Where systematic investigation is to be carried on, however, one of the ordinary

thermostats with regulator should be obtained, or, failing this, one of the small egg-hatching machines, of which there are several in the market, may be used.

Sterilized Vessels for the Reception of Various Media.

Ordinary test tubes, flasks, and other special glass apparatus, are first carefully washed with soap and water, then with boiling solution of permanganate of potash, to which a few crystals of oxalic acid are added. They are then rinsed with distilled water, and are allowed to drain on a rack for some time, after which they are carefully plugged with cotton wool, care being taken that the wadding inside the neck is perfectly smooth and firm, the tuft outside being large enough to overlap well the lip of the test tube. These plugs *in situ* may be covered with paper, which keeps off the dust. They are heated for an hour in the hot air chamber at 150° C. For the reception of fluid media, Salomonsen recommends the use of a small flask or test tube of which the neck or upper part is so drawn out that it has a comparatively narrow mouth. The mouth is closed by a piece of indiarubber tube a couple of inches long. The tube is washed with bichloride of mercury solution, then with distilled water, is wrapped in hard parchment paper and sterilized at 100° C. in the steam sterilizer. It is then filled for half its length with cotton wadding that has been sterilized at 150° C. in the hot air chamber. The flasks are sterilized as above. For these stoppers the following advantages are claimed :—(1) In opening and closing the flasks, the wadding and dust that is collected on it are not touched. (2) The apparatus is opened and closed at a point which can always be easily kept free from dust. (3) The opening through which the inoculation is made is smaller than in the case of the ordinary test tubes. Chamberland has devised flasks and test tubes which differ from these only in having glass in place of indiarubber caps.

Preparation of Fluid Culture Media.

Beef Broth—Bouillon.

To prepare beef extract for the nutrition of micro-organisms, take a pound of lean beef, mince it fine ; add to this a litre of pure water, mix thoroughly, and allow to stand in a cool place for twenty-four hours ; again mix thoroughly and squeeze through a cloth, passing sufficient additional water through the meat to again make up the quantity of fluid to a litre ; boil the extract thus obtained for half an hour, render it neutral, or very slightly alkaline, by adding a saturated solution of mixed sodium hydrate, sodium carbonate, and sodium phosphate ; with a bit of gummed paper fasten a strip of neutral litmus paper and one of turmeric paper to the end of a glass rod ; as soon as the faintest alkaline reaction is obtained, add no more of the alkaline solution ; boil for an hour ; allow to cool, and remove the fat ; again filter into a large stock flask or into test tubes that have been plugged with cotton wadding and sterilized as above. These vessels, with their contents, should now be boiled in a potato steamer or other steam sterilizing apparatus for a quarter of an hour on each of two or even three successive days, the wadding plugs being protected from dust by several layers of paper tied over them, or by means of thin indiarubber caps that have been washed in a solution of corrosive sublimate. The meat extract may be modified by the addition of various materials, such as .5 per cent.

of common salt, recommended by Miquel, or of 5 per cent. glycerine, which is added before the nutrient fluid is finally sterilized (first used by Roux and Nocard). This glycerine meat extract is, as we have already seen, an excellent medium for the growth of the bacillus tuberculosis. Albumen peptone, cane or grape sugar, acetic acid, mannite, &c., have all been added in various proportions and for various purposes. Liebig's extract of beef, in the proportion of five parts to one thousand, or Cibil's extract, twenty grammes to a litre, may also be used. These latter require to be very carefully sterilized by Tyndall's method of discontinuous heating, in which the fully developed organisms are killed off in a very short time on exposure to a comparatively low temperature. Some of the spores that remain in these develop into the vegetative form during the next twenty-four hours. This crop is again killed by a second heating. The remaining spores, if any, are again encouraged to develop, and then this crop is also killed off, usually leaving the fluid sterile, though in some cases the process may have to be repeated three, four, or even five times. Various infusions and decoctions of wheat or hay, of different fruits or vegetables, yeast water, beer wort (the latter especially for the culture of the mucors and yeasts), a mixture of beer wort and prune juice (especially useful for the growth of the various aspergilli) may be used. These should all be sterilized by discontinuous heating at 100° C. in the steam sterilizing apparatus for twenty minutes on three or four successive days. Urine, aqueous humour, or other fluids of the body drawn with antiseptic precautions, may all be used as cultivation media for certain organisms.

Milk.

Milk may also be used as a culture medium, but although it is a substance easily obtained, it is a somewhat difficult matter to render it absolutely sterile. If heated under pressure to 120° C., milk may be sterilized in from ten to fifteen minutes; but in the steam sterilizing chamber, at 100° C., it is necessary to heat it for an hour on the first day, and from twenty to thirty minutes on each of the two following days.

Solid Culture Media.

Bread Crumb.

One of the simplest of the solid culture media, bread crumb, is prepared by taking the crumb of a loaf, drying it in small pieces, spreading it out on a sheet of clean paper in an oven, or on the top of a stove, or even in front of a warm fire; then rubbing it through a fine sieve, or passing it through a coffee-mill. A small quantity of this dried crumb, sufficient to cover the bottom of a flask to about the depth of a quarter of an inch, is put into a wadding-stoppered small sterilized flask; distilled water is added until the bread crumb is thoroughly moistened, no superfluous water, however, being left unabsorbed. If after allowing the bread crumb to stand for about a quarter of an hour, it is found to be properly moistened, the flasks containing it are heated in the steam chamber on each of three successive days for half an hour. In place of using water, the crumb may be moistened with beef extract, sugar solution, dilute glycerine, or any other of the fluid media already referred to. This medium is used chiefly for mucors and should be rendered slightly acid by the addition of a small quantity of Tartaric or other organic acid.

Soyka's Ground Rice Medium.

A medium which can be used instead of bread paste is that described by Soyka. It is composed of ground rice, 10 grammes; milk, 15 cc.; neutral beef bouillon, 5 cc. These ingredients are thoroughly mixed, and put into small covered glass dishes or small glass flasks, which are sterilized, as is the bread paste. It forms a beautiful solid white opaque mass, on which coloured organisms may be even more easily studied than when they are grown on bread paste.

Hueppe's Method of Cultivating on Egg Albumen.

Eggs may also be used as culture media. The yolk is broken down and mixed with the white by means of thorough shaking (or the white only may be used, in which case the yolk is left unbroken); the shell is then disinfected with bichloride of mercury solution, a hole is chipped at one end, and the membrane cut through with a pair of sterilized scissors. The inoculation is made with a pipette or a platinum needle, and then the opening is covered with a piece of sterilized cotton wadding or paper, which is painted over and sealed with surgical collodion.

Potatoes.

The simplest and most effective way in which potatoes can be used as solid culture media is by introducing small wedge-shaped strips into sterilized test tubes. All that is here necessary is to clean the potato thoroughly, then steam it for five minutes, allow it to cool, and, with a cork-borer or apple-corer, cut out a cylinder from the longest diameter, remove the two ends of the cylinder with a knife, and cut obliquely across from end to end, so that two wedge-shaped portions are formed; each of these is put into a test tube, prepared as follows:—Into a plugged test tube, sterilized by dry heat, a small piece of sterilized cotton wadding, well moistened with distilled water, is introduced and pushed to the bottom; the potato wedge is then introduced so that the base rests on the surface of the moist wadding. The whole is boiled in the steam sterilizer for at least three quarters of an hour (better an hour or an hour and a half), when it is ready for use.

If test tubes are not readily obtainable, a soup-plate and a basin, well washed and rinsed with a 1 per 1,000 solution of bichloride of mercury may be used as a sterile chamber, a couple of layers of blotting-paper soaked with the bichloride solution being placed in the soup-plate, on to which any organisms in the air under the basin may fall after the sterilizing process has been completed and the parts of the apparatus placed in position. A clean and smooth-skinned potato is thoroughly scrubbed, and the eyes and any diseased portions are removed with a sharp-pointed knife; it is then soaked for fifteen minutes in a 1 per 1,000 solution of bichloride of mercury, after which it is washed in water, wrapped in paper, and steamed for half an hour; at the end of twenty-four hours it may again be steamed for fifteen minutes, and allowed to cool. (A single steaming is, however, usually sufficient.) The hands are carefully washed first in soap and water, then in a 1 per 1,000 sublimate solution. A knife is sterilized by heat in a naked flame, or in the hot-air chamber at 150° C. (in the latter case it should first be wrapped in cotton wadding or in paper), and then allowed to cool. The potato is taken in the left hand, and, with the sterilized knife, is cut through the middle, and the two halves are intro-

duced under the basin with their cut surfaces, on which the inoculations are to be made, uppermost. Various modifications of these methods may be made by individual workers, but in most cases the potato used in the test tube is the most convenient.

Koch's Gelatinized Meat Peptone Medium.

To prepare Koch's peptone meat jelly or solid gelatine medium, cover one pound of beef, freed from fat and finely minced, with 1,000 cc. of water, to which a drop or two of hydrochloric acid may be added; allow the mixture to stand in a cool place for twenty-four hours, and squeeze through a cloth, as described in the preparation of meat extract. To this fluid add 10 grammes of albumen peptone, 5 grammes of common salt, and 100 grammes (75, or even 50, if the weather be cool) of Coignet's *gold label* gelatine. Mix in a two- or three-litre flask, boil for half an hour, or until the gelatine is thoroughly dissolved, neutralize with the alkaline solution (p. 399), and again boil for nearly an hour. This length of boiling in some cases appears to be too prolonged, the gelatine afterwards not becoming properly solidified. In such cases it is necessary to add a little more gelatine, boil, and again neutralize. The mass is then filtered through a layer of fine white filter-paper, moistened with hot water in a funnel, which must be kept heated, to prevent solidification of the gelatine on the filter. Salomonsen recommends a very ingenious device, a device that may be used at almost any time, especially where only small quantities of gelatine are to be filtered. He pours a little of the warm gelatine into a filter which has been previously sterilized, warmed, and moistened as follows: a layer of water is poured into a flask to the depth of half an inch, in the mouth of which rests a funnel with a clean filter-paper. The top of the funnel is covered with several thicknesses of filter-paper, over which a sheet of asbestos or a plate of zinc is laid. By heating the water to boiling-point for a few minutes, flask, funnel, and filter are at once sterilized, warmed, and moistened. The hot water is then poured from the flask, the funnel is replaced, its cover carefully removed, and the gelatine is poured into it. If the flask be placed on a non-conducting surface, a very considerable quantity of gelatine may be thus filtered. Where larger quantities have to be filtered, it may be necessary to obtain a double walled metal funnel, or, better still, an enamelled funnel with the limb bent at an angle. This funnel is fitted into the top of the steam sterilizing apparatus, the bent tube coming through the side. This, of course, can be heated for any length of time, and is useful when large quantities of gelatine or agar-agar are dealt with. (This apparatus, which is comparatively cheap, may be obtained from Frazer, Teviot Place, Edinburgh.) Before filtering, it is sometimes necessary to clarify the gelatine by a process well known to cooks. The gelatine is allowed to cool to about 50° C., the white of an egg is broken into 100 grammes of water; this, along with the broken-up shell, is added to the gelatine, with which it is thoroughly mixed. The whole is then boiled until the albumen coagulates and a perfectly clear liquid appears between the flakes; the mass is then filtered.

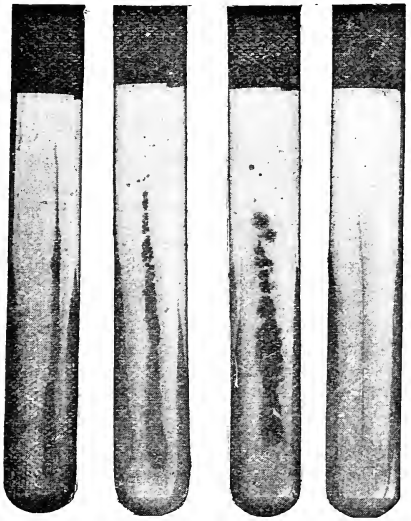
Gelatinized Milk Serum.

One litre of fresh milk is warmed to 60° or 70° C. Add from 70 to 100 grammes of gelatine (according to external temperature) and dissolve. Boil for a few minutes, until all the casein is precipitated, and pass through a fine muslin strainer. The fluid is allowed to

stand for about twenty minutes at the temperature of the body, in order to allow the fat to come to the surface, after which it is allowed to cool, and the layer of cream is carefully removed. To the resulting slightly opalescent fluid, 1 per cent. of albumen peptone is added, and the whole is neutralized, boiled, filtered, and sterilized and used as nutrient gelatine. This material, like agar-agar (which was first used by Frau Hesse), was introduced into bacteriological work by a lady, Madame Raskina.

Agar-Agar Peptone Meat Jelly.

In place of 10 per cent. gelatine, we may use 1.5 per cent. of agar-agar. This is prepared in much the same manner as the gelatine jelly,



Photographs. Growths on oblique Agar-Agar surface of—
1. White Torula.
2. Yellow Torula.
3. Aspergillus Albus.
4. Scheuerlein's Cancer bacillus.
Numbered from left to right.

except that it requires a more prolonged boiling before it can be properly filtered. It is prepared in the following manner :—Cut into pieces, about half an inch long, 15 grammes of agar-agar, place in a porcelain basin, and soak in a strong solution of common salt for twenty-four hours, pick out the coarse particles of dirt and wash thoroughly, then drain off the water ; to the washed agar-agar add one of the following solutions : (1) Water, 1,000 grammes; beef, 1 pound ; peptone, 10 grammes; common salt, 5 grammes; or (2) water, 1,000 grammes; Liebig's extract of beef, 5 grammes; albumen peptone, 30 grammes; common salt, 5 grammes.

In (1) the beef is first extracted, as in the preparation of gelatine. The

preparation after this is the same in both cases. Boil the mixture thoroughly over a naked flame for about an hour (great care being required at first to prevent the mixture boiling over in consequence of the large amount of air contained in the agar-agar, which is, however, gradually boiled out). As a small quantity of the agar is dissolved, the boiling point is gradually raised until the temperature of the mixture is about 105° C., instead of 100° C., and the complete solution of the agar-agar is more readily brought about. As soon as the whole of the agar is dissolved, which is usually in about an hour, the mass should be filtered as quickly as possible through a very hot filter.

To Make Glycerine Agar.—After filtration of the agar jelly, add from 5 to 8 per cent. of glycerine, *i.e.*, 50 to 80 cc. per 1,000, sterilize and put into test tubes. After sterilizing, allow to cool in the oblique position in order to obtain a larger surface. This also applies to the other agar-agar media.

For the preparation of Agar-Agar and Gelatine (*see p.* 392.)

Koch's Sterilized Blood Serum.

