Micro-organisms and disease : an introduction to the study of specific micro-organisms / by E. Klein.

MICRO-ORGANISMS

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AN

INTRODUCTION TO THE STUDY OF SPECIFIC MICRO-ORGANISMS

BY

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TO

SIR JOHN SIMON, K.C.B., D.C.L., LL.D., F.R.S.

THIS BOOK

Is Respectfully Dedicated

BY

THE AUTHOR

PREFACE TO THE NEW EDITION

TEN years have passed since the last (third) Edition of this book appeared. Great changes have taken place since that time. The phenomenal extension of the study of Bacteriology, its application to the study of Public Health, and to Medical and Surgical Practice; the remarkable extension of our knowledge of the chemical activity of Micro-organisms; the results achieved in the battle against infectious diseases by means of accurate bacterioscopic analysis and diagnosis, and by the brilliant application of serum therapeutics, are matters obvious to the Biologist, the Chemist, the Sanitary Expert, the Physician and Surgeon. To get an idea how much the study of micro-organisms in their relation to disease has extended we may state that when Baumgarten's *Jahresbericht*—an annual review giving a tolerably complete account of the work done in pathological mycology—first appeared (Vol. I.) in 1886 its size was 185 pages; in 1892 (Vol. VII.) it had reached the size of 862 pages, and it has been steadily increasing since. Since 1886 several most important periodicals have come into existence

which are almost entirely devoted to the publication of original work in bacteriology: *Zeitschrift für Hygiene*, *Annales de l'Institut Pasteur*, the *Journal of Pathology and Bacteriology*; in addition the *Hygienische Rundschau*, the *Fortschritte der Medicin*, the *Archiv für Hygiene*, the *Proceedings of the Royal Society*, various medical and veterinary periodicals in this country and abroad, continue, as before, to publish occasional papers on bacteriological subjects. It must be obvious that it is an impossibility to give, even in its essential outlines, an account of all this enormous progress in a small handbook like the present volume, but I have striven to add the more important results of the work of the last decade, principally so far as they refer to the relation of micro-organisms to disease. For this purpose it was found necessary to revise and to rewrite some, and to materially alter others, of the chapters of the old edition.

A considerable number of illustrations taken from photograms have been added; these have been, for the most part, prepared from my own preparations, unless otherwise stated, by Messrs. A. Pringle and E. Bousfield.

E. KLEIN.

March, 1896

CONTENTS

CHAPTER V

CHAPTER VI

CHAPTER VII

CHAPTER VIII

CHAPTER IX

CHAPTER X

CHAPTER XI

CHAPTER XII

CHAPTER XIII

CHAPTER XIV

CHAPTER XV

CHAPTER XVI

CHAPTER XVII

CHAPTER XVIII

CHAPTER XIX

CHAPTER XX

CHAPTER XXI

MICRO-ORGANISMS AND DISEASE

INTRODUCTION

The relation of micro-organisms to disease is admitted to be very intimate; with special regard to infectious diseases there exists now no doubt that specific microbes are the *causa causans*, but also in a number of diseases not infectious in the ordinary term—*i.e.* not communicable from individual to individual—an important relation between a specific microbe and the disease itself has been proved to exist. Amongst the infectious or communicable diseases there are still some in which the satisfactory demonstration of specific microbes has not been achieved yet, as in hydrophobia, variola, syphilis, measles, whooping-cough, &c.; but in a large number of maladies which affect man and animals, and which from the ascertained etiological data are of the nature of communicable diseases, the demonstration of the specific microbes is an established fact. Amongst the diseases which do not strictly belong to the communicable diseases, there are some in which it has been either proved or made highly probable that microbes have an important

bearing on their production in particular individuals specially predisposed.

To mention two series only: it has been shown that there occur in some individuals a variety of localised inflammatory and suppurative foci, which are intimately associated not necessarily with pus-forming cocci—the microbes of typical suppuration—but with one or the other kind of microbes —*e.g.* proteus vulgaris, bacillus coli, pneumococcus, and other microbes. These organisms have in these particular cases only caused disease; under healthy conditions their presence in the various tissues is insufficient to do so. Or, to take another series of disorders: fatal summer diarrhœas in children and in adults. Bacillus coli or proteus vulgaris are under ordinary normal conditions found in the intestine, in the large intestine and in the lower ileum, as also in many other conditions associated with putrid proteid decomposition. Under certain abnormal conditions of the intestine caused, in the first instance, by fermentative changes, such as the lactic or acetic fermentation or others, the intestinal tract, specially the small intestine, is rendered highly favourable for the multiplication of those microbes, so that it practically contains a pure culture of them. Bacillus coli and proteus vulgaris, being both endowed with the power of intensive proteid decomposition, would under these favourable conditions of multiplication produce copiously poisonous alkaloids, ptomaines or allied toxins, which, absorbed into the circulation, might produce fatal results.