A large stoppered glass jar, well washed with soap and warm water, is sterilized either by being wrapped up in paper and heated for an hour at a temperature of 150° C., or by being rinsed with a 1 to 1,000 solution of bichloride of mercury, and then with absolute alcohol to remove the mercury salt. The stopper is greased with pure vaseline. When the animal from which the blood is to be collected has been stabbed the first few drops of blood that come should be rejected, and that which follows must be collected as carefully as possible. The jar is then placed in cold water until the blood coagulates, removed to the laboratory and placed in an ice box; and the first few drops of coloured serum that soon make their appearance on the surface of the clot are carefully removed with a pipette. It is then left for from twenty-four to thirty-six hours, at the end of which time a quantity of clear serum will be found to have been expressed. In the meantime a pipette has been prepared by exposing it to the action of boiling water for ten or fifteen minutes, or by thoroughly cleaning it with hot water and rinsing with bichloride of mercury and alcohol and then passing it through a flame for the purpose of driving off the alcohol. This pipette is used to transfer the clear serum from the space around the clot to the sterilized plugged test tubes, care being taken that the serum is not allowed to smear the sides of these tubes. The test tubes, with the contained clear serum, are placed for an hour in a water bath, which is kept at a temperature of 58° to 60° C. every day for a week. Each day the serum becomes clearer, and a small precipitate of grey powdery material collects at the bottom and a thin film of cholesterin forms on the surface. This water bath may be constructed very cheaply. It is made of tin, is about twenty-seven inches high and four inches in diameter, with a collar projecting about half an inch. Over this collar a piece of iron netting serves both as a lid and as a support to the thermometer, which is fixed in a cork passing through a hole in the centre; or a bulb thermometer may be used. This is supported by a second piece of wire gauze resting at some little distance—about three-quarters of an inch—from the bottom of the bath. When the water has reached a temperature of 58° C. it is easily maintained there by means of a small spirit flame. In place of this simple apparatus any of the small incubating chambers sold by the chemical apparatus makers may be used for this discontinuous sterilization at comparatively low temperature. Special serum

sterilizers and inspissators may also be obtained from the apparatus makers. Blood serum so sterilized may be used in various ways. It may be rendered solid with the tube in an upright position, or when a large surface is required in an oblique position, by placing the tube on the sloping floor of a warm chamber. This slope should be such that the blood serum does not come within an inch of the wadding plug. To bring about the solidification the temperature should be kept between 65° and 68° C., at which temperature solidification takes place slowly, but the serum remains comparatively clear. Sheep's blood coagulates more rapidly than that of the calf, the time required varying from half an hour to an hour, according to the temperature that is maintained. In place of test tubes, watch glasses or covered glass dishes may be used to hold the serum. As the serum cools there collects in the tube a quantity of condensed vapour; this, however, may be prevented by adding a small quantity (1 per cent.) of gelatine or 6 to 8 per cent. of glycerine. This latter prevents the formation of a dry scaly surface, and at the same time absorbs a considerable proportion of the condensed vapour. When glycerine is added the temperature required to bring about solidification is over 75° or 78° C.

Löffler's Serum Medium.

Löffler added to three parts of blood serum one part of the flesh infusion already described, 1 per cent. of peptone, 1 per cent. of grape sugar, and 5 per cent. of common salt. The meat broth, prepared as above described (p. 399), is allowed to cool down to 50° C., and is then mixed with the serum.

Hueppe's Agar Serum.

Hueppe uses a mixture of blood serum and agar-agar for plate cultivations, especially where he wishes to use the plate method for the separation of the tubercle bacilli. He takes the sterilized serum at a temperature of 37° C., inoculates it, and shakes it thoroughly in order to distribute the organisms through it; he then pours it into the fluid agar-agar meat peptone solution at 42° C.; the mixture is again well shaken to continue the distribution of the germs equally through the fluid, and then either plate, tube, or flask cultivations are made; the mass is allowed to become solid, after which it is kept at the temperature of the body (about 37° C.), at which temperature it remains perfectly solid.

To Fill Test Tubes or Flasks with Fluid Media.

Before transferring fluid gelatine or agar-agar meat broth or other fluid media to test tubes or flasks, these should be previously plugged and sterilized, as mentioned on p. 399. With a sterilized pipette (see p. 389) the fluid is run into these vessels, the plug is returned, and the vessels with the contained nutrient medium are sterilized for ten minutes or a quarter of an hour on each of two successive days. As soon as the vessels are taken from the steamer the plugs are covered with sheets of sterilized paper, which are kept in position by string or indiarubber bands.

In place of the sterilized pipette a small carefully-sterilized funnel may be used. The stem of this is simply inserted into the flask or test tube, and the amount of the medium required is poured in from small stock flasks. A burette with a small funnel may be used in the same way, or better still, the stock flasks may be fitted with an indiarubber bung in which are bored two holes. Through one of these holes is introduced a glass tube, at one end

of which is a thistle-head funnel filled with sterilized cotton wadding. The other end dips down *nearly* to the level of the nutrient medium. Through the other hole a short glass tube passes just to the other side of the bung; its outer end is slightly bent, and is fitted with a piece of indiarubber tubing, on which a pinch clip fits. Into the other end of the rubber tubing a wash-bottle nozzle is fitted. The gelatine is first filtered into this flask, and the whole is sterilized as usual, a piece of cotton wadding or paper tied in position serving to keep dust away from the nozzle. When tubes or small flasks are to be filled the gelatine is melted, the flask, inverted, is fitted into a retort stand ring, the nozzle is allowed to fall into the mouth of the vessel to be filled and the pinch clip is compressed. If care be taken to cover the nozzle carefully each time that the flask is used, there is very little danger of contamination from without. Fluid media may also be kept in these flasks, but Lister's flasks are perhaps adapted better for this purpose.

Apparatus used for Inoculating Animals or Artificial Nutrient Media.

Inoculating needles are made of pieces of platinum wire mounted in glass rods; these are of various shapes—(1) perfectly straight, (2) with a loop for inoculating liquids, or (3) with a hook or short rectangular limb for inoculating extensive free surfaces, such as "oblique" blood serum, agar-agar or gelatine, potatoes, &c. These are usually allowed to stand upright in a wide-mouthed bottle in which a piece of cotton wadding is placed. Before it is used the wire should be heated to a white heat; the glass rod is also thoroughly heated; the needle is then allowed to cool and a small quantity of the substance to be inoculated is taken on the end of the wire and introduced as required. Where the inoculations have to be made into narrow-mouthed flasks or tubes, short fragments of platinum wire are often used. These, held in a pair of forceps, are heated in a flame. When they are quite cool, one of them is dipped into the inoculating fluid and then dropped into the fluid culture medium. Capillary tubes, five or six inches long, pipettes, or glass needles, may be used for the same purpose. Where large quantities of fluid have to be inoculated, the Pasteur pipette, which consists of a piece of tubing with one end drawn out into a fine capillary tube, the other being plugged with sterilized cotton wadding, is often used. These capillary pipettes are always kept closed at the ends. All that is necessary before using them is to snip off the end with a pair of sterilized forceps, pass the glass once or twice through the flame, allow it to cool, and then draw up the amount of inoculating fluid required and inject into the media, tissues, or vessels.

Cantani uses a similar pipette, but fits it up as follows:—Over the end of the pipette that is filled with cotton wadding he fits a piece of india-rubber tubing. Into the other end of this latter is placed a piece of glass tubing, in which is a hole at the side; then comes another piece of india-rubber tubing, then a glass mouthpiece, and the apparatus is complete. When the apparatus is to be used, the end of the pipette is broken off after being carefully heated and allowed to cool; it is then introduced into the inoculating fluid, a finger is placed over the orifice in the side of the middle glass tube, and by suction the required quantity of fluid is drawn into the pipette; the finger on the opening controlling the pressure or vacuum. To inject, reverse the process. As soon as the finger is removed the pressure within the tube becomes equal to that outside, however much suction or blowing there may be, and the operation stops. It is a valuable apparatus,

because when it is used there is never any danger of drawing toxic fluids into the mouth.

Glass needles are especially useful when anærobic organisms are being dealt with, as the smooth surface of the glass does not allow of oxygen being carried down with it along the track, which closes up as soon as the needle is withdrawn.

To Inoculate Solid Culture Media.

The test tube or flask is held inverted in the left hand, and the plug of cotton wool is twisted once or twice in the mouth of the test tube to break down any adhesions between it and the neck of the vessel. If the plug is at all dusty, it is well to singe the surface by passing it rapidly through a flame before removing it from its position. Wadding burns very rapidly, and must be extinguished at once. The plug is removed and held between two of the unoccupied fingers of the left hand, great care being taken that no part of the plug that passes into the test tube shall come in contact with any source of infection other than the air itself. At the same time this portion of the plug is directed downwards, in order to avoid any falling germs that may be present in the atmosphere. The platinum or glass needle with its charge of seed material, is plunged straight into the gelatine mass, then carefully withdrawn and the plug replaced. Where the seed material is also in solid gelatine, the two tubes may be held inverted in the left hand, one between the thumb and finger, the other between the first and second, the plugs being held between the second and third and third and fourth fingers.

Methods of Cultivating Anærobic Organisms.

When it is necessary to cultivate such an organism as that of malignant œdema or of tetanus the experimenter has to resort to the methods used for keeping out the air. To isolate these organisms, Koch first used the ordinary plate cultures, placing at irregular points on the surface of the gelatine, thin sheets of mica that had previously been sterilized (p. 172). Fraenkel, to render the exclusion of oxygen more perfect, ran a little melted paraffin round the margins of these plates. By filling a tube with gelatine or agar, Liborius succeeded in obtaining cultures of anærobic organisms at the lower part of his culture medium. If the tube is filled with the solid medium close up to the plug, and the air is carefully excluded by means of melted paraffin poured over the plug, this method often succeeds exceedingly well. It is, however, difficult to obtain pure inoculating material from such growths without removing the contents of the tube or breaking it up altogether.

One of the simplest methods of isolating anærobic bacteria in gelatinized media is that described by Fraenkel, who uses a test tube fitted up like a wash-bottle, with a long and a short glass tube passing through an india-rubber cork. This, after being carefully sterilized, has about 10 cc. of nutrient gelatine poured into it. An inoculation is made, and the india-rubber cork, with its two tubes, is pushed home into the mouth of the test tube, and the whole is luted down with paraffin, great care having been taken to sterilize, by heating in a flame, those parts of the tubes that are to go down into the test tube. A stream of hydrogen, purified by being washed in an alkaline solution of pyrogallic acid, is passed through this liquefied gelatine (kept at 35° C.) for four or five minutes. The tubes are then

sealed in a flame, and a "tube plate" cultivation is made by distributing the gelatine on the walls of the test tube. In this way excellent anærobic cultures may be obtained. The advantage of this method is that fresh inoculations may be readily made from the growths that appear on the thin layer of gelatine that covers the wall of the tube. Anærobic cultures may also be obtained by drawing the media, that have been de-oxygenated by means of hydrogen or by means of the air pump, into capillary tubes, which are then sealed so as to prevent the access of any fresh oxygen. Fluid media, of course, may be used in exactly the same way. Where an air pump or water exhaust apparatus is obtainable, an ordinary tube, with thick walls and a long neck, may be used in which to make the culture; but a combination of these methods, as a rule, gives the best results. To such a tube may be fitted a "T" piece with a stop-cock, through which a vacuum is first created; then hydrogen is allowed to come in, the process being repeated several times until all the oxygen is removed. One very simple method of obtaining a solid medium in which some anærobic organisms will grow is to add a small quantity of glucose (1 to 2 per cent.) to agar-agar or gelatine. For the more complicated methods we refer the reader to special treatises.

Various methods have been utilized for preserving anærobic cultures or of obtaining "needle" cultivations of such cultures in gelatine, &c. In some of the earlier experiments on abiogenesis a layer of warm oil was used to prevent the access of air to the nutrient medium, and this method is still sometimes used when inoculating a solid medium with an anærobic organism. A puncture cultivation is made with a glass needle, and then a layer of a couple of inches of boiled and cooled olive oil is carefully poured over the surface. Roux used two methods—one physical, the other biological—for the attainment of this object. For the former he draws out one end of a pipette into a long capillary tube, then a short distance from the other end a constriction is made; the whole is carefully heated to 150° C. in the hot air chamber, or by a Bunsen burner, the point of the pipette being closed by fusion, and the other end plugged with sterilized cotton-wadding. This pipette is then filled with sterilized nutrient gelatine that has been brought to the boiling point in order that as much air as possible may be driven off. To do this the capillary point is broken with sterilized forceps; the fluid is drawn up to the level of the constricted portion of the tube by means of suction; the capillary end is then fused, after which the tube is also fused at the constricted part at the other end. Such tubes may be kept for an indefinite period. To inoculate the gelatine in this tube one end is broken, a fine glass needle on which is the material to be inoculated, is carefully introduced and the end is again sealed. Under these conditions no air can make its way to the organism, or to the medium into which it is inoculated.

Roux's second, or biological, method is to boil a quantity of nutrient agar in a test tube, and then cool it as quickly as possible in cold water. It is then inoculated with the anærobic organism on a smooth glass needle. A layer of melted nutrient gelatine is poured on the surface, and when this is cooled a drop of a broth cultivation of "Bacillus subtilis" is run on to the surface from a capillary pipette. The tube is then fused, or the cotton-wadding plug is rendered impervious by being luted with warm paraffin. As the Bacillus subtilis develops and grows it uses up the oxygen at the surface. The organism below receives none, and

is thus placed under favourable (anærobic) conditions for its growth. To obtain inoculating material from such a tube it is necessary to break it at the bottom when the growth is easily taken from the lower part of the medium. Salomonsen uses a modification of this method, placing one tube within another. A small inner tube containing gelatine or agar is inoculated with the anærobic organism, the outer tube, the neck of which is sealed, or the opening of which is closed with paraffin, contains bouillon which is inoculated with a strongly ærobic organism, such as the Bacillus subtilis. In place of the bouillon and Bacillus subtilis a solution of one part of pyrogallic acid to ten parts of a ten per cent. solution of caustic potash may be used. Buchner, in making use of this method, plugged his inner tube in the ordinary fashion with cotton wadding and supported it on a kind of stage, closing the orifice of the larger tube with an indiarubber cork. This is a very convenient plan, as it enables the observer to gain access to the cultures very readily indeed. All these methods may be used with both solid and fluid media; and now that potatoes can be used within test tubes they also may be used for the cultivation of anærobic organisms. Here all that is necessary is a strong test tube with a lateral stem. The inoculation is made through the open mouth of the tube which is then sealed, after which, by means of an exhaust apparatus, the air is extracted from the potato and then from the tube; the lateral tube is then sealed.

To Separate Bacteria from their Products.

To obtain the products of bacteria apart from the bacteria, and, therefore, in a sterile condition, several methods of filtration have been suggested, all of them depending on the close porous nature of unglazed porcelain and baked clay. When fluid that originally contains bacteria is aspirated or forced through such a filter, all the organisms are kept back, and a perfectly clear sterile fluid comes through on the opposite side. The best form of filter is a tube of this unglazed porcelain, one end of which is closed, and the other so constricted that a thick walled indiarubber tube may be affixed. The Chamberland filter consists of a wide tube, as above described, the upper piece of which is composed of a glazed funnel-shaped end with a nipple on which a piece of indiarubber tubing can be fastened. The other end of this tubing is fitted to the long glass tube of an ordinary strong wash bottle or flask which acts as a receiver; the second or short tube of the wash bottle is attached to an aspirating apparatus, either in the form of a siphon bottle placed at a lower level, or of a Geissler water exhaust pump. The wash bottle, the filter, and the glass tube, are all carefully plugged, covered with paper or cotton-wadding and sterilized for an hour at 150° C. The indiarubber stopper of the wash bottle and the tubing are sterilized by being well soaked in a 1 per 1,000 solution of bichloride of mercury, washed with sterilized distilled water, and then boiled for twenty minutes in the steaming apparatus. As soon as everything is cool the hands are thoroughly cleansed, and the apparatus is put together as above described, a small cotton-wadding plug being left in the short tube of the wash bottle to prevent the return of unfiltered air when the exhaust apparatus is removed. When all is ready, the filter is lowered into a tall glass jar with a firm base, which contains the fluid to be filtered. The aspirator or other exhaust apparatus is set to work (care being taken that the exhaust is not too great), and the fluid is drawn into the flask which is gradually filled.

Another flask may be inserted, and so on until the whole of the fluid is filtered. At the end of the process, such fluid as remains in the filter is withdrawn by means of a sterilized pipette, and may be used along with the other, though it is better to use a fresh filter for each flask, as then the indiarubber communication between the flask and the filter may be clamped and the flask removed. The fluid within the flasks so removed may be kept sterile for a considerable length of time. Another very convenient filter is Kitasato's, which consists of a large thistle shaped funnel attached by an indiarubber connection to a piece of very thick pipe stem of unglazed porcelain, which is well-plugged with baked porcelain at the bottom. This pipe stem passes down through the indiarubber cork into a bottle which serves as a receiver. From the side of the neck of the receiver a lateral tube is given off, to which the exhaust apparatus may be attached. The apparatus is thoroughly sterilized as above. The fluid to be filtered is placed in the funnel, the exhaust is applied and the filtrate passes down into the bottle. Here also it is well to place a small plug of cotton-wadding in the lateral tube. In all cases where the water exhaust pump is used it is well to have a bottle intervening between the receiver and the pump, so that should there be any backward flow of water it may pass into this flask and not into the receiver. This filter requires a somewhat stronger exhaust than the Chamberland pattern, and an ordinary aspirating siphon bottle is not sufficient to obtain the required suction. Various modifications of this apparatus will at once suggest themselves to an ingenious worker, but one that may sometimes be used, especially where the culture fluids have to be kept at a low temperature, is that recommended by Miquel. It consists of a flask with a long wide neck; at some distance above the bulb (about halfway) this neck is considerably constricted, and between the constriction and the bulb is a long narrow lateral tube which is pointed somewhat downwards. To prepare this as a filter, the tube above the constriction is packed with asbestos, above this is poured some thoroughly dried and sterilized plaster of Paris made into a cream-like paste with boiled distilled water, this should be nearly an inch in thickness. The plaster is allowed to "set," and the filter is ready for use. A small quantity of water is put into the flask and thoroughly boiled, the steam escaping from the lateral tube. As soon as the water is boiled away, but while the flask is still full of steam, the lateral tube is sealed. The whole apparatus is now thoroughly sterile: an indiarubber bung with a funnel passing through the centre is fitted into the tube above the plaster of Paris; into this the fluid to be filtered is poured. As the air in the flask cools (and eventually ice may be packed round the flask) the fluid is drawn through the plaster of Paris; this flask can seldom be more than about half filled in this manner. After the indiarubber cork has been removed, the flask may be kept in ice, the contents remaining sterile for an indefinite period.

Hanging Drop Cultures.

The best form of moist chamber for making drop cultures is an ordinary slide into which is cut a deep round groove which surrounds a central pillar or disc of glass which has been ground and polished so as to be slightly below the level of the remainder of the slide. This, after careful sterilization, is used as follows: A small drop of the fluid is placed upon the lower central pillar (a small portion of the fluid may also be allowed to run into

the groove), the cover glass, after being thoroughly heated in the flame, is allowed to cool; a ring of vaseline is painted on the slide around the groove; on to this the cover glass is lowered, where it compresses the drop of culture fluid, which thus presents a flat surface and allows of the development of the bacteria being exceedingly well followed. The groove around the disc forms a kind of air chamber. If it is required that the oxygen should be absorbed this groove may be partially filled with the alkaline pyrogallic acid solution.

Various modifications of the hanging drop culture method are described. Buchner dries the spores upon a sterilized cover glass, then places on this a drop of his culture fluid and inverts the cover glass; fragments of cover glass are then arranged around the drop so as to support the cover glass on which it is hanging and the whole is cemented down to form a perfectly air-tight cell in which the development of the organisms may be readily watched.

Salomonsen describes a moist chamber constructed of a thin sheet of cardboard which is sterilized by boiling, and is rendered so soft that it fits accurately to the slide and allows of the cover glass with its hanging drop of inoculation fluid being pressed down so as to form an air-tight chamber; the moisture from the cardboard serving to prevent the evaporation of the fluid. It is difficult to keep these cardboard supports sufficiently moist and sterile without the use of somewhat complicated moist sterilized chambers. The best way of keeping them, however, is under bell jars, the air of which is saturated with moisture. These hanging drop cultures may be dried, and the organisms are then stained as in the case of an ordinary cover glass preparation. Watson Cheyne has utilized this method in a most ingenious manner for obtaining a permanent record of the various phases of development of micro-organisms. He makes a series of hanging drop cultures of any organism that is to be studied, and then dries and stains them, taking them at stated intervals, say, five, ten, fifteen, twenty minutes, and so on through a whole series. In this manner the whole cycle of development may be watched and referred to again and again, especially if different series of these cultures be grown at different temperatures.

Methods of Staining Bacteria.

Methylene Blue.

Methylene blue may be kept as a saturated alcoholic solution; a few drops of this filtered into water will give a very beautiful stain in cover glass preparations. The sputum, &c., on the cover glass, after being dried and passed three times rapidly through a spirit lamp or Bunsen flame (see page 206), is floated on the surface of the methylene blue, it is allowed to stain for five or ten minutes, washed in water (sometimes first in alcohol), tilted on edge and allowed to dry. It is then mounted in xylol balsam.

Kühne's Methylene Blue Method.

One of the best general stains for bacteriological work is Kühne's methylene blue solution. 1.5 grammes of methyl blue is dissolved in 10 cc. of absolute alcohol and 100 cc. of a 1 to 20 watery solution of carbolic acid. Specimens are stained in this for from five minutes to two hours, although sections may be left in it for a whole day without becoming overstained. They are then carefully washed in water, then with acidulated water made by adding a couple of drops of hydrochloric acid to 100 cc. of water. As soon

as the sections become a pale blue colour they are transferred to a solution of lithium water made of the strength of about 1 part of lithium carbonate to 20 of water; they are then thoroughly washed in pure water, dehydrated in absolute alcohol in which a little methyl blue may be dissolved, placed in aniline oil, which may or may not contain a small portion of methyl blue in solution, and rinsed in pure aniline oil. They are after this treatment transferred to terebene, where they are left for about a couple of minutes. They are then washed in two lots of xylol and mounted in Canada balsam. Almost any organisms may be stained by this method, even the glanders bacillus coming out fairly distinctly.

Another great stand-by of bacteriologists is fuchsin. With this reagent almost every bacillus may be brought into prominence. It is especially useful for the bacilli of tubercle, leprosy, and mouse septicæmia.

Ziehl-Neelsen Carbol-Fuchsin Method for Tubercle Bacilli.

The Ziehl-Neelsen method of staining the tubercle bacillus is a modification of the Weigert-Ehrlich method. The sections or cover glasses are stained in Neelsen's solution, made as follows:—Fuchsin 1 part, is dissolved in 10 parts of absolute alcohol, to this solution are added 100 parts of a 5 per cent. watery solution of carbolic acid, and the mixture is heated until steam rises pretty freely. Cover glass preparations are stained in three or four minutes, or even less; sections are usually sufficiently deeply stained in seven or eight minutes. In the cold they may be left for twelve or even twenty-four hours. The superfluous fluid is drained off and the preparations are placed for a second or two in alcohol (90 per cent.), then in a 25 per cent. solution of sulphuric acid, when the pink tinge should immediately be replaced by a yellowish brown. The preparations are then washed in alcohol, and if they are sufficiently decolorized they are transferred to a solution of lithium carbonate. They may afterwards be stained with a watery solution of methylene blue, cleared up with clove oil or with aniline oil, terebene, and xylol, and mounted in Canada balsam. Exceedingly good results are obtained by this method, which is preferable in many respects to the aniline oil method. In place of sulphuric acid nitric or hydrochloric acid may be used.

The Kühne-Gram Staining Method.

Kühne's modification of Gram's method is, perhaps, superior to the original. Instead of using Weigert's saturated alcoholic solution of methyl violet (or gentian violet) in 100 parts of aniline water and 10 parts of absolute alcohol, he stains with a 2 per cent. solution of gentian voilet in dilute alcohol, to which has been added one sixth of its bulk of a 1 per cent. watery solution of ammonium carbonate, or with a similar solution of Victoria blue without the ammonium carbonate, for about five or ten minutes. The preparations are then rinsed in water, and are placed in Gram's solution made up of iodine 1 gramme, iodide of potassium 2 grammes, distilled water 300 cc. for two or three minutes; they are again washed in water, dehydrated with fluoresceine alcohol, which is prepared by dissolving 1 gramme of yellow fluoresceine in 50 cc. of absolute alcohol, the part undissolved being allowed to settle at the bottom of the bottle. The section is washed in pure alcohol, then with aniline oil, and mounted in xylol balsam.

The Kühne-Unna Method.

Kühne describes a modified method of making dry preparations adopted

from Unna's method. The preparations are stained in methylene blue, and up to the stage of the washing with lithium carbonate are treated exactly as above The section is then spread out on a cover glass, and the moisture is allowed to run off the edge on to blotting-paper, the upper surface being carefully wiped with a cloth. With a balloon syringe a stream of air is then directed down on to the section as it lies on the cover glass, and beginning at the centre of the section gradually driving the water towards the margins, and then on to the cover glass, whence it may be removed by scraps of blotting-paper. The sections are then placed on a plate of glass which is gently warmed over a lamp to the body temperature, the sections gradually becoming transparent and glassy, the heating is continued for about five minutes after this occurs, after which they are treated with terebene, then with xylol, and mounted in balsam in the usual fashion. For other methods the reader is referred to works specially devoted to the treatment of this subject.

To demonstrate Tubercle bacilli in Milk.

To demonstrate tubercle bacilli in tuberculous milk the best plan is to pass the milk through a centrifugal apparatus and to take the sediment for examination, as almost the whole of the bacilli that were originally in the milk will be found along with the mucus and solid particles in this sediment. Where it is not possible to obtain the use of such apparatus the milk should be allowed to stand for from twelve to twenty-four hours in a glass "separator" such as is used by chemists or in a conical or funnel-shaped vessel surrounded by ice. The sediment with the contained bacilli is drawn off from the separator by the tap placed at its lower part, or the cream and the upper layers of the milk may be carefully removed by means of a siphon, then with a pipette a few drops of the milk from the bottom of the funnel are taken, dried on a cover glass, and examined in the ordinary way. In place of the separator or other funnel-shaped vessel, I have used, at Mr. Coghill's suggestion, a long wide burette in which to place the milk. In drawing off the sediment from the separator or burette the first few drops are rejected, the fluid from immediately above the stop-cock, which contains most of the bacilli, being taken.

To demonstrate Flagella on Bacilli.

Make a potato broth composed of two parts cooked potato mashed and boiled in ten parts of distilled water; carefully sterilize; on this make a cultivation of the required organism. A drop of the culture is then diluted from five to ten times with distilled water. If the organisms will not grow on this potato broth they may be cultivated in meat bouillon, which must be diluted forty or fifty times before it is used for microscopic examination, or on gelatine, which must be diluted about one hundred times. A drop of the diluted fluid is spread on a cover glass; on this a drop of 10 per cent. alcohol is allowed to fall; the whole is dried in the open air or in a warm room at a temperature of 40° C.; the bacilli are then stained in a solution made up as follows:—10 per cent. tannin solution 20 parts, water 80 parts, cold saturated solution of sulphate of iron 5 parts, fuchsin or methyl violet 1 part; to this mixture a drop of hydrochloric acid in some cases, or of an alkaline solution in others, will bring out flagella most beautifully. Acetic or sulphuric acid may be used. Cholera bacillus, vibrio Metschnikoff, spirillum rubrum, spirillum concentricum, and proteus

vulgaris all stain on the addition of larger or smaller portions of acid. With alkali the bacillus crystallosus, micrococcus agilis, and the typhoid bacillus all show flagella. The glanders bacillus, although said to be motile, has apparently no flagella.

Identification of Species of Bacteria.

The Organism is a Micrococcus.
The „ „ Bacillus, see p. 421.
The „ „ Spirillum, see p. 438.

The Organism is a Micrococcus.

I. The gelatine is not liquefied.
II. The „ is liquefied, see p. 418.
III. No growth on gelatine, see p. 421.

The gelatine is not liquefied.

A. The colonies are white.
B. The „ „ yellow, see p. 417.
C. The „ „ red, see p. 418.
D. The „ „ black, see p. 418.

A. The colonies are white.

a. The colonies are small, but confluent, growing slowly.
b. Colonies confluent, growing luxuriantly, p. 416.

a. The colonies are small, not confluent, growing slowly.

(1) *Streptococcus pyogenes*.—On plates grow as small punctiform masses ½-mm. in diameter, at first appear white, pale yellow, and then brown, under low power of microscope; no tendency to run together in either plate, puncture, or stroke cultivations, except on blood serum, or agar-agar, where the mass is thicker in the centre; terraced towards edges, and then again discrete as in gelatine cultivations at the extreme margins; no growth on potatoes; Cocci 1μ. in diameter arranged in chains or diplococci; not pathogenic to mice or healthy rabbits; frequently found in pus in human subject and in lymphatics, near the spreading margin of a suppurating area.

(2) *Streptococcus erysipelatosus*.—Very like the above, but differs in that in stroke cultivations the colonies have a somewhat greater tendency to run together; these appear whiter and more opaque, and have at the periphery numerous outgrowths which consist of projecting chains, which give to the cultivation the appearance of a fern-leaf; found in the lymphatics of the spreading zone of an erysipelatous area; it sets up erysipelatous inflammation when inoculated into the ear of a rabbit; sets up typical erysipelas and not suppuration in man.

(3) *Streptococcus pyogenes malignus*.—Cultivated by Flügge (also described by Krause), from necrotic masses in a leucaemic spleen. Colonies only visible at end of forty-eight hours; stroke cultivations, like those of No. 1, fatal to mice and rabbits in about four days. Symptoms at first like those obtained with 1 and 2, but soon followed by suppuration and general infection.

(4) *Streptococcus articulorum.*—Found in the mucous membrane and tonsils of cases of diphtheria and scarlatina; colonies grow slowly; appear as transparent watery greyish drops with delicate feather-like protrusions at the margins; chains have here and there larger cocci; slight indications of transverse division; often kills rabbits and mice with formation of pus in joints in which these streptococci are found; this occurs specially when cultivations are injected directly into the veins.

(5) *Diplococcus albicans tardissimus.*—Grows very slowly on nutrient jelly, the track being only about 1mm. broad after several weeks; grows more rapidly on blood serum at the body temperature, when colonies form as greyish-white points; these have a peculiar moist appearance and an irregular outline; identical in form with the gonococcus (see p. 421), but individuals are more adherent and form small masses.

(6) *Streptococcus septicus.*—Colonies grow very slowly indeed; seen as fine points on fourth and fifth days in plate and puncture cultivations; cocci have a special tendency to form chains or diplococci; fatal to mice in forty-eight to seventy-two hours, to rabbits in three or four days, when injected into veins; vessels in various organs plugged with organism, this leading to the formation of purulent or necrotic foci.

(7) *Micrococcus or Diplococcus of Trachoma.* (Sattler).—An organism found in the contents of the follicles of the eyelids in cases of acute conjunctivitis met with in Egypt; in the contracted follicles met with in trachoma. It grows on plates in the form of whitish clouds; in gelatine tubes it grows as pearly white tufts, little beads running along the line of the needle; later, these become slightly yellow; on agar-agar, potatoes, and blood serum we have a similar growth on the surface, which is usually somewhat viscid; grows best at the body temperature; is a diplococcus, but the line of division is not very distinctly marked; the only motion that has been noticed is a rotatory, or oscillatory one; gives rise to trachoma when inoculated into the eyelids of the human subject, but does not effect rabbits.