In order to pass in review all the ascertained facts and observations in this vast and constantly growing field of pathology, and to appreciate and to assign their true value to the many observations bearing on this relation of micro-organisms to disease, it is necessary that the reader, and still

more the worker in this field, should be enabled to criticise the observations and facts brought forward by the numerous writers on this subject, for otherwise he would probably take as proved what has really not passed beyond the stage of possibility. And it is this point which requires the most careful attention—viz., to be able to see at a glance that, owing to the imperfect or faulty methods employed, or that, owing to certain inferences incompatible with the general laws and general tendency of the well-founded and experimentally proved facts, the statements set forth in a particular observation or series of observations are not to be accepted.

In all investigations of the relation of micro-organisms to disease it is necessary to bear in mind that, as Koch[1] has pointed out, no observation can be said to be complete, or, one should rather say, in no instance can it be said to have been *satisfactorily proved*, that a particular morbid process is due to a particular micro-organism if any one of the following conditions remains unfulfilled:—(1) It is absolutely necessary that the micro-organism in question is present either in the blood or the diseased tissues of man or of an animal suffering or dead from the disease. In this respect great differences exist, for in some infectious diseases the micro-organisms, although present in the diseased tissues, are not present in the blood; while in others they are present in large numbers in the blood only or in the lymphatics only. These points will be considered hereafter in the special cases. (2) It is necessary to take these micro-organisms from their nidus, from the blood or the tissues as the case may be, to cultivate them artificially in suitable media—*i.e.* outside the animal body, but by such methods as to exclude the accidental introduction into these media of other micro-

[1] *Die Milzbrand-impfung*, Cassel and Berlin, 1883.

organisms; to go on cultivating them from one cultivation to another for several successive generations, in order to obtain them free of every kind of matter derived from the animal body from which they have been taken in the first instance. (3) After having thus cultivated the micro-organisms for several successive generations it is necessary to re-introduce them into the body of a healthy animal susceptible to the disease, and in this way to show that this animal becomes affected with the same disease as the one from which the organisms were originally derived. (4) And, finally, it is necessary that in this so affected new animal the same micro-organisms should again be found. A particular micro-organism may probably be the cause of a particular disease, but that really and unmistakably it is so can only be inferred with certainty when every one of these desiderata has been satisfied.

Now, at the time when Koch laid down these principles, which being of the nature of exactness were accepted by all, there still existed amongst medical men a considerable number who doubted that microbes have any primary relation to disease, the doctrine of *contagium vivum* was still to them an unproven view, although the practice in sanitary science had long accepted the correctness of that view. And it may be said to have been one of the most complete and exact achievements of Koch to have been the first who by exact methods conclusively demonstrated the correctness of the view in the case of malignant anthrax. The same principles led him to the demonstration of the causative relation between certain microbes and septi-cæmic infections in animals, and he may be said to have crowned the edifice by his brilliant discovery of the tubercle bacilli. In all these instances it was possible for him to absolutely and by exact methods and without cavil to

establish the correctness of the theory of *contagium vivum*. Many facts have since come to light which necessitate a relaxation of these rigid rules. As then so also now these principles hold good, if the true causative relation between a particular microbe and a particular disease is to claim unequivocal acceptance, but there are a variety of conditions under which such rigorous proof is impossible. Koch himself has seen this in the case of cholera Asiatica. As he himself so also others before and after him have accepted as correct the everyday experience that Asiatic cholera is a disease of the human subject only, that domestic animals under natural conditions, in localities where cholera is endemic or in localities where epidemics prevail, have never been known to have been subject to this disease. It is therefore obvious that since no animal can be said to be susceptible to cholera in the sense in which man is, the third and fourth points above stated, in furnishing proof positive, cannot be fulfilled. True, under certain modes of experimentation with cultures of the cholera vibrio (see the Chapter on Cholera), guinea-pigs are capable of developing an acute fatal disease; but under the same methods of experimentation other microbes, not connected with cholera or any other disease, produce the identical results or results differing only in degree.