(8) *Micrococcus of Cattle pneumonia* (*Micrococcus der Lungenseuche der Rinder*). (Poels and Nolen).—This organism grows on plates as sharply circumscribed white rounded colonies with a delicate yellow tinge; in gelatine tubes it grows very much like Friedländer's pneumonia bacillus, but in place of being white it has a delicate cream colour; has a similar growth on agar-agar; on potatoes it forms a moist yellowish layer; on blood serum it is at first white but gradually assumes the cream colour above mentioned; this organism, which grows best at about 37° C., consists of cocci of various sizes of an average diameter of .9μ; it is single, or may be arranged in short chains of from two to six cocci; is usually surrounded by a somewhat deeply stained capsule; pure cultures introduced into the trachea of rabbits, guinea-pigs, dogs, and cattle produce pneumonia.

(9) *Micrococcus of Mastitis* (*Micrococcus der Mastitis der Kühe*).—Obtained by Kitt from the inflamed udder of the cow. On gelatine plates it grows as little opalescent white rounded well-defined drops from the size of a pin's head to a lentil; in gelatine tubes it grows as a white opaque fungus-like mass along the needle track; on potatoes it occurs as a prominent layer, whitish or dark yellow in colour, which after several days becomes moist and glistening looking; grows in milk at the temperature of the body, and gives rise to a lactic acid fermentation; a micrococcus .2μ to .5μ in diameter, usually in pairs, masses, or chains.

b. Colonies confluent, growing luxuriantly.
(α) Cocci arranged irregularly.
(β) Cocci occur as diplococci, or dumb bell shaped organisms.
(γ) Cocci arranged as sarcinæ.

α. Cocci arranged irregularly.

(1) *Micrococcus candicans* forms irregular masses, small yellowish white discs with smooth margins in substance of gelatine; opalescent or milk white moist flat colonies 2mm. or more in diameter, with indented and sinuous margins at the surface; dark brown in the centre when seen by transmitted light, but transparent near the thin margin; nail-head appearance in puncture cultivations; micrococci quite round, moderately large.

(2) *Micrococcus ureæ.*—Grows as miliary points like mother-of-pearl, smooth on surface, sharp margins; these grow rapidly, are well formed in twenty-four hours; project above surface of gelatine, colony gradually divided by fissures; along the track of the needle in a tube culture there appear long delicate threads; there is a large surface growth; has a peculiar paste-like odour; grows best at higher temperatures, (30° C.), coccus 0.8 to 1μ in diameter, occurs as diplococci, tetrads, or chains; along with other organisms, causes decomposition of urea into ammonium carbonate.

(3) *Staphylococcus cereus albus* grow moderately rapidly; white points on gelatine during the first few days; in stroke cultivations forms a wax-like layer, with slightly thickened irregular margins along the needle track; grows on blood serum and potato; found in pus; usually saprophytic in its action.

β. Cocci arranged as diplococci, or dumb-bell shaped organisms.

(1) *Diplococcus lacteus faviformis* grows rapidly along track of needle in small points, which run together to form milk white colonies; found in the sputum and certain secretions as isolated diplococci; in cultivations occurs as parallel bands of diplococci, each organism being about 1.25μ in length and consisting of two hemispheres, between which there is a distinct but narrow fissure.

(2) *Diplococcus albicans amplus.*—Very like No. 1, found in the same positions, but grows in thick white lines along the track of a stroke inoculation, organism is comparatively large, measuring 2.25μ in diameter.

(3) *Diplococcus der Pferdepneumonie.*—Obtained from the lungs of a horse affected with acute pneumonia. Has only been cultivated on gelatine and agar-agar at the temperature of the room; forms small white, somewhat transparent rounded colonies in agar-agar; along the line of the needle track in a gelatine tube culture there is seen a row of small white granules, which gradually become larger and coalesce, but there is no special surface growth; this is an oval micrococcus which sub-divides in its shortest diameter, two of them usually lying together surrounded by a clear homogeneous capsule; pathogenic for mice, guinea-pigs, rabbits, and dogs.

γ. Cocci arranged as sarcinæ.

(1) *Micrococcus tetragonus* forms small white points in gelatine in from twenty-four to twenty-eight hours; under lens deep colonies have a faint yellow tinge; mulberry-like surface; is somewhat raised on the surface of the gelatine along track of needle in puncture inoculation; first there appear rounded points, which run together; these grow most readily at surface,

spreading into cracks and forming a layer of considerable thickness; micrococci about 1 to 1.5μ in diameter, dividing into four, which remain united by a gelatinous envelope, or there may be a large round cell in which there are found indications of division; kills white mice, but not ordinary mice; produces local abscesses or septicæmia in guinea-pigs; rabbits and dogs are unaffected; unlike sarcina in dividing in two planes only.

B. The colonies are yellow.

a. The colonies form raised drops.
b. The colonies form flat deposits.

a. The colonies form raised drops.

(1) *Staphylococcus cereus flavus.*—Forms white points in two days; colonies spread on surface with irregular margins gradually assuming a dark citron yellow colour; in the early stages the growth is almost like micrococcus cereus albus; cocci 1.15μ in diameter, single, in groups, or in short chains, found in pus; set up no pathogenic action.

(2) *Micrococcus flavus tardigradus.*—Grows very slowly (four to six days); in gelatine occurs as rounded or oval, dark chrome yellow coloured points; on the surface these have smooth wax-like surface, and project slightly, especially near the centre; colour is always darker in the deep layers; along track of needle in puncture occurs as minute yellow isolated points, which do not make their appearance for six or seven days; large coccus, sometimes with peculiar dark poles.

(3) *Diplococcus citreus conglomeratus* occurs in certain forms of pus and in dust; on gelatine plates forms citron yellow colonies, raised at the margins, at first moist and slimy, gradually becoming cracked and scaly, forming tuberculated masses which when crushed and diluted with water are seen to be made of cocci resembling gonococcus, or tetrads; average diameter 1.5μ.

(4) *Sarcina lutea* grows rapidly on gelatine plates; appears in two days as yellow points with somewhat irregular and scalloped outlines, yellow in the centre, grey in the intermediate zone and transparent at the periphery; occurs in air; made up of rounded cells 1.2μ in diameter, dividing in three axes, thus giving rise to the well-known corded packets.

(5) *Sarcina aurantiaca* (*Orange sarcina*).—Forms small colonies in plate cultures with smooth outlines; along the track of the needle of a gelatine tube culture it grows very slowly, but best at the ordinary temperature of the room, as a whitish growth; at the surface it forms an orange yellow layer; cocci which look as if cut in two, arranged in twos or fours, or in regular packets.

b. The colonies form flat deposits.

(1) *Micrococcus versicolor* grows rapidly, forming white points in twenty-four hours, which twenty-four hours later become yellow; spherical growths in the gelatine, outline sharp, substance yellowish green in colour, and opaque; on the surface growth is irregular or square; has a peculiar gelatinous consistence, and a yellowish green iridescent shimmer; although the growth is flat it may be slightly raised in the centre; along the needle puncture the yellowish colonies are developed separately; small cocci are arranged in pairs, or in masses.

C. The colonies are red.

(1) *Micrococcus cinnabareus* forms cinnabar red drops, grows very slowly, only just visible at the end of four days in the deeper gelatine, and colonies are very small on the surface; at the end of eight days appear as small wax-like drops on the surface of the gelatine, these gradually deepen in colour; the deeper cultivations along the needle track remain white; on plate cultivations superficial colonies when seen magnified by a lens, are light brown, rounded with somewhat irregular outline, and slightly nodulated surface; margins transparent.

(2) *Micrococcus roseus*, a rose-coloured growth, flourishes luxuriantly on the surface of gelatine and at the ordinary temperature; somewhat raised, especially at the margins; moist and granular with distinct rosy red colour; arranged as diplococci with a broad division between the two halves, 1 to 1.5μ in diameter.

(3) *Pink Torula* (not a micrococcus, but frequently met with). A coral pink mass, growing freely on the surface of gelatine. Small white or grey points along the needle track. On bread paste grows as a rose-coloured succulent film. It consists of rounded or slightly oval cells 5 to 8μ in diameter; these contain pigment of delicate yellow colour, under microscope, pink only in mass.

D. The colonies are black.

(1) *Black Torula* (not a micrococcus, but sometimes met with in air). Grows on gelatine as a black heaped-up mass. Along the track of the needle it forms small black nodules. On potato and bread paste grows as a dull sooty crust with a dry slightly furrowed surface. In milk it forms a black crust, with a dusky grey tint on the upper surface. The milk itself becomes of a muddy colour from an invasion of the deeper layers by colonies of the organism. Under the microscope is like the Pink Torula, but with a dark brown pigment.

II. The gelatine is liquefied.

A. The colonies are white.

B. The colonies are yellow, see p. 419.

A. The colonies are white.

(1) *Staphylococcus pyogenes albus.*—Grows rapidly in plate cultivations; colonies seen under lens are dark in the centre, with smooth borders; liquefy the gelatine on the second or third day, forming a little clear cup, with a white mass at the bottom; liquefying centres gradually run together. Along track of needle white mass is formed; liquefaction commences at surface and extends along the whole track; at the bottom of the liquefied gelatine is a greyish or white deposit; a micrococcus .8 to .9μ in diameter; occurs as irregular masses, diplococci, tetrads or short chains; is fatal in large doses to mice, guinea-pigs and rabbits, if injected into the veins or into the peritoneal cavity, otherwise usually forms abscesses; appears to be especially associated with suppuration, pyæmia, ulcerative endocarditis, osteo-myelitis and similar diseases; it is found in pus, necrotic tissues and in capillary vessels of internal organs.

(2) *Micrococcus ureæ liquefaciens.*—In plate cultivations forms small white points, somewhat opalescent with well-defined margins which appear in two days; grows more rapidly near the surface; surface granular, and as the

gelatine becomes liquid the border becomes wavy ; along the track of a needle in puncture cultivations there is first a continuous growth, liquefaction takes place along this track commencing at the surface, and in the later stages the gelatine is liquefied to the depth of the needle track, a sediment of light yellow deposit being thrown down, the liquefied gelatine is somewhat turbid but uncoloured; organism rounded 1.25 to 2μ in diameter, occurs singly or in short chains ; this organism is supposed to convert urea into carbonate of ammonia by a kind of fermentation.

(3) *Sarcina alba.*—An organism obtained from the air. Grows slowly on plates as small white colonies along the needle track ; in gelatine grows slowly and forms a white projecting head on the surface of the gelatine, causing very slight liquefaction near the surface ; on agar it forms a whitish yellow layer, which surrounds the point of inoculation ; grows like a small coccus arranged in twos, fours, or packets.

B. The colonies are yellow.

a. Gelatine liquefies slowly and imperfectly.

b. Gelatine becomes completely liquid.

a. Gelatine liquefies slowly and imperfectly.

(1) *Micrococcus flavus desidens* occurs in the dust of the atmosphere ; organism grows slowly in the depth of gelatine where the colony has somewhat irregular outline, grows more rapidly at the surface ; it is then dull yellow or brown in colour, is smooth and almost slimy in consistence ; gelatine underneath is softened, and there is slow sinking of the surface growth, the soft jelly becomes opaque ; the organism is a small coccus ; may be arranged in diplococci, in triangles or in short chains ; is non-pathogenic.

(2) *Micrococcus ærogenes* (Miller).—Found in the intestinal tract. On gelatine plates forms dark coloured round scalloped colonies with smooth outlines ; under the microscope these may be either opaque or transparent ; in gelatine tubes the growth occurs along the track of the needle as a brownish yellow mass, forming on the surface a flat greyish white porridge-like layer of some thickness; liquefaction takes place at a later stage ; the same yellowish white porridge-like layer is seen on both agar-agar and potato growths ; it is a large non-motile oval coccus.

b. Gelatine becomes completely liquefied.

a. Colonies remain limited to the centre of the liquefying area.

β. Colonies are found occupying both in the centre and the periphery of the liquefying area, see p. 420.

a. Colonies remain limited to the centre of the liquefying area.

(1) *Staphylococcus pyogenes aureus* (Probably identical with Micrococcus of Osteomyelitis).—Grows rapidly in plate cultivations ; seen under microscope as light brown circular masses, darker in the centre and with smooth borders ; these become yellow and liquefy the gelatine on the second or third day, forming a little clear cut funnel with an orange yellow mass at the bottom ; liquefying areas gradually run together ; along puncture track of needle in gelatine tube a white mass is formed, which only becomes yellow on the access of air, liquefaction commencing at the surface and extending the whole length of track ; micrococcus .8 to .9μ in diameter ; occurs in irregular masses, diplococci, tetrads or short chains ; is fatal in large doses to mice, guinea-pigs and rabbits if injected into the veins or into the peritoneal cavity ; inoculated subcutaneously usually gives rise to abscesses,

but to little other disturbance. Like the Staphylococcus pyogenes albus appears to be associated with suppurative processes and is found under similar conditions.

(2) *Staphylococcus pyogenes citreus.*—Found in the pus of acute abscesses; differs from No. 1 only in the fact that instead of being dark orange yellow it remains bright citron yellow in colour.

(3) *Diplococcus subflavus.*—Grows rapidly on nutrient jelly and blood serum, first as whitish points which gradually become yellowish and then deep yellow; in large quantities produces abscesses; occurs in several secretions as a diplococcus from 0.5 to 1.5μ in diameter; it is made up of two hemispheres with a central division and resembles the gonococcus somewhat in appearance, but retains the aniline dyes much more tenaciously than that organism.

(4) *Streptococcus coli gracilis.*—Occurs in the intestinal canal and fæces of the carnivora; on plates it forms small sharply outlined dark colonies in the centre of an area of clear liquefied gelatine; these, later, become somewhat crenated at the margins; in a gelatine tube the medium is liquefied rapidly along the track of the puncture and after six or eight days there is precipitated a white finely granular mass; on agar-agar, potatoes and blood serum there is very little superficial growth even at the body temperature, which is most favourable to its growth; it is a coccus from .2 to .4μ in diameter; in fresh gelatine cultures it forms curved chains consisting of from six to twenty cocci.

β The colonies are found occupying both the centre and periphery of the liquefying area.

(1) *Micrococcus coronatus.*—Appears on the second day in plate cultivations as whitish yellow points; deep colonies, under the microscope appear as opaque sharply-defined plates; superficial growths project slightly, this is made more marked by a slight zone of depression surrounding the gelatine; at intervals tooth-shaped processes advance beyond the general circular periphery; the older growths are dark in colour, newer growths are yellow or yellowish brown; liquefaction takes place around the growth in presence of air; the coccus, 1.1 to 1.2μ in diameter, occurs singly in short chains or in irregular masses.

(2) *Micrococcus radiatus.*—Growths visible in twenty-four hours; 1 mm. in diameter in two days; white or yellowish green, sharply defined, granular, or with outgrowths like the rays of a star fish; colonies sink as gelatine becomes liquid, and a series of circles of rays formed of delicate threads project radially, this zone increasing in breadth towards the periphery; one, two or three of these circles are seen according to the age of the growth, the rays of the outer circles always being shorter than those of the inner ones, each circle forms in about two days; in a puncture cultivation isolated points form along the track of the needle; from these, lateral branches project; a funnel-shaped area of liquefaction is formed very slowly, it extends for a short distance only into the gelatine; micrococci .8 to .9μ in diameter; usually grouped in small masses but sometimes in short chains.

(3) *Micrococcus flavus liquefaciens.*—Occurs on gelatine plates as small yellow circular, oval, or irregular finely-toothed colonies; superficial colonies distinctly yellow, cause liquefaction. Smaller colonies are found at the border of the liquefying area which has a very sharp outline; lines of cocci radially disposed run from the centre to the periphery in the clear liquefying area, giving an appearance that is said to resemble the wheel of a waggon;

in puncture cultivations yellow points are seen in two days, these become confluent and rapidly liquefy the jelly, which remains clear with a yellow deposit below; it is a comparatively large coccus, occurring in irregular masses or in twos or threes.

III. There is no growth on gelatine at 22° C.

(1) *Micrococcus gonorrhœa.*—Grows on blood serum at 37° C. as a thin greyish yellow layer with moist smooth surface; organism consists of two hemispheres slightly concave on the opposed sides with a clear line of division between them; it is from 0.8 to 1.6μ in length and from 0.6 to 0.8μ in diameter; unlike most other organisms it is contained within the protoplasm of the tissue cells, and is readily decolorized by Gram's method.

(2) *Diplococcus intracellularis meningitidis.*—Found in fresh exudation of cases of acute cerebral meningitis. Grows on a mixture of agar-agar and gelatine at the temperature of the body; the growths in the deeper layers are very small, those on the surface are larger, and are somewhat grey; at first they are round when seen under the microscope, they then become irregular, are finely granular and yellowish brown, the centre is usually darker than the periphery; on the surface of agar this organism grows well but not along the track of the needle; it forms a grey, viscid growth as the various colonies run together; it only remains virulent for about six days, affects mice, guinea-pigs, rabbits and dogs; is probably very closely allied to the diplococcus of pneumonia; grows as a coccus sometimes singly but usually arranged in pairs, fours, or small masses; in single cocci a line of division may usually be seen; is almost invariably found within the cells contained in the exudation.

(3) *Micrococcus pyogenes tenuis.*—An irregular coccus, larger than the staphylococci, and not forming masses; found in a certain proportion of unopened abscesses by Rosenbach; cultivated on agar, chain-like micrococci in Endocarditis ulcerosa; micrococci found in disease of the hands and fingers of butchers and tanners, but not yet fully studied.

Micrococci have also been described in small-pox pustules and in the various internal organs in the lymph of vaccinal vesicles, in scarlatina by Crooke, and in measles, in diphtheria, in inflammation of membranes of the brain, in influenza (doubtful), in ozœna, in hæmophilia neonatorium, in acute yellow atrophy of the liver, and in many other diseased conditions.

In addition to these may be mentioned Pathogenic micrococci, in the blood of patients suffering from "Clou de Biskra or Bouton d'Alep," which excite gangrene when injected subcutaneously into rabbits, or death sixteen hours after they are injected into the blood.

The Organism is a Bacillus.

I. The nutrient gelatine is not liquefied.

II. The nutrient gelatine is liquefied, see p. 429.

III. Organisms do not grow on nutrient jelly, and only on other media at higher temperatures in the presence of air, see p. 434.

IV. Organisms will only grow under conditions of anærobiosis, see p. 436.

V. Organism has not yet been artificially cultivated outside

the body, *i.e.*, it does not grow under ordinary conditions, see p. 437.

I. The nutrient gelatine is not liquefied.

A. Colonies white, nutrient gelatine near growth not stained.

B. Colonies colourless, nutrient substratum near growth stained, see p. 427.

C. Colonies cream coloured, see p. 428.

D. Colonies of a yellow colour, see p. 428.

A. Colonies white, nutrient gelatine near growth not stained.

a. Colonies form minute small translucent drops on plates, delicate growths in stroke and puncture cultivations.

b. Colonies form thin films on plates, and on the surface of tube cultures, see p. 423.

c. Colonies form white nail-head projections on plates, and nail-shaped growths in tube cultures, see p. 425.

d. Colonies are branched, not circumscribed, see p. 426.

a. Colonies form minute small translucent drops on plates, delicate growths in stroke and puncture cultivations.

(1) *Bacillus choleræ gallinarum* (Fowl cholera).—Grows on gelatine as small, round, white, superficial, finely-granular colonies, light yellow in the centre, a dark zone further out, outlines irregular; on potatoes do not grow at the ordinary temperature of the room, but at 37° C. grow slowly as yellowish grey transparent drops. Under the microscope average 1.2 to 1.5μ in length, and are seen as short rods with rounded ends, which are always more deeply stained with aniline colours at the ends than in the middle, so that they appear like diplococci. Fatal to fowls in from 24 to 36 hours, also to mice and rabbits (probably identical with Koch's bacillus of rabbit septicæmia). Not fatal to guinea-pigs, sheep, and horses, but causes abscess formation.

(2) *Bacillus* (*Bacterium*) *der Wildseuche* (Described by Kitt and Hueppe).—Grows on plate cultivations as white or greyish-white colonies, about the size of a pin head, which under the microscope appear to be slightly granular; in the needle track in puncture cultures we see small isolated colonies which run together to form a greyish-white line; on the surface there are small, white, rounded layers, which grow up from the surface; on agar-agar they have much the same appearance, but are greyer and more transparent; on potatoes forms greyish yellow, slightly prominent layers; on blood serum it has a peculiar iridescent appearance; grows best at the temperature of the body; occurs as short rods two to three times as long as they are broad, with somewhat rounded ends; sometimes appear as cocci, or may be ellipsoidal; Arthrospores are said to be present. Said to be the cause of certain forms of infective pneumonia. It gives rise to most marked symptoms in a number of animals, and is classed by Hueppe with the organisms of

swine erysipelas, rabbit septicæmia, and fowl cholera, all of which give rise to hæmorrhagic septicæmias.

(3) *Bacillus septicus agrigenus.*—A bacillus found in cultivated ground. On plates it grows as rounded, finely-granular colonies, with sharp outlines, centre of colony light yellow, margin darker. Under the microscope it is exceedingly like the bacillus of fowl cholera. In the body it adheres to the red blood corpuscles, and is fatal to mice and guinea-pigs. Colonies form thin films on plates and on the surface of test tube growths.

b. Colonies colourless, form thin films on plates and on the surface of tube cultures.

a. Cultivations odourless.

β. Cultivations giving off an odour, see p. 424).

a. Cultivations odourless.

(1) *Bacillus acidi lactici.*—Found by Hueppe in sour milk. It grows on gelatine plates as small, white points, which gradually become opaque and moist looking, forming a thick layer of from 1 to 2mm. in diameter. Under the microscope these colonies appear to be dark yellow in the middle. The margins are irregularly indented and toothed. In tubes, growth appears as small granules along the line of puncture; surface growth thick, moist, and opaque; grows very slowly, and in milk can only develop at a temperature above 10° or 12° C., and below 45° C.; occurs as short, plump, motionless rods, 1 to 1.7μ in length, and .3 to .4μ in thickness; usually arranged in pairs, sometimes, but rarely, in chains of four; well-marked refractile bodies which are regarded as spores, which are usually placed at the end of the rods.

(2) *Bacillus of typhoid fever* (p. 194) *and Pseudo typhoid bacilli* (p. 202).

(3) *Bacterium coli commune.*—Is found in the intestinal canal of man and animals, especially at the lower end. It grows on gelatine in the form of superficial colonies, from 2 to 4mm. in diameter, which are granular, or may be slightly wrinkled; in the deeper layers of the gelatine appear as yellow granular discs; grow pretty rapidly. Organism occurs as thin rods, about 2 to 3μ in length, and .4μ in breadth; sometimes it occurs as short ovoid, or even rounded forms; rods are slightly curved, and may be slightly motile. When injected into the veins of rabbits or guinea-pigs, kills these animals with symptoms of violent diarrhœa and fever, but guinea-pigs are not quite so susceptible as rabbits. Does not form spores.

(4) *Brieger's Bacillus or Bacillus Cavicida.*—Found in fæces and putrefying fluids. The growth on plates occurs as colonies 2 to 4mm. in diameter, composed of white concentric rings, like the scales on the back of a tortoise; grows rapidly as dirty yellow masses on potatoes; small rods about twice as long as broad; injected into guinea-pigs, cause death in about 72 hours. They act like ordinary putrefactive bacilli, produce propionic and other acids which give characteristic odour; do not cause death of rabbits or mice.

(5) *Bacillus diphtheriæ columbarum.*—An organism separated from the false membrane of the diphtheria of pigeons. The colonies are from 2 to 4mm. in diameter, and occur as white nodules in the deeper layers of gelatine, but grow on the surface as whitish or brownish yellow films. On potatoes it is sometimes difficult to distinguish it, as it is almost the colour of the potato itself, having, however, a slightly greyer colour; bacilli are

longer and thicker than those of fowl cholera, but the ends are somewhat rounded as in that organism; are usually grouped together in small masses. Kills pigeons, sparrows, rabbits, and mice, but does not affect fowls, guinea-pigs, rats, and dogs.

(6) *Bacillus of Diphtheria of Rabbits* (*der Darmdiphtherie der Kaninchen*).—In rabbits, as in pigeons, there has been described an organism which grows in the "diphtheritic" processes of the intestine. On gelatine plates is seen as small transparent grey colonies, which gradually become brown; the surface is finely granular, and has a peculiar pearly shimmer; growth in tubes along the track of the needle is comparatively slight, as the organism requires a considerable amount of oxygen for its growth; but on the surface it forms a slowly growing whitish layer; rods 3 to 4μ in length, and 1 to 1.4μ thick, rounded at the ends, arranged in pairs or in long chains; in rabbits causes an inflammatory exudation in the alimentary canal.

β Cultivations give rise to a strong odour.

(1) *Bacillus ureæ*.—Found in ammoniacal urine; grows on gelatine plates as small, semi-transparent points, which make their appearance on the second day; on the tenth day they are about the size of a sixpence. These are described as having the appearance of a ground glass plate that has been breathed upon; the growth extends in the form of concentric rings, the outer one of which has a somewhat zigzag outline; in gelatine tubes it grows along the track of the needle very slowly as an exceedingly delicate grey film; on the surface it grows rather more rapidly, and in old cultivations gives rise to a characteristic trimethylamine or herring brine odour. The organism occurs as plump rods with rounded ends, 2μ in length, and half as broad as long; it converts urea into carbonate of ammonia.

(2) *Bacillus pyogenes fœtidus*.—First obtained from a phlegmonous abscess. Occurs in a very short time (24 hours) as white points, which rapidly spread out as greyish-white films over the surface, and may gradually become confluent; the margins are usually somewhat more translucent looking than the centre, which is thicker; along the needle puncture in gelatine are delicate, greyish-white points of various sizes, whilst on the surface there is formed a layer similar to those described as occurring on plates; the gelatine is not liquefied; it becomes slightly opaque in older cultures; on potatoes, is seen as glistening light brown growth; occurs as short rods with rounded ends, about 1.45μ in length, and .6μ in breadth; sometimes in chains of two or more, and is slightly motile; causes death of mice and guinea-pigs, when injected into the abdomen, in about 24 hours; the bacilli are then found in the blood, but not at the point of inoculation; spores may be indistinctly made out in these bacilli.

(3) *Schottelius' intestine bacillus* (*Bacillus Coprogenes fœtidus*).—Found in the intestinal canal and liver and spleen of pigs suffering from swine erysipelas. This organism grows on gelatine as light yellow rounded deep colonies, or as a fine transparent grey layer on the surface; does not give rise to liquefaction of the gelatine; on potatoes it forms a dry, clear layer; the organism is about as thick as the hay bacillus (2μ), but is only 4 or 5μ in length; it has rounded ends, and is motile; the spores appear in distinct rows along the course of the threads. It does not cause any affection in pigs, and is only toxic in large doses to rabbits.

(4) *Bienstock's putrefactive bacillus* (*B. Putrificus Coli*, "*Drumstick*" *bacillus*).—Was first separated from fæces. On gelatine it has first a peculiar opalescent appearance, but later it becomes yellowish; on agar-agar it has much the same appearances; is an extremely motile organism, which occurs in longer or shorter threads; usually the long threads break up into shorter rods, about 3μ in length, at one end of which may be seen a spore similar to that described in the tetanus bacillus; this terminal spore giving to the bacillus the characteristic drum-stick shape.

(5) *Bacillus or leptothrix epidermidis*.—Occurs in the fragments of epidermis taken from between the toes; grows very sparsely on gelatine and on agar-agar, where it forms only a superficial growth; on potatoes, at a temperature of 15 to 20° C., it occurs in the form of transparent fluid drops, which gradually run together, become thicker, and form a characteristic superficial skin; also forms a similar skin on blood serum; is a bacillus of about 2.8 to 3μ in length, and .3μ in diameter; forms spores from 1.2 to 1.5μ in length, and .3 to .4μ in breadth; this spore formation goes on best at a temperature of 25° C.

c. Colonies form white nail-head projections on plates, and on the surface of tube cultures.

α. Colonies microscopical with a granular border.

β. Colonies with smooth borders, see p. 426.

α. Colonies microscopical with a granular border.

(1) *Bacillus pneumoniæ* (Friedländer).—Found in the lung and in the rusty-coloured sputum of croupous pneumonia. Occurs in plates as small, round, well-defined, darkish yellow or olive green granular colonies in the deeper layers of the gelatine; on the surface appears as white, thick, well-defined projecting points; in the gelatine needle cultures the growth has the characteristic nail (with rounded head) appearance, the superficial growth almost appearing like a very white, split, porcelain bead, that has been dropped on the surface (after a time there is usually slight coloration of the gelatine, and small bubbles of gas are formed if the gelatine is not too solid); grows best at a temperature of from 16° to 43° C.; at the higher temperature (on potatoes) it forms a moist, yellow mass, in which little bubbles of gas may be seen; grows very rapidly, and is not strictly ærobic. Under the microscope seen as short, thick bacilli, with rounded ends, or oval cocci, which are frequently arranged in pairs. When found in the lung tissue or sputum it is usually surrounded by a delicate capsule, which gives it a very characteristic appearance; but this capsule is not, as a rule, found in cultures. The organism is non-motile; sometimes gives rise to pneumonia in mice, guinea-pigs, and dogs, but does not affect rabbits. No spores have been demonstrated.

(2) *Bacillus crassus sputigenus*.—Found in the sputum and in the "fur" scraped from the tongue. Forms on gelatine plates greyish-white viscid drops, which project above the surface of the gelatine; colonies seen with a lens are greyish brown, coarsely granular, and have a somewhat irregular margin; in needle cultures have the same characteristic nail appearance as No. 1, and on potatoes also grow like No. 1; short, thick rods, with slightly rounded ends, sometimes described as being like bent sausages; said to form spores at a temperature of about 35° C. Kills mice in about 48 hours, and in larger doses may kill rabbits and dogs in a very short time.

(3) *Bacillus pseudopneumonicus.*—Very like the true bacillus of pneumonia; but it has been found in pus taken from abscesses. The colonies seen through the microscope are dark grey in colour, and finely granular; puncture cultures in gelatine have the characteristic nail appearance; grows rapidly, and differs from the two previous forms in that it causes rather dark coloration of the gelatine, and gives rise to a slight putrefactive odour; grows well on potatoes as a white, viscid layer, but no gas is formed even at a temperature of 37° C. Requires air for its growth; microscopically it is very like the pneumonia bacillus, 1.16μ in length, and .9μ in diameter. It is only slightly, if at all, pathogenic.

β. Colonies with smooth borders.

(1) *Bacillus oxytocus perniciosus.*—Obtained from milk that had been allowed to stand for a considerable time. Occurs on plate cultures as small colonies, with smooth borders and circular outlines; under the microscope appears to have a light brown colour; growing on the surface may attain a size of 1.5 mm.; colonies are greyish white, and are usually round; needle cultures have, at first, the characteristic nail appearance but, after a time, the growth along the needle track is comparatively small, whilst the surface growth becomes very extensive; gives a peculiar acid reaction to milk but no odour is developed; under the microscope it is seen as a short rod with rounded ends, somewhat thicker and shorter than the lactic acid bacillus. In large doses is fatal to rabbits.