Again, take the case of typhoid fever: the microbe which is found in the tissue of the spleen and mesenteric glands in large numbers in cases of typhoid fever only is, from its constant occurrence and its special biological characters, justly considered to be the microbe of that disease, but since animals are not susceptible to typhoid fever, the two conditions mentioned *sub* 3 and 4 cannot be verified. The pathogenic action which this microbe is capable of exerting on guinea-pigs when injected sub-

cutaneously or intraperitoneally, is not of a specific nature and is not of the nature of typhoid fever in man. As a last example we may mention leprosy. No one doubts that the bacilli so peculiar in their morphological and biological characters and in their distribution, which are found crowding the cells and tissues of the leprosy nodules, are the real microbe of leprosy, but no one has succeeded as yet in producing leprosy in an animal.

It will be my aim in the following pages, first to describe the methods that may be employed with success in investigations bearing on the relation of micro-organisms to disease; secondly, to describe in conformity with reliable observations the morphology and physiology of the micro-organisms that bear any relation to disease; and thirdly, to enumerate the principal observations that have been made in recent years to prove the existence of such an intimate relation. Last, but not least, we shall consider the precise relation of the principal micro-organisms and their chemical products to the causation of disease.

CHAPTER I

MICROSCOPIC EXAMINATION

For the examination of micro-organisms good high powers are essential, at the least a power magnifying 300 to 400 linear diameters. Zeiss' D or E and Zeiss' or Leitz's or Reichert's oil immersion 1-12th or 1-16th inch (2 mm.) will be found sufficient for all purposes. In the case of tissues stained with aniline dyes a good substage-condenser such as Abbé's or Powell and Lealand's, is invaluable. I use Zeiss' or Leitz's stand with Abbé's condenser, open diaphragm, and plane mirror. As Koch [1] pointed out, and what is now universally acted upon, stained specimens mounted in Canada-balsam solution or Dammar varnish, when examined over an Abbé's condenser, show the micro-organisms with extreme clearness and sharpness.

The examination of the morphological characters of an organism is carried out on fresh unstained, as well as on fresh stained, microscopic specimens. Although the latter method is, for reasons hereafter to be mentioned, by far the most perfect and reliable one, it is nevertheless important to

[1] *Die Aetiologie d. Wundinfectionskrankheiten*, p. 34, Leipzig, 1879. Translated as *Traumatic Infective Diseases* (New Syd. Soc.), London, 1880.

ascertain as far as possible the motility, chemical reactions, and general morphology of living fresh specimens. Blood, juices, tissues, and fluids in which the micro-organisms are present, are subjected directly, without any previous preparation, to microscopic examination. In the case of artificial media in which micro-organisms have been growing, the examination of fresh specimens is of great importance, for the reason that the organisms can be easily identified and their size and general morphological characters be more correctly ascertained than after drying, hardening, and staining. Besides, the chemical reactions can be satisfactorily studied in fresh specimens only. All one has to do is to draw up with a capillary pipette or to take up with the point of a platinum needle a drop or particle of the material, to place it on an object-glass, and to cover it up with a thin cover-glass. Where one has to deal with liquids, such as artificial nourishing fluids, blood serum, tissue-juices, secretions, transudations and exudations, no addition is required. In the case of more solid material, such as solid artificial nourishing material, bits of tissue, &c., the addition of a drop of neutral previously well-boiled saline solution (of 0·6 to 0·75 per cent.) is advantageous although not absolutely necessary, since by pressing down the cover-glass a layer of the material sufficiently thin for examination can be obtained. In some instances a bit of tissue can be teased out into fine particles by means of two clean needles. Where it is a question of micro-organisms sufficiently conspicuous by their shape, size, and general appearance, their identification in the fresh condition is not difficult; this is the case with bacilli, vibrios, actinomyces, and mycelia, but in the case of micrococci, especially when isolated or in couples, and lying in blood, juices, or tissues, their recognition is often extremely difficult. When in large

clumps, such as larger or smaller masses of zoogloea, or when in the shape of chains, the identification is not difficult; but in the more isolated state they are not easily recognised, owing, as a rule, to the presence of granules or particles of various kinds, from which morphologically their distinction is well-nigh impossible. In such cases there are certain rules of thumb, if I may say so, which assist, although they do not absolutely insure, the diagnosis. These are the uniform size and shape and micro-chemical reactions. The addition of liquor potassæ leaves micro-organisms quite unaltered, whereas fatty and most albuminous granules alter or altogether disappear by it. Acetic acid from 5 to 10 per cent. strong does not affect micro-organisms, but albuminous and other granules become in most instances altered. These two re-agents, I think, are as reliable as any others; if they fail, then others like alcohol, chloroform, sulphuric ether, &c., are not of any greater help, but the latter re-agents may be used, for instance, when it is a question between fat-granules and micrococci, or crystals and bacilli.

Micro-organisms have a great affinity for certain dyes, especially aniline dyes, and therefore these are used with great success to demonstrate their presence, and to differentiate in many instances morphological details which in the unstained condition are not discernible. The staining is effected on fresh unaltered organisms, or after they have been dried. In the first instance the process is carried out thus :—A microscopic specimen is made, and to it is added afterwards drop after drop of the dye, passing it through the specimen in the usual way of applying fluids to a microscopic specimen—*i.e.* by adding with a capillary pipette the dye at one margin of the cover-glass and sucking it up with a strip of filter-paper applied to the opposite margin of the cover-glass. When the staining has taken

place the excess of the dye is washed away with salt solution, water or alcohol, or both, as the case may be (see below). Unless the organisms are embedded in continuous masses of solids, this method gives good results. In the latter case, say if they are embedded in a microscopic lump of tissue, or in a particular spot of a fine section of a fresh tissue, it is necessary, after having placed the lump or section on an object-glass, to drop the dye on to this previous to putting on the cover-glass. After some minutes the dye is allowed to run off by inclining the object-glass, and then the washing is proceeded with till all the excess of the dye is removed; the mounting is then done by placing a drop of water or salt solution on the specimen and covering it with a cover-glass. In the case of sections through fresh and hardened tissues containing micro-organisms, the method of staining and of permanently mounting them as a whole is more complicated, and will be detailed presently.

When one has to deal with coherent masses of micro-organisms, present either in natural media (*i.e.* animal tissue) or artificial cultivations, such as zooglea and pellicles of *micrococcus* or *bacillus*, these can be bodily transferred to a watch-glass, stained, washed, and mounted without much difficulty, either for immediate or permanent use. The permanent specimens are made in this way:—Place the section or pellicle in a watch-glass containing the dye, leave it there till deeply tinted, take out with a needle, section lifter, or the like, wash in water, then in alcohol, leave here for sufficient time till most of the excess of the colouring-matter is removed, then lift it on to an object-glass, spread well out, place on it a drop of xylol or clove-oil, and after a minute or two drain off, add a drop of Canada-balsam solution (in chloroform or xylol), and cover

with a cover-glass. In some special instances, such as the bacilli of leprosy and tuberculosis, double staining is required. With other organisms, such as the bacilli of glanders or tuberculosis, the washing is carried out, not with water but with acid (acetic acid and nitric acid respectively). All the details will be stated when dealing with these special organisms.

The method extensively and successfully used for the demonstration and preservation of microscopic specimens of micro-organisms in fluids, in blood, pus, mucus, and juices, is that of Weigert and Koch, which consists in spreading out on a glass slide or cover-glass a very thin film—the thinner the better—of the fluid (artificial or natural culture medium), blood, pus, or juice, and drying it rapidly by holding it for ten to twenty seconds over the flame of a spirit-lamp or gas-burner. The most successful preparations are obtained when the heating is carried on for such a time that the film, having become opaque at first, rapidly turns transparent. Several drops of the aniline dye to be used are then poured over the specimen, or the film is placed over the dye contained in a watch-glass, and after remaining in contact from half to thirty minutes or more, according to the nature of the microbes and the dye, the specimen is removed.

The cover-glass specimen is then well rinsed with distilled water, dried over the flame, and mounted in Canada-balsam solution or Dammar varnish—of course always bearing in mind on which surface of the cover-glass the film has been spread. If the film has been well heated in the first instance washing in water is quite sufficient, but if the drying has been insufficient a good deal of diffuse staining of the ground substance has taken place, and then the cover-glass specimen must be also washed in alcohol

sufficiently long to remove this undesirable staining, then washed in water, dried and mounted. In some instances, washing with alcohol removes also the dye from the bacteria, but as a rule it is better to first over-stain the cover-glass specimen, then wash well in alcohol so as to remove the dye from all except the bacteria, but do not wash with alcohol too long, then rinse in distilled water, dry and mount.

A method extensively used and yielding the best specimens is the one known as the method of making impression specimens—*Klatschpræparate* of the Germans. This method aims at representing in stained films the impression of bacteria in the actual position on a solid culture medium. Be the bacteria growing in a streak or in isolated colonies on the surface of gelatine or agar in a plate cultivation (see below), by pressing a clean cover-glass on to the surface of the growth an impression is obtained of the growth, the cover-glass is heated, and stained and treated as before. When it is successful the bacteria are seen in the exact position which they occupied in the culture, be that in a streak or in separate colonies; the manner in which they arrange themselves and the manner in which the growth proceeds at the margin is well shown. Care must be taken to make impression specimens of young growths, for if late the impression is too thick; but even in such cases the second or third impression of the same colony gives the desired result.