(2) *Bacterium lactis aërogenes.*—Found in the small intestine of mammals and sometimes even in milk. Colonies have smooth borders; do not spread out much, but are usually of considerable thickness, like little white porcelain points. In needle cultures it grows luxuriantly in the nail form; along the line of the needle, small rounded points occur at regular intervals, so that the growth looks almost like a string of beads; forms white layers on potato, in which bubbles of gas are frequently developed, sometimes this layer has a peculiar creamy appearance; grows rapidly at about 37° C.; causes diarrhœa and collapse in rabbits and guinea-pigs, but does not affect mice; short thick rods with rounded ends 1 to 2μ long and .5 to .8μ broad; usually occurs in pairs, or may be arranged in irregular masses; the organism is non-motile.

(3) *Weisser bacillus.*—Obtained from water. Grows on gelatine plates as round smooth white pin-head-like colonies; in gelatine tubes it grows slowly, forms a whitish mass along the track of the needle and a white head on the surface; on potatoes it forms a yellowish-white growth; grows slowly; the organism is a short motile bacillus with truncated ends, often joined to form chains.

d. Colonies are branched, not circumscribed.

(1) *Bacterium Zopfiii.*—First found in the intestine of fowls. It grows on plates almost like a mucor; in needle cultures appears as a thick, pale, yellow string, from which white branches radiate into the surrounding gelatine; is strongly ærobic, and grows very rapidly, especially at a temperature of about 20° C.; spores are formed which are extremely resistant to heat; the organism is from 2 to 5μ in length and from .7 to 1μ in breadth; is motile; occurs in long threads, which in gelatine show numerous bends or spirals.

(2) *Bacillus of Mouse Septicæmia.*—Originally found in garden earth and

in putrifying fluids. Grows on plates, in the deeper layer of the gelatine only, as exceedingly slowly growing delicate white clouds ; along the track of a needle culture delicate, branching, almost cloud-like growths are seen, which are always more marked in the deeper part of the tube than in the upper part. (N.B.—If the gelatine is exceedingly alkaline there may be slight liquefaction of the medium.) On agar-agar, pale yellow sharply-defined colonies are formed. The organism is non-motile, is exceedingly small, being only about 1μ in length and from .1 to. 2μ in thickness ; two of them are frequently adherent to one another ; they contain spores. Mice inoculated die in from 40 to 60 hours when bacilli are found in the blood, especially in the capillaries of the kidneys and spleen.

(3) *Bacillus of swine erysipelas* (*Schwein Rothlauf*).—Has been obtained from the spleen and blood of pigs that have died from this disease. It is extremely like the previous organism except in the following points :—The cloudiness in the needle culture is not quite so diffuse, and the bacilli are slightly longer and thicker ; causes death of mice in from 2 to 3, pigeons in from 3 to 4, and rabbits in 6 days ; is also fatal to pigs.

B. Colonies colourless, nutrient substratum near growth stained.

α. Substratum stained greenish.

β. ,, ,, blue or greyish brown.

γ. ,, ,, violet, see p. 428.

α The substratum is stained greenish.

(1) *Bacillus fluorescens putidus*.—Obtained from putrefying fluids. In the deeper layers of gelatine plates it forms small dark colonies. At the surface it appears as round wafers with irregular outlines ; the surrounding gelatine has a peculiar greenish fluorescent appearance ; in needle cultures there is distinct cloudiness along the needle track and green coloration of the gelatine, which is always more marked when oxygen has access to the growth ; strong herring brine odour ; grows rapidly on potatoes, forming a thin brown or greyish layer ; is a short, thin, very motile bacillus with rounded ends.

(2) *Bacillus erythrosporus*. — Obtained from putrefying albuminous fluids, drinking water, &c. Occurs on plates as whitish colonies, which gradually spread over the surface ; around them in the gelatine a peculiar fluorescence appears ; the centre of the colony is usually opaque and brownish, the outer zones are light yellowish green, not so opaque, and there is a slight radiate marking ; along the needle track and at the surface is a well-marked growth ; the surrounding gelatine is green by transmitted, and yellow by reflected light ; on potatoes forms reddish or nut-brown localized patches ; grows moderately quickly, especially at the ordinary temperature of the room ; occurs as slender bacilli with slightly rounded ends, single or in threads ; in these threads are from two to eight dirty red spores, which are very distinctly seen, sometimes have almost the appearance of a string of beads.

β. The substratum it stained blue or greyish brown.

(1) *Bacillus cyanogenus* (or Blue milk bacillus).—On gelatine plates forms rounded, dirty white, finely granular colonies with smooth outlines ; the surrounding gelatine takes on a light green or greenish brown colour ; in a needle culture in gelatine it has the "nail" appearance, with a milk white head, the surrounding gelatine becoming greenish blue or

even dark brown or black ; on agar-agar is seen as a similar growth, which, however, is somewhat grey in colour, and the green is never so well made out as in the early gelatine cultures ; on potatoes it forms a yellowish layer near the point of inoculation, the surrounding potato being stained greenish blue ; on blood serum it gives rise to no coloration ; is an ærobic and exceedingly motile bacillus from 1 to 4μ in length and .3 to .5μ in breadth with slightly blunted or, if spores are being formed, club-shaped ends, though very frequently spores appear to be formed in the middle of the organism also ; in alkaline milk gives rise to a slate colour, but if grown in the presence of lactic acid to an intense blue ; this colour is most freely developed at from 15° to 18° C., at 37° C. no colour is formed at all.

γ. The substratum is stained violet.

(1) *Bacillus janthinus* (Violet bacillus).—Differs from the bacillus violaceus which liquefies gelatine. (Grows very slowly, and later causes liquefaction of the gelatine.) It was first found in water ; when grown on gelatine, milk white points appear, which later become violet, especially at the margins ; the surrounding gelatine also becomes deep violet, but the organisms only develop the colour where there is a free supply of oxygen ; in this case, as in the last, the colour is probably formed through the breaking down of the proteid ; under the microscope the organism is found to consist of motile rods, some longer some shorter, but these gradually break up into shorter lengths.

c. Colonies are cream coloured.

(1) *Bacillus of septic pneumonia.*—Poels has described an organism as occurring in septic pneumonia of calves. It is a short rod-shaped organism with a constriction in the middle, which gives it the appearance of a diplococcus ; grows on gelatine as a rough layer ; forms small rounded colonies around the point of inoculation, which gradually run together ; along the track of the needle in puncture cultures small rounded whitish or cream coloured colonies are formed ; on agar-agar it forms a shining smooth layer exceedingly thin and sharply defined in from 10 to 15 hours ; on sterilized blood serum forms a creamy layer similar to that already described, and on the surface of sterilized potatoes spreads over a large area ; kills rabbits, guinea-pigs, mice, calves, pigs ; sheep and dogs not affected, Poels thinks that it is closely allied to the mouse septicæmia group of bacilli.

d. Colonies of a yellow colour.

(1) *Bacillus luteus.*—Forms in the superficial layer of gelatine plate cultivations small yellowish points 2 to 3mm. in diameter, often of a light brown colour with a whitish translucent margin ; deeper down they are much smaller, are usually the shape of a lentil, and can only be made out with the aid of a microscope, when they appear to be irregular in outline and of a brown colour ; a yellowish growth is formed along a surface needle track ; it is a short non-motile bacillus of medium thickness.

(2) *Bacillus fuscus.*—Obtained from a putrefying infusion of maize, and also found as an accidental impurity in certain cultures. Quickly forms rounded brownish colonies ; deeper it forms dark brown nodules surrounded by a highly refractile border ; at the surface of puncture cultures it usually forms a wrinkled brownish red deposit around the point of entrance of the needle. The organism is a motile rod.

(3) *Bacillus Fitzianus.*—Obtained from the dust of hay, and supposed to be really a variety of the Bacillus subtilis. The colonies are brownish yellow in colour with a dark opaque centre and sharp outline, those lying on the surface of the gelatine are like brownish yellow gelatinous drops; organisms are from 1 to 2μ and upwards in length and about 1μ in thickness, the longer rods are frequently bent at the ends; there is distinct spore formation; sets up æthylic alcoholic fermentation, especially when glycerine is present.

II. The nutrient jelly is liquefied.

A. Colonies are white; nutrient substratum remains uncoloured.
B. Colonies or nutrient substratum coloured, see p. 433.

A. Colonies are white; nutrient substratum remains uncoloured.
a. Colonies branched or with processes.
b. Colonies circumscribed without branches, see p. 431.

a. Colonies branched or with processes.

α. Colonies are non-motile.
β. Colonies motile and swarming, p. 430.

a. Colonies are non-motile.

(1) *Bacillus anthracis,* see p. 272.

(2) *Bacillus ramosus liquefaciens.*—Roundish colonies on plates with radiating processes, the rounded disc looking as if it were surrounded by a zone of hairs; superficial colonies are oval or pear-shaped; there is slight liquefaction around the growth and a deep circular funnel is formed, which is surrounded by concentric rings, which gradually increase in size; running off from the funnel at right angles are a number of branches longer near the surface and becoming shorter as the deeper layers are reached; the organism is a medium size slightly motile bacillus with blunted ends.

(3) *Bacillus subtilis* (*Hay bacillus*).—Obtained from hay infusion that has been boiled. Grows on plate cultures as white rounded colonies with radiating processes; liquefies gelatine on plates rapidly; along the track of needle causes liquefaction which commences at the surface; first occurs as small whitish colonies, which under a low power have a yellowish brown colour with the hair-like margin, outside this is a narrow clear zone, beyond which again is a greyish layer composed of radiate lines; on potatoes and on agar it forms a whitish moist creamy layer, which afterwards becomes somewhat granular and dry; is dryer and more wrinkled looking on agar than on potatoes. Liquefies blood serum; grows rapidly about 30° C.; is strongly ærobic; is a motile organism about 6μ in length and about 2μ in breadth with slightly rounded ends, it divides and multiplies exceedingly rapidly; large well defined spores are formed (when the supply of nutrition is gradually cut off), about 1.2μ in length and .6μ in breadth.

(4) *Bacillus pneumonicus agilis* (or Bacillus of vagus pneumonia of rabbits).—Grows on gelatine plates as round dark granular colonies with slightly roughened surface and margins; after from 20 to 24 hours there are marked movements in the middle of the colonies, and liquefaction takes place rapidly; in needle cultures in gelatine tubes rapid liquefaction of the medium occurs, and a *shallow* funnel-like space, in which the gelatine is liquefied, is formed; growth on potatoes, spreads very rapidly over the whole surface as a "chamois" red layer; on blood serum grows much more slowly and only

causes slight liquefaction ; the organism occurs as an elliptical coccus or diplococcus, or as a short thick bacillus ; is fatal to rabbits when pure cultures are used.

(5) *Bacillus mesentericus fuscus.*—On gelatine plates form whitish colonies with sharp outlines, later these become yellowish brown and take on a granular surface, rays running out from the periphery ; growth liquefies the gelatine, especially near the surface, after a whitish opacity has grown along the track of the needle ; the liquefied gelatine is at first turbid or has whitish flakes floating in it ; on potatoes a smooth yellowish growth appears on the first day and spreads rapidly, this soon becomes dry and wrinkled ; small and short motile bacilli usually occur in twos and fours and contain small refracting spores, which as a rule are somewhat irregularly arranged ; colonies pear-shaped with thick processes at the pointed end, in puncture cultivations like "sparks" of fluid.

(6) *Bacillus alvei.*—(Cheshire and Watson-Cheyne) found in the disease known as foul brood of bees. Grows on plates as small oval or pear-shaped colonies, which under the low power appear to have thick processes at the pointed end, presenting the appearances above described ; small lateral projections make their appearance along the track of the needle in stroke cultivations, these gradually curve and form well-marked circles, from these, new circles or pear-shaped masses project, and so on ; the gelatine becomes fluid immediately around these, forming canals, following the course of the masses of bacilli ; ultimately the gelatine becomes liquefied around the whole colony ; on potato the organism grows slowly in the form of a yellowish deposit, best at the body temperature but even then somewhat slowly ; liquefies gelatine very rapidly. The bacillus in the honey combs of foul brood hives is about 3.6μ in length and .8μ in breadth ; the cultivated organism varies between 2.5 and 5.μ in length ; the ends of the bacilli are rounded or pointed ; they are sometimes motile and form large spores 2.1μ in length and 1.7μ in breadth.

(7) *Bacillus mycoides* (*Earth Bacillus*).—Obtained from the surface of the earth of cultivated fields or gardens. Colonies without distinct centres in the form of a mass or mycelium-like network of threads ; in gelatine plates we have a whitish turbidity, in which fine threads, irregularly branched and interwoven, may be seen ; grows exceedingly rapidly, and resembles the mycelium of a fungus, so much so that it is often mistaken for one ; when they come to the surface the threads become much thicker ; near the surface in needle cultures cause liquefaction of the gelatine, but this is preceded by a growth of little spikes, which pass from the track of the needle at right angles into the surrounding gelatine. On potatoes rough granular parchment-like growths gradually spread over the surface ; organisms are about the size of the anthrax bacillus, which they resemble very greatly, and sometimes occur in threads ; they are motile, and contain highly refractile spores, which are usually situated in the middle of the bacillus ; non-pathogenic.

β. Colonies motile and swarming, giving rise to rapid liquefaction.

(1) *Proteus vulgaris.*—Found in putrefying organic matter, in ulcers, in meconium-fæces, in water, &c. Grows on gelatine plates very rapidly as whitish-grey turbid masses which are distinguishable at the end of about eight hours. From the central colony little projections pass outwards, the shape of these constantly varying as the bacilli are re-arranged ; the whole surface

gradually becomes covered with these motile colonies, and then liquefaction takes place rapidly; this is completed at the end of forty-eight hours. There is a foul odour and a marked alkaline reaction; in the track of the needle in pure cultures colonies may be seen which have a peculiar radiate formation, the liquefaction always extending wherever these colonies appear. Liquefaction takes place more slowly when oxygen is cut off; short ciliated rods and threads 1·25 to 3.75μ in length, and about ·6μ in thickness. The threads are usually twisted and convoluted; grows at about 20° to 24° cc., and causes very rapid liquefaction of the gelatine; no spore formation; involution forms are found—spherical bodies, about 1.6μ in diameter; is pathogenic.

(2) *Proteus mirabilis.*—Something like the preceding organism, but liquefies the gelatine much more slowly. (No liquefaction takes place when oxygen is cut off.) The threads are much longer, and the colonies have a finely granular brownish appearance under the microscope, especially towards the centre; the organism is about the same thickness as the above, but may be somewhat shorter; distinguished especially by the fact that spherical or pear-shaped involution forms are more frequently met with, these being from 3.75 to 7μ in diameter; zooglœa forms are also very numerous.

(3) *Proteus Zenkeri.*—In plate cultures forms thick whitish-grey layers, but gives rise to no zooglœa forms; in gelatine tubes a thick layer is formed at the point of inoculation, which by regular steps becomes thinner towards the periphery; from the margin threads shoot out; at the end of twenty-four hours there are large moving islands similar to those already examined, but liquefaction only takes place immediately at the surface, the deposit gradually becoming thicker and more opaque; the long thread forms are seldom met with; the bacilli are 1.65μ in length and .4μ in breadth, or they may be more rounded, or even a little longer; they are motile. (Although classed with the liquefying organisms, this liquefaction is sometimes so slight that it can scarcely be made out.) There is little or no odour given off from gelatine or blood serum cultures, but there is a strong smell given off when the organism is cultivated in meat infusions.

b. Colonies circumscribed without branches.

a. Bacilli 2.5μ in breadth.