In the case of liquefying bacteria impression preparations must be made from gelatine growths at an early stage before liquefaction commences (*vide* Fig. 44) of a young colony of anthrax bacilli on gelatine.

The most useful dyes in the examination of animal tissues for bacteria are those aniline dyes that are soluble in water;

these are preferable to those soluble in alcohol only. They have all great affinity for cell nuclei (Hermann) and belong to the group of neutral or basic aniline colours. Methyl-blue, methyl-violet, vesuvin, Bismarck-brown, magenta, fuchsin, gentian-violet, Spiller's purple, rosaniline, Humboldt's red (purple), are the dyes most commendable.

For staining of cover-glass specimens, as well as for sections made of fresh tissues, the above dyes can be advantageously used in the following manner: 2 to 5 grammes of the solid dye are rubbed up in a mortar with 10 ccm. of absolute alcohol; add then gradually, while mixing, warm distilled water, to bring up the total to 100 ccm.; filter and keep in stoppered bottle. For use, filter a little of the dye into a watch-glass. For staining film preparations or sections of hardened tissues, the above dyes prepared with aniline oil are preferable; they are prepared thus: (*a*) Make a saturated watery solution of pure aniline (aniline oil) by mixing in a bottle one part of aniline oil with three parts of distilled water; shake well every half hour for four to six hours, decant the water as the oil settles to the bottom. The decanted fluid is the saturated watery solution of aniline. Of this take 100 ccm. Add to this (*b*) a saturated alcoholic solution of either fuchsin, gentian-violet, Humboldt's red, methyl-blue or methyl-violet, 11 ccm.; mix well, filter into stoppered bottle. The sections are left in this dye for from a few minutes to several hours (Humboldt's red requires only a few seconds). Different bacteria require different periods to stain. As a rule warming the dye facilitates the staining of the bacteria; occasionally, also, the addition of a few drops of liquor potassæ. All sections, after having been sufficiently stained, are transferred to and washed in water, then methylated spirit, then in absolute alcohol, then clarified in xylol or clove-oil, and finally

mounted in Canada-balsam (dissolved in chloroform, or better still in xylol) or in Dammar varnish.

After a very extensive experience in staining film specimens and sections, carried on for nearly eighteen years, I have come to the conclusion that for all purposes of bacteriological work the following stock of dyes is sufficient: (*a*) methyl-blue, and (*b*) gentian-violet, both these prepared with a saturated watery solution of aniline oil as described on the previous page; for staining of cover-glass film specimens use this gentian-violet aniline water, and absolute alcohol in equal volumes in a watch-glass, and allow the specimen to remain in this mixture for a few seconds to half a minute; afterwards wash well in water, dry and mount in balsam; (*c*) carbol-fusin prepared after Ziehl[1]; and (*d*) Löffler's methyl-blue: of a 2 per cent. watery solution of methyl-blue a little is mixed with equal volume of potassic hydrate 1 in 10,000; the staining must be of a prolonged character; after staining, wash well in water acidulated with acetic acid. (*e*) Alcoholic solution of eosin $\frac{1}{6}$ per cent.; (*f*) watery solution of rubin 2 per cent.; (*g*) watery solution of Bismarck-brown 2 per cent.

In order to bring out by the dye more conspicuously the bacteria present in fluids or tissues various methods are used, all of which are based on the principle that the bacteria have an affinity to the dye which is greater than that of the tissue-elements. Hence after staining, the tissue-elements may be decolourised without abstracting the colour from the bacteria. Cover-glass specimens or sections, after having been well stained with a dye, are subjected to various decolourising re-agents, whereby the tissue-elements become deprived of the dye, but the bacteria retain it. Although in

[1] 1·5 grammes fuchsin, 10 cc. absolute alcohol, 100 cc. of a 5 per cent. watery phenol solution.

some instances this is not easy of achievement, since by such decolourising processes also the bacteria are liable to lose the stain, it nevertheless is possible in the majority of instances. In many cases prolonged washing in alcohol absolutus and in clove-oil is sufficient to abstract the dye from the tissue-elements, but in some special cases, owing to peculiar chemical properties possessed by certain bacteria, the decolourising process requires special methods. Of these the following are the most useful :—

1. In some instances the specimens (cover-glass specimens and particularly sections) are stained in one dye, then washed in alcohol till quite pale, then transferred to a contrast dye. As contrast dyes are to be regarded blue and red, or red and brown, or blue and brown, or violet and brown. In some cases only the bacteria retain the first dye, the tissue-elements become stained by the second dye. A similar result is often obtained by mixing the two dyes, and then using them like a single dye ; hereby occasionally the bacteria are found to take one colour, while the tissue-elements take the contrast dye.

For double-staining of film specimens or sections the following methods will be found most practicable for general purposes :—(*a*) As a first stain methyl-blue aniline water is used ; after well staining the specimen it is well washed in water and then placed in $\frac{1}{6}$ per cent. alcoholic solution of eosin for from half to one minute, then washed in water and prepared for mounting in balsam as usual ; (*b*) a 2 per cent. watery solution of rubin is used as first dye, then well washed in water, then placed in methyl-blue aniline water for half to one minute, washed in water and proceeded in the usual manner for balsam mounting.

The number of methods for successfully and differentially double and treble staining normal and pathological tissues is legion, and those who consult the excellent books by

Behrens, and by Kanthack and Drysdale, will find all they require not only with reference how to prepare the dyes and how to apply them, but particularly in what cases and for what tissues they were first employed and found most useful. Without wishing in the slightest degree to convey that those engaged in pathological work should not avail themselves to the full of every method that is recommended and that has been found useful, I venture to say here that the methods of staining, mentioned in this book, which after many years' experience have been successfully employed in my laboratory, have been found quite sufficient in bacteriological work.

2. One of the most useful methods for staining bacteria in sections of hardened tissues or in films is Gram's method. Film specimens or sections are kept for five to ten minutes in absolute alcohol, are then placed in any of the above mixtures of aniline water and dye (fuchsin, magenta, Humboldt's red or gentian-violet, methyl-blue or methyl-violet), and kept there for from two to five minutes or more ; they are then washed in alcohol for from one to three minutes, and are then transferred into the following solution : one part of iodine, two parts of iodide of potash, 300 parts of distilled water ; they are kept here till their colour completely changes (as a rule into dark purple), they are then transferred into alcohol till all colour has apparently gone. If successful, such sections when examined under the microscope, show only the bacteria stained, while the tissue-elements are quite colourless. To bring out these latter more strikingly the sections are stained in a contrast dye, vesuvin or Bismarck-brown, if red, violet, or blue has been used as the first dye.

This method is of great diagnostic value, inasmuch as it represents an important distinction between species which otherwise may be difficult to distinguish ; one kind becom-

ing decolourised by the iodine, while another retains the first dye after passing through the iodine.

3. Ehrlich's method, used specially for demonstrating tubercle-bacilli and leprosy-bacilli.—The specimens, after having been well stained with carbol fuchsin (by heating in a watchglass till the fluid begins to bubble), are transferred for 10–30 seconds into 30 per cent. watery solution of nitric acid; according to Friedländer a mixture of three parts of nitric acid in 100 parts of alcohol is equally good. A 10 per cent. watery solution of nitric acid is quite strong enough. All bacteria except the tubercle-bacilli and leprosy-bacilli lose the dye by this treatment. The preparations are then stained for contrast in methyl-blue vesuvin or Bismarck-brown.

4. Koch's method.—According to this the sections, after having been stained, are transferred to a saturated solution of carbonate of potash to which previously an equal volume of water has been added. The preparations remain here for from five to ten minutes, are then washed in water, alcohol, clove-oil, and finally mounted in Canada-balsam solution or Dammar varnish.

5. Lustgarten's[1] method, used for the demonstration of the syphilis-bacilli.—The sections are stained for from twelve to twenty-four hours at ordinary temperature, and then for an additional two hours at 40° C. in aniline water gentian-violet; they are then washed for a few minutes in absolute alcohol, and then transferred to a 1·5 per cent. solution of permanganate of potash for ten seconds, then for the same period into a watery solution of pure sulphurous acid; wash in distilled water, repeat the above process of placing the sections first into the permanganate of potash solution, then into the sulphurous acid water till they become apparently

[1] Lustgarten, *Med. Jahrbücher der K.K. Ges. d. Aerzte*, Vienna, 1885.

quite colourless. Only the syphilis-bacilli, tubercle-bacilli, and leprosy-bacilli, are able to retain the dye; other bacteria lose it by being subjected to the permanganate.

De Giacomi[1] has improved this method of decolourising by oxidation. Cover-glass specimens made of syphilis material are stained with warm fuchsin for a few minutes, are then washed in water to which a few drops of solution of iron perchloride have been added, then placed into concentrated solution of iron perchloride till the preparations have lost all colour; they are then stained for contrast in vesuvin or Bismarck-brown.

A. Gottstein[2] places sections of syphilis material for twenty-four hours in fuchsin or aniline water genetian-violet; wash with distilled water, then place them for a few seconds into a pure or dilute solution of liquor ferri, then wash in alcohol, clarify in clove-oil, mount in Canada-balsam.