β. Bacilli at most 1μ in breadth.

a. Bacilli 2.5μ in breadth.

(1) *Bacillus megaterium.*—First found on boiled cabbage-leaves. Occurs on plates as small round liquefying colonies; grows on gelatine very rapidly, liquefying in a funnel shape from the surface downwards; develops as a whitish layer on agar-agar, the surrounding material becoming somewhat darker; grows rapidly on potatoes at 20° C., as yellowish-white cheesy points near the seat of inoculation; proliferates by transverse division and by end spores; distinctly an ærobic organism; occurs as slightly bent motile rods, 10μ in length and 2.5μ in thickness; the ends are somewhat rounded; sometimes form chains of from two to ten bacilli. The cell contents are frequently granular.

β. Bacilli at most 1μ in breadth.

i. Development of clostridium forms before spore formation, see p. 432.

ii. No clostridium forms, see p. 432.

iii. Giving rise to the formation of bubbles of gas.
iv. Giving rise to a strong putrefactive odour, see p. 433.

i. Development of Clostridium forms before spore formation.

(1) *Bacillus butyricus*) (Hueppe).—One of the forms of bacilli giving rise to butyric fermentation; found in milk and in fleshy roots, such as turnips, &c., grows on plates in the deeper layers of the gelatine as delicate yellow masses; which later assume a brown granular appearance; these rapidly liquefy the gelatine and run together; on agar-agar they grow as viscid superficial yellow layers; in gelatine tube puncture cultures rapidly cause liquefaction along the track of the needle, the fluid becoming cloudy; the superficial layer is greyish-white or yellow, forming a delicate felted mass; grows very rapidly, especially at a temperature of from 35° to 40° C.; digests the casein of milk, interferes with the lactic acid fermentation, and gives a bitter taste; large, thick, very motile rods, with rounded ends of from 3 to 10μ in length, and 1μ in breadth; frequently forms chains; gives rise to well-developed spores.

(2) *The Clostridium butyricum or Bacillus amylobacter of Prazmowski* is morphologically exceedingly like the above organism, but has the characteristic of giving off on solid nutrient media a large quantity of gas which has the butyric acid smell; it is also markedly anærobic, transforms starch, sugar, dextrine, and lactates into butyric acid, setting free CO_2 and hydrogen; the threads may be unjointed; gelatine is liquefied, a regular felted scum forming on the surface; it grows at a temperature of from 35° to 40° C. Although one of the organisms first described, this bacillus has not yet been fully investigated.

ii. No Clostridium forms.

(1) *Bacillus mesentericus vulgatus.*—(Potato bacillus.) Forms rounded or oval colonies, with sharp margins; on plate cultivations, first somewhat transparent, afterwards slightly yellow; in needle cultures causes liquefaction along the track of the needle from above downwards, this is always more marked near the surface; a scum forms on the surface, and the growths along the track of the needle sink to the bottom of the funnel; the fluid is usually turbid; on potatoes grows extremely rapidly in the form of a wrinkled moist layer; later becomes somewhat dried, and rather like a crumpled felt; the organism, which is strongly ærobic, is slightly motile, and occurs as small thick rods with rounded ends, arranged in pairs, or sometimes in fours.

2. *Bacillus Ærophilus* is very like the above as regards its growth in gelatine, but the colonies are oval and have sharp margins; on potatoes it grows as a smooth yellow layer, which later becomes crumpled at the margins; slender spore-bearing rods and threads, about 1.4μ in diameter.

3. *Bacillus liodermos* (Described by Flügge).—Colonies form small irregular heaps in gelatine, and on potatoes a smooth slimy layer; short bacilli with rounded ends, which are actively motile; in other respects the growth is very like No. 1.

iii. Giving rise to the formation of bubbles of gas.

(1) *Gasbildender* (*or gas-forming*) *bacillus.*—An organism found in water. It liquefies gelatine very rapidly, and on plates forms moderately large lenticulate spaces, in which greyish points may be seen; these sometimes contain gas; on the surface the gelatine becomes liquefied, the greyish mass being seen in the centre; in gelatine tube cultures there is a tube-

shaped liquefaction area along the line of the needle, and in the gelatine that still remains solid, clear bubbles of gas may be seen. The organism that produces these changes is a very minute, exceedingly motile rod.

iv. Giving rise to a strong putrefactive odour.

(1) *Liquefying bacillus of Water* (*Verflüssigender bacillus of the Germans*). —Very rapidly forms round colonies with smooth walls; in the centre of the liquefying area is seen a white viscid mass; after a time an offensive putrefactive odour is given off; in a gelatine tube puncture cultivation there soon appears along the track of the needle a white granular mass, which is followed by a funnel-shaped area of liquefaction; short, somewhat thick rods with rounded ends.

B. Colonies or nutrient substratum coloured.
a. Colouring matter, red.
b. Colouring matter green, see p. 434.
c. Colouring matter violet, see p. 434.

a. Colouring matter red.

(1) *Bacillus prodigiosus* (*Micrococcus prodigiosus*, *Monas prodigiosus*). —Grows very rapidly on plates at from 20° to 22° C., in the deep layers as grey points, superficial growths small grey rounded colonies, about 1 mm. in diameter; these sink into the gelatine, which is rapidly liquefied, but remains quite clear; under the low power deep colonies are seen to be rounded or oval, and to have sharp outlines, but those at the surface are granular and have an irregular outline; when liquefaction has set in a beautiful red colour makes its appearance, but this is best seen on agar cultivations, where there is free access of oxygen to the growing organism; on potatoes a beautiful moist blood-red layer is formed, which is perfectly characteristic; the bacilli themselves are colourless; the pigment is insoluble in water, soluble in alcohol; occurs as egg-shaped non-motile cells, about 1μ in diameter, especially when the liquefaction is rapid; sometimes, when the growth is slower, the organism is distinctly rod-shaped, or it may occur in the form of short threads.

(2) *Bacillus indicus ruber*.—Found in the contents of the stomach of a monkey. Was the organism with which Koch made some of his experiments in connection with the destruction of micro-organisms in the alimentary canal. The colonies on plates are first of a yellow colour, and have a wavy outline; the superficial growths bring about the liquefaction of the gelatine, this first appearing as a little zone round the colony, and giving rise to a liquefied funnel-shaped area; in gelatine needle cultures a waxy or brick-red colour forms at the surface, but along the track of the needle the growth is somewhat grey or white; on potatoes forms a localized brick-red or waxy layer; grows best at a temperature of about 35° C., as a very thin and short bacillus with rounded ends; injected in large quantities into rabbits it causes death from diarrhœa in from three to twenty hours.

(3) *Red bacillus*.—Found in water. On plates forms finely granular colonies with smooth surfaces; red coloured points accumulate in the centre of small liquefied areas in gelatine tubes, as the gelatine is gradually liquefied, and a brownish-red coloured mass sinks to the bottom; grows on agar-agar, potatoes, and blood serum with the same characteristic brownish-red colour; is an exceedingly motile bacillus of medium size, and with somewhat truncated ends, sometimes united to form long threads.

b. Colouring matter green.

(1) *Bacillus pyocyaneus* (*Bacterium æruginosum, Bacillus of blue or green pus*).—On plates forms microscopical colonies, which send out radiating threads and give rise to funnel-shaped liquefaction of the gelatine about the second or third day; the margin is clear and granular; in tubes the gelatine commences to liquefy at the surface in twenty-four hours; there is a funnel-shaped liquefying area limited to the neighbourhood of the needle-track, the surrounding gelatine remaining solid, but assuming a beautiful fluorescent colour; on potatoes this organism grows as dry colonies with a dirty rusty colour, the surrounding potato being stained slightly green; if a drop of ammonia is added to this, a green, if a drop of acid, a red colour is obtained; the organism grows extremely rapidly, and is strongly ærobic. It is a very minute, short, thin rod, which is sometimes mistaken for a micrococcus; does not form any spores.

(2) *Bacillus fluorescens liquefaciens* (*Grüngelber bacillus of the Germans*).—Is found in putrefying substrata, water, &c.; colonies seen under lens are at first circular, later have irregular outlines; the centre is dark brown, finely granular; outside this is a transparent liquefying zone; the whole gelatine gradually becomes green; in puncture cultures in gelatine a white line is seen along the track of the needle, but near the surface there is a little funnel-shaped depression, which gradually increases in size, a little air-bubble frequently being formed near the surface; the gelatine around the liquid has a greenish-yellow fluorescent appearance, an appearance that is not so marked in the liquefied gelatine itself; on potatoes a yellowish-brown layer is formed, around which there is slight discoloration of the potato; the organism consists of a short active bacillus, arranged in pairs and usually constricted in the middle.

(3) *Bacterium graveolens.*—Found in the fragments of epidermis taken from between the toes; obtained by Bordoni Uffreduzzi. Grows at the ordinary temperature of the room on gelatine plates in the form of irregular whitish-grey specks, which rapidly liquefy the surrounding gelatine; these give off the peculiar smell of the feet, and give rise to a greenish-yellow coloration; on potatoes grow very rapidly, and form a greyish mass with an exceedingly offensive odour; these organisms are about .8μ in length, and nearly as broad as long.

c. Colouring matter violet.

Bacillus violaceus.—Is found in water; on plates it grows as small round colonies, which liquefy the gelatine very rapidly; these are first white, but they very rapidly assume a beautiful violet colour, the mass sinking to the bottom of the liquefied gelatine; in tube puncture cultures the gelatine is liquefied very rapidly, usually in a funnel shape, at the bottom of which a beautiful violet granular mass collects, the liquefied gelatine remaining clear; the same beautiful colour is formed on agar-agar, potatoes, and blood serum; grows somewhat slowly and best at the ordinary temperature of the room; is a motile rod about four times as long as broad, with rounded ends, and often contains spores; it also grows out into longer threads.

III. Organisms do not grow on nutrient jelly, and only on other media at higher temperatures and in the presence of air.

(1) *Bacillus tuberculosis* grows on blood serum at 37° C., see p. 206.

(2) *Bacillus septicus sputigenus* (*Diplococcus Pneumoniæ*).—Forms a transparent layer on the surface of blood serum; difficult to obtain a growth of this organism in gelatine plates, as it requires about 20 per cent. of gelatine to keep it solid at 24° C.; it then grows very slowly as small, rounded, sharply defined, slightly granular, whitish colonies; is an oval or coccus-like bacillus, resembling the pneumonia bacillus; the short rods are frequently joined together in chains of five or six links: are usually taken from the sputa of cases of lung disease, from the rusty-coloured sputum of pneumonic patients, from severe cases of empyæmia, and from the fluid from cases of cerebro-spinal meningitis; they have a kind of capsule, similar to that met with round the pneumonia bacillus; this capsule never makes its appearance in cultivations; mice, guinea-pigs, and rabbits die in 24 to 48 hours after inoculation with this organism, when the blood is found to contain a large number of the encapsuled bacilli.

The other organisms belonging to this group may be cultivated at a somewhat lower temperature, viz.:

(3) *Bacillus mallei* (*or Glanders bacillus*), see p. 264.

(4) *Bacillus diphtheriæ* (*or Klebs-Löffler bacillus*), see p. 299.

(5) *Bacillus saprogenes.*—Under this name Rosenbach describes three separate bacilli:

No. 1.—Obtained from offensive secretions and from the white casts taken from the recesses of the mucous membrane of the wall of the pharynx; grows slowly on the surface of agar-agar as a dirty grey opaque line along the track of the needle; it is, however, slightly transparent when held up to the light; forms a growth of considerable thickness, and an opaque, tenacious, viscid consistence; later this surface assumes an almost shell-like look; causes putrefaction of albuminous substances, and gives rise to a very offensive odour; for this, however, it apparently requires the presence of oxygen; is a somewhat large bacillus, in which end spores may frequently be seen; apparently non-pathogenic.

No. 2.—Obtained from the foul-smelling sweat of the feet; grows much more rapidly than No. 1 as a delicate superficial transparent growth, which gradually becomes whitish-grey and of a tough, gelatinous consistence; is also an ærobic organism, but it can give rise to its peculiar foul sweaty odour even when oxygen is excluded; is a bacillus thinner and shorter than No. 1; when injected into serous cavities of rabbits it sets up suppurative inflammation.

No. 3.—Obtained from cases of suppuration of bone in a patient suffering from septic poisoning; grows moderately rapidly, the growth at the temperature of the room taking about eight days to form an ash-grey, almost fluid, semi-opaque layer, with wavy outlines; is a short, thick bacillus, with rounded ends. (Although it is ærobic, it gives rise to putrefactive changes much more rapidly when growing anærobically.) On injection into a joint it induces a peculiar yellowish-green infiltration, with surrounding inflammation, giving off at the same time a very offensive putrefactive smell.

(6) *Bacillus necrophorus.*—Found by Löffler when he inoculated small particles of flat condylomata into the anterior chamber of the eye of a rabbit. It does not grow on gelatine or agar-agar, and only very slowly on blood serum, but in neutralized rabbit broth it forms a white fluffy mass around the particles of the substance that has been implanted in the

broth, from these smaller masses break off and float in the fluid; the organism occurs as long threads consisting of bacilli, these bacilli of various lengths are usually of the same thickness, and are frequently very much bent, or curved, or even intertwined; causes death of rabbits in about eight days, giving rise to a peculiar necrotic process at the seat of inoculation; kills white mice in about six days.

(7) *Bacillus of psuedo septicæmia of mice.*—Described by Bienstock. An organism found in fæces. Grows on agar-agar very slowly, forming a scarcely visible layer on each side of the inoculation stroke; is a non-motile organism very like the bacillus of mouse septicæmia, but is somewhat thicker, being sometimes half as broad as long; is occasionally mistaken for a micrococcus; found mostly in the œdematous fluid and not in the blood of animals that are inoculated; kills both rabbits and mice.

(8) *Bacillus of conjunctivitis.*—Has been described by Weeks as obtained from the conjunctival sac in cases of conjunctivitis; will not grow on gelatine, but in .5 per cent. of agar-agar it grows slowly on plates, forming in about forty-eight hours small pearly growths at the point of inoculation; about the fifth or seventh day the growth is complete, and at the end of a month the organisms appear to have lost their power of growth; it grows exceedingly well in fluid flesh broths, but not in solid media; grows best at from 34° to 37° C.; a bacillus from 1μ to 2μ in length and $.25\mu$ in thickness; like the tubercle bacillus, often forms long threads. As another organism was present with this when inoculations into the human subject were made it is not yet quite proved that this organism is the actual cause of conjunctivitis.

(9) *Xerose bacillus.*—From the xerotic masses of the conjunctiva of a child suffering from keratomalacia, conjunctivitis and hypersecretion of the Meibomian glands. This organism does not grow on gelatine or potatoes, but grows at the temperature of the body on blood serum, and afterwards (though not primarily) on agar; on blood serum it forms fine dull grey streaks along each side of a surface needle track; is sometimes floated off by the water of condensation; is a bacillus about the length of the mouse septicæmia bacillus; usually collected into little groups; a peculiar fatty substance forms a kind of capsule which makes it appear to vary in thickness according to the method of staining used.

(10) *Bacillus der Akne contagiosa des Pferdes* (Bacillus of horse-pox).—Grows best on blood serum at 37° C.; on the surface along the stroke track of the needle grows as white points, which later become yellowish grey, some of this growth is washed away by the condensation water, but sinks to the bottom leaving the water quite clear; also grows *in* gelatine as small white round points, which gradually increase in size, and *on* gelatine as minute strongly refractile points; small rods 2μ in diameter, usually straight or slightly curved; when injected subcutaneously the bacillus gives rise to the so-called acne in the horse, calf, sheep, and dogs; causes erysipelatous swelling and pus formation in the rabbit; when injected subcutaneously is fatal to guinea-pigs and mice.

IV. Organisms will only grow under conditions of anærobiosis.

(1) *Bacillus of malignant œdema* (*Bac. odematis maligni, vibrion eptique*).—Grows deep down in gelatine as small "bubbles" with fluid

contents; in agar-agar as smoke-like opacities not sharply defined from the surrounding agar; in needle cultures there is a similar turbidity along the track of the needle; grows best in this latter substance when one or two per cent. of grape sugar is added, and at the temperature of the body; liquefies gelatine, and is an exceedingly motile organism from 3 to 3.5μ in length, and 1.1μ in breadth; usually a couple of rods are linked together, or they may form threads 14 to 40μ in length; have rounded ends, are comparatively stiff, may be broken or looped and twisted around each other; spores are formed either at the end or in the middle, giving rise to drum-stick shape or spindle shaped forms, these spores are most readily formed at the temperature of the room; they differ from the anthrax bacillus in having the rounded ends, being somewhat smaller, and in being strongly anærobic.

(2) *Pseudoedema* (*Pseudoödem*) *bacillus* (Liborius).—Found in the œdematous fluid of the tissues of a mouse that had been inoculated with garden earth. On plates forms small globes with fluid contents, at the lower part of which there are usually white deposits; above this is fluid and then a little bubble of gas; in agar-agar containing sugar little oval or lentil-shaped bubbles with irregular outlines are formed; in puncture cultures in agar-agar there occurs a cloudiness along the needle track and gas is formed, this frequently bringing about the cleaving of the medium; the organism grows slowly, and does not liquefy the gelatine; under the microscope it is a bacillus somewhat thicker than the œdema bacillus; one or two spores may be formed in each bacillus; when injected into the veins or into the subcutaneous tissue of mice and rabbits it causes death in a very short time.

(3) *Bacillus of symptomatic anthrax* (*Charbon symptomatique*, *Rauschbrand bacillus*).—Is found in the serous fluids, bile, and muscular tumours in cases of "quarter evil." Can only be cultivated anærobically; it has been cultivated in fowl broth to which small quantities of glycerine and sulphate of iron have been added, the air being driven from the upper part of the vessel in which the culture is made by means of CO_2 or hydrogen; grows best at the temperature of the body; is a motile organism from 3 to 5μ in length, and from .5 to .6μ in breadth; the organism very frequently contains spores at the ends, and is usually motile.

(4) *Bacillus butyricus* (Liborius).—Is an anærobic organism very like the bacillus butyricus of Prazmowski, even as regards the method of formation of the spores; when grown in gelatine from which the whole of the oxygen has been driven off, it appears as whitish, not very sharply defined, colonies, which on about the third day are surrounded by a narrow zone of liquefaction, this gradually increases in size, and the whitish mass sinks to the bottom of the globe so that we have a clear globe with a small precipitate at the bottom, bubbles of gas gradually passing into the upper layers of gelatine, driving out the oxygen and allowing the organism to grow in this position, which it never does in the first instance; this gas has a disagreeable odour.

(5)? *Clostridium butyricum or bacillus butyricus of Prazmowski*, see p. 432.

(6) *Bacillus of tetanus*, see p. 287.

V. Organisms described in the tissues, but has not yet been artificially cultivated outside the body, *i.e.*, they do not grow under ordinary conditions.

(1) *Bacillus leprœ*, see p. 247.

(2) *The bacillus of syphilis.*—Has been demonstrated in tissues by Lustgarten, who stained his sections of syphilitic new growths in Weigert's aniline gentian violet solution (see p. 412), decolorized by means of a solution of permanganate of potash, and then washing with sulphurous acid, this is repeated until the sections are colourless, when the bacilli stand out prominently. The bacilli of leprosy and tubercle are stained by the same method, but they may be distinguished from the syphilis bacillus by the fact that the latter loses its stain on the sections being washed with a mineral acid. The organisms are somewhat S-shaped, are about 4.5μ in length, and have frequently a slight swelling at the ends; they are somewhat wavy or slightly indented, and along the line of the bacillus may be seen two or four clear spaces, probably spores. It has more recently been found, however, that other bacilli take on a similar stain.

(3) *Bacillus of rhinoscleroma.*—An organism found in the tissues of patients suffering from a disease very rarely met with in this country. In sections of the thickened skin or mucous membrane stained in methyl violet for forty-eight hours and then decolorized for forty-eight hours in absolute alcohol a bacillus may be demonstrated; short rods, 1.5 to 3μ in length, and .5 to .8μ in breadth, with rounded ends, each containing stained granules, and surrounded by an oval capsule which stains much more delicately than the organism itself.

(4) *Bacillus septicus*, see p. 344.

(5) *Bacillus diphtheriæ vitulorum.*—Described by Löffler as occurring in the diphtheria of calves. Long bacilli 2.5 to 3.6μ in length, and .5 to .6μ in breadth, frequently united to form long threads; found in the deeper tissues under the diphtheritic deposits in the mucous membrane.

The Organism is a Spirillum.

The more important of the spirella may be distinguished by their appearance as they occur in nutrient gelatine at a temperature of from 20° to 24° C.

I. The gelatine is liquefied.

II. The gelatine is not liquefied, see p. 439.

III. The organisms have not yet been cultivated on artificial media, see p. 440.

I. The gelatine is liquefied.

(1) *Koch's cholera bacillus or spirillum choleræ Asiaticiæ.*—Plate cultures, colonies light yellow, have irregular outlines liquefying the gelatine slightly and sink to the bottom, leaving a clear surrounding space. Grows on potatoes as a brownish film at 30° C. or higher, see p. 153.

(2) *Finkler and Prior bacillus.*—On gelatine plates grows rapidly and appears as small white points; under a lens these are yellow or yellowish-brown in colour, and have a sharp well defined circular outline; the surface is not so refractile nor so granular as in the case of the true cholera colonies. Liquefaction takes place at an early date and goes on very rapidly. The liquefied fluid becomes turbid, whilst in the cholera bacillus it will be noted that the upper part remains perfectly clear. In gelatine tube cultivations this is still more marked. Liquefaction occurs in the form of a funnel-shaped tube, the fluid gelatine being exceedingly turbid. On nutrient agar yellowish white films are formed. Blood serum is rapidly peptonized and

liquefied. This organism grows on potatoes at the ordinary temperature of the room as a yellowish white layer. Organisms slightly larger than Koch's bacillus, frequently somewhat pointed at the ends. The spirals are as a rule not so long and not so perfect as the cholera bacillus, involution forms being more frequently met with. The odour is very disagreeable. The organism is more resistant than the cholera bacillus; it is not nearly so fatal to guinea-pigs, though a certain number usually succumb to its action. It was supposed to be obtained from cases of cholera nostras, but it is probable that it is merely one of those spirilla which are met with in the alimentary canal under ordinary conditions, similar to those described as organisms of the mouth.

(3) *Deneke's cheese bacillus* (*Spirillum tyrogenum*).—Plate colonies under microscope similar to those of the Finkler and Prior bacillus, but brownish in colour. It grows very rapidly as plate cultivations, and gives rise to liquefaction of the gelatine, not so rapidly as the Finkler and Prior bacillus, but more rapidly than the cholera bacillus. It also forms a yellowish-white layer on agar-agar and blood serum. It was first described as giving rise to no growth on potato at any temperature, but is now found to grow as a yellow layer on this medium. This bacillus is somewhat smaller than the cholera bacillus. Forms long spiral threads, in which the spirals are close and very perfect; like the previous bacillus, it is exceedingly motile. When introduced into the duodenum of the guinea-pig, according to Koch's method (p. 164), kills about twenty per cent. of the animals, but the organism under ordinary conditions is probably non-pathogenic.

(4) *The Vibrio Metschnikoff* was first observed by Gamaleia in the contents of the intestine of a fowl. Its growth on gelatine plates resembles that of the Finkler-Prior bacillus; although it does not liquefy gelatine quite so rapidly, it gives rise to the same peculiar cloudiness. It sometimes resembles the Finkler-Prior, at others the Koch comma bacillus, and sometimes the cheese bacillus cultures. It grows in gelatine, on agar, and on potatoes, as does the cholera bacillus, but in bouillon it causes turbidity of the fluid at an early date, and a thin film soon appears on the surface. It gives the cholera red reaction on the addition of sulphuric or hydrochloric acid, just as does the cholera bacillus. It is an organism very like the cholera bacillus in many respects, and is certainly closely related to it. It occurs as a somewhat curved bacterium, shorter and thicker, but rather more bent, than Koch's comma bacillus. In fluid media it forms regular spirals. It is provided with long delicate wavy flagella, and is motile. Metschnikoff has described it as being capable of passing through a whole series of changes in form, and as being one of the best examples of polymorphism. Apparently it does not form spores. It is pathogenic in the case of hens, guinea-pigs, and pigeons, but does not affect mice.

II. The gelatine is not liquefied.

(1) *Emmerich's bacillus* is a special form of bacillus which was described in the cholera epidemic of Naples (1884). It occurred along with the true cholera organism, and was obtained in the alimentary canal and was said by Emmerich to occur in the blood of patients who had died from cholera.

On plate cultivations colonies grow deep down as small white points.

These come to the surface, spread out as thin yellowish opalescent growths, which do not liquefy the gelatine. Under the microscope small characteristic deep growths, with a dark brown colour and having somewhat the concentric appearance of the Spirillum concentricum, are seen. The superficial growths are usually paler in colour, and are slightly yellow in the centre. The margins are toothed, and the whole surface has somewhat the appearance of a network.

In tube cultures it grows almost like the typhoid bacillus. Yellowish-white points make their appearance along the line of the needle, whilst on the surface there is a greyish-white glistening layer with scalloped margins. The surrounding gelatine becomes somewhat cloudy, but there is no liquefaction. On agar-agar it grows as a white moist layer, which has no special characteristics. On potatoes it forms a yellowish-brown viscid layer which has a somewhat characteristic appearance.

It occurs as short rods with rounded ends, which usually remain single, but are sometimes united into long threads. It forms spores, and can grow anærobically.

It is said that it is also found in the fæces of healthy individuals, in the air, and in putrefying fluids, and that it is not necessarily found in cases of cholera. It is certainly not found in all cases of cholera, and it is now thought that it may be nothing more than the ordinary fæces bacterium.

(2) *The Spirillum rubrum* was first obtained by Von Esmarch from the body of a mouse. It grows extremely slowly, and cannot be made out in plate cultivations for about five days. Under the microscope the points, which are exceedingly small, have a yellowish-red appearance, are finely granular, and have sharp margins. In the presence of oxygen no colouring matter is formed, but in the deeper part of the needle track in a puncture culture there is a beautiful wine colour developed, the surface growth having a moist appearance and well-defined margins. It is a somewhat thick, transparent, homogeneous, screw-like spirillum with three or four to forty spirals; is extremely motile, and is provided with flagella. Its vegetative multiplication is by transverse division. It probably forms spores, as it is resistant to the action of drying, although it cannot withstand a temperature of more than 50° C. It multiplies at a temperature of between 16° and 40° C., but most actively at the temperature of the body. In solid cultures the rods are short, but in fluid media the spirals are well developed. It is non-pathogenic.

(3) *Spirillum concentricum* occurs in putrefactive blood; it was first described by Kitasato, and probably belongs to the same group as the larger spiral bacteria that are frequently found in that substance. It grows rapidly at the temperature of the room on plate cultivations, forming greyish-white, round, smooth, well-defined colonies, each of which appears to grow with concentric marking, and looks almost like a cockade; hence the name. In gelatine it gives rise to no liquefaction, and grows better on the surface than deeper down along the needle track. It does not grow on potatoes, and is apparently non-pathogenic.

Like the Spirillum rubrum, it forms short spirals on nutrient media, but long spiral threads on fluid media. It is motile, and is provided with flagella.

III. The organisms have not yet been cultivated on artificial media.

(1) The *Spirillum Obermeieri* is an exceedingly delicate, flexile spiral of from ten to twenty turns. It is found in the blood of patients suffering from relapsing fevers. It is from 16 to 40μ in length, and is usually less than half the diameter of the cholera bacillus. According to Koch and Vandyke Carter these organisms can only grow in the blood inside the body, although they may be preserved alive for a considerable length of time in blood serum or in normal salt solution. When the blood of patients suffering from relapsing fever (during the febrile stage) is inoculated into the long-tailed macacus monkey an attack of fever is set up, during which the spirillum can be demonstrated in the blood. It may also be demonstrated in the blood vessels of organs removed from animals killed during this febrile attack. There are no relapses in the monkey as in man, but one attack of the disease does not protect against a second.

(2) *Spirochæte plicatilis* is an organism of extraordinary length—110 to 225μ. It is extremely motile, and occurs in stagnant pools in which there is decaying organic matter. The threads are arranged in long wavy lines, the long waves appearing to be cut into shorter waves; these, of course, are merely the spiral turns.

(3) *Spirillum tenue* is an exceedingly short spiral of from 1.5 to 5 turns of a screw. The length of the organism is from 4 to 15μ. It usually occurs in vegetable decoctions, in which it darts about with very great rapidity

(4) *Spirillum serpens* consists of thin threads, which interlace to form a kind of network. The organisms are from 11 to 28μ in length, and usually have from three to four regular wave-like turns. It is often found in stagnant pools and where there is decaying vegetable matter. It is about 1μ in thickness. Where the felting is very marked it may sometimes appear to be united in chains. It moves about rapidly.

(5) *Spirillum undula.*—Sometimes thicker than the above, but not so long, though the average length is greater. Length 8 to 12μ, and breadth 1.1 to 1.4μ. The spirals are well marked, but they seldom consist of more than about three turns. There are usually distinct flagella at the ends (fig. on p. 29). Like the other forms, it occurs in putrefying fluids.

(6) *Spirillum volutans* is much longer and thicker than any of the preceding forms. It is 1.5 to 2μ in thickness and 20 to 30μ in length. The ends are somewhat thinned and rounded. The protoplasm contains a number of dark granules; there is a distinct flagellum at each end. This spirillum may be motile, but very frequently it is perfectly motionless. It has been found in marsh water, and also in a decoction of dead fresh-water snails.

(7) *Spirillum rugula* consists of short cells or single wavy bacilli 6 to 8μ in length and .5 to 2.5μ in thickness. They have usually a single bend or a short flat turn; may hang together in chains or form a felted mass. They are extremely motile, and rotate round their own axis. They are provided with flagella, and form spores at their ends very much as does the tetanus bacillus, having then very much the appearance of a comma. Found in marsh water and very frequently in the alimentary canal; probably anærobic in character, and, according to Prazmowski, sets up decomposition of cellulose.

Literature.

To Zopf, Flügge, &c., the reader is referred for full descriptions of Crenothrix, Beggiatoa three forms (B. alba, B. roseo-persiciana, and B. mirabilis), Cladothrix, Dichotoma, Streptothrix Foersteri, and various forms of Monas, Spiromonas, and Rhabdomas.

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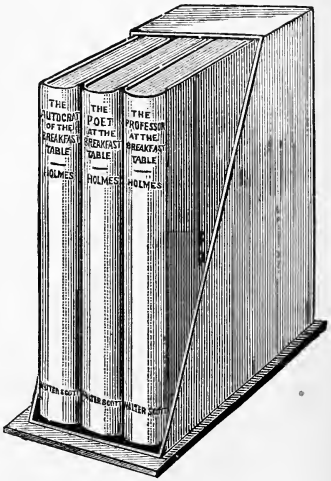

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