It may not be unnecessary to point out, that if sections are kept for many hours in the staining fluid, there may be found in them micro-organisms (particularly bacilli) which have been accidentally introduced into them by the solutions of aniline dye. Many of these, particularly when used alkaline, contain organisms, and if the sections are kept in them for many hours, notably in warm weather, bacteria will be found to have not only invaded the tissue but to have multiplied therein.

In examining fresh or hardened tissues for micro-organisms it is necessary to make thin sections, which can be easily done with the aid of any of the microtomes in common use, amongst which Williams's microtome for ice or ether freezing, Cathcart's for simple ether freezing, Minot's microtome and the Cambridge rocker with ordinary razor for cutting riband sections from paraffin-embedded hardened materials, are easiest to manipulate. As a matter of fact we now use

[1] De Giacomi, *Schweizer Correspondezblatt*, xv. 12.
[2] A. Gottstein, *Fortschritte d. Medizin*, Berlin, 1885, No. 16, p. 545.

either Williams's or Cathcart's microtome, and above all for hardened materials, the Cambridge rocker. By this latter the most exquisite and uniformly thin sections in a riband are obtained.

As regards hardened material, it is necessary to remember that the hardening must be carried out properly, small bits —about a half to one cubic inch—of tissue being placed in alcohol, or better, in Müller's fluid, and kept there ; in the first instance, for two to five days ; in the second for from one to three weeks or more. Then small bits are cut out, of which it is desired to make sections. Those hardened in spirit must be soaked well in water to enable the material to freeze, then superficially dried with blotting-paper, and then used for cutting sections with the microtome. Those hardened in Müller's fluid are at once superficially dried with blotting-paper and cut. When making sections with Williams's freezing microtome it is necessary to soak the material first in gum mucilage and then to freeze and to cut. Fresh tissues are at once cut with the freezing microtome, the sections placed in a 0·6 per cent. saline solution, floated out and well spread out, and then stained by transferring them in this condition—*i.e.*, well spread out, into a watch-glass containing the dye. The sections of hardened tissues are floated out in water, well spread out, and then transferred to the dye or dyes as the case may be.

It is necessary to prevent too much shrinking of the sections, especially those of fresh tissues ; for this reason it is advisable to float the sections in the saline solution or water, as the case may be, on a broad lifter or spatula, to spread them well out upon it, and to transfer them carefully into the dye, then into the dish with water used for washing off the excess of the dye, to transfer them, well spread out on the lifter, to alcohol, then after several minutes to oil of

cloves, and finally on to a glass slide, on which they are mounted in the usual manner with Canada-balsam solution, the excess of clove-oil being previously drained off.

It is advisable, although not absolutely essential, to keep the sections in a well-spread-out condition for a few seconds in alcohol before placing them into the dye.

For the preparation of mounted stained specimens by the rocking microtome the following method is used in my laboratory:

Small pieces of tissue are put in Müller's fluid[1] and changed on the second and third day, are then allowed to stand for seven days, then put in two-thirds methylated spirit and one-third water, change after twenty-four hours to absolute alcohol, and allow to properly harden.

Embedding in Paraffin.

Selected piece of tissue is then treated as follows:

1. Absolute alcohol, twenty-four hours.
2. Absolute alcohol and cedar wood oil, equal parts, twelve hours.
3. Cedar wood oil, twelve hours.
4. Paraffin liquid, 50 to 52° C., bath No. 1, twelve hours.
5. Paraffin liquid, 50 to 52° C., bath No. 2, twelve hours.
6. Pour paraffin in a small box of brass or lead and place tissue in it, when set take the paraffin block out of the box, trim and fix on rocking microtome. Cut number of sections and float into warm water at 30° C. Fix sections on cover-glasses, and allow to dry in incubator at 37° C.
7. Xylol, five minutes.

[1] Glanders tissues are best placed at once in absolute alcohol.

8. Absolute alcohol, five minutes.
9. Methylated spirit, five minutes.
10. Stain specimens in selected dye.
11. Wash in water.
12. Wash in methylated spirit.
13. Absolute alcohol.
14. Oil of cloves.
15. Xylol.
16. Mount in xylol Canada-balsam.

Demonstration of flagella.—Löffler (*Centrabl. f. Bakt. und Parasitenkunde*, Bd. VI. Nos. 8, 9) has shown by a new method that flagella of bacteria can be stained. Although motile bacteria have been known or supposed to owe this motility to the presence of flagella, these have in most cases eluded demonstration, till Löffler by using a mordant of tannin and ferrosulphate solution, previous to the stain, showed with extreme clearness the actual presence of flagella even in the weakest motile bacteria. Moreover, he showed the quite unexpected and remarkable fact that while in some only one flagellum at one end is present there are others in which there are several such flagella, and even the body of the bacteria may be completely invested in flagella —*e.g.* in the case of the typhoid bacilli. Löffler's beautiful photographs created deservedly great sensation amongst bacteriologists, and a host of workers have devoted at once careful attention to the subject, hence resulted several useful modifications of the composition, reaction, and duration of action of the mordant. We shall limit ourselves to the single statement that by the flagella staining alone a diagnostic differentiation between bacillus coli and bacillus typhosus has become possible, the former possessing two to eight, at any rate a limited number of flagella, whereas the latter possesses quite a mass of wavy spirilla-like flagella ex-

tending over the whole body at each end and at right angles from the cylindrical body.

The method of flagella staining which both in Dr. Kanthack's laboratory, and owing to his initiative also in my laboratory, is used with facility and with unequivocal success, is that described by van Ermengem, which I copy from Dr. Kanthack's manual. It ought to be stated that I have seen specimens, prepared by beginners, of culture of bacillus coli and of typhoid bacilli, which showed the flagella in a manner and quantity that can without exaggeration be described as striking. But in all these cases it must be added that they were prepared without deviating in any essential point from van Ermengem's prescription. The flagella appear not as prolongations of the protoplasm of the bacteria as generally supposed, but seem to be part or outgrowths of the sheath itself.

The method is this [1] :—

STAINING OF FLAGELLA (Van Ermengem).

Prepare the following solutions :

(α) Osmic acid (2 per cent. solution) . . . . . 1 part.
Tannin (10 to 25 per cent. solution) . . . . . 2 parts.

To each 100 cc. of the tannin solution add four or five drops of glacial acetic acid.

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

(γ) Gallic acid . . . . . 5 grammes.
Tannin . . . . . 3 grammes.
Fused acetate of soda . . . . . 10 grammes.
Distilled water . . . . . 350 cc.

Boil the cover-slips to be used in the following solution :—

Potassium bichromate . . . . . 60 grammes.
Concentrated sulphuric acid . . . . . 60 grammes.
Water . . . . . 1000 cc.

[1] From *Practical Pathology*, Kanthack and Drysdale, pp. 38 and 39.

Then wash them repeatedly in water. Keep them in absolute alcohol and before use allow them to dry, without wiping, by placing them in a vertical position, protected from dust.

BACILLUS OF TYPHOID FEVER AND VIBRIO CHOLERÆ ASIATICÆ.

Carefully suspend one or two loops of an Agar-Agar culture (ten to eighteen hours old) in a watch-glassful of distilled water.

(*a*) With a single loopful of this "suspension" prepare a cover-glass film and allow it to dry in the air.

(*b*) Fix it by passing it three times through the flame, holding the specimen in the fingers, so as to avoid over-heating.

(*c*) Pour a few drops of solution (α) on the film and allow them to act for half an hour.[1]

(*d*) Wash very carefully in a large excess of distilled water, and then in alcohol.

(*e*) Now keep it for three to five seconds in solution (β).

(*f*) Without washing, pass quickly through solution (γ).

(*g*) Wash again in a fresh quantity of solution (β), moving the specimen about gently and withdrawing it when the solution begins to turn black.

(*h*) Wash it thoroughly in several changes of distilled water.

(*i*) Dry it carefully between blotting-paper.

Mount it first in water and examine it with $\frac{1}{12}$ in. oil immersion, and if the specimen be satisfactory, mount it permanently in xylol balsam.

If the flagella are not sufficiently stained, float the cover-slip off the slide and begin again at (*f*).

Care must be taken to change the nitrate of silver solution as soon as any precipitation shows itself.

This is an easy and very trustworthy method.

Mr Mervyn Gordon has succeeded in producing a uniform and perfect dark staining of the flagella of the typhoid bacilli—far more exquisite than I have seen it produced previously, by introducing in the above method the following alterations :

(*c*) Solution (α) is allowed to act for *one hour*, instead of half an hour.

(*d*) The cover-glass is left for *five minutes* in alcohol.

(*e*) It is kept for *two minutes* in solution (β).

(*f*) It is drained on blotting-paper, and left for *one and a half to two minutes* in solution (γ). [The last half minute determines the degree of staining.]

[1] At a temperature of 60° C. five minutes is sufficient.

CHAPTER II

PREPARATION OF CULTURE MATERIAL

ARTIFICIAL cultivations of micro-organisms in suitable nourishing media in the incubator (Figs. 1 and 2) at tem-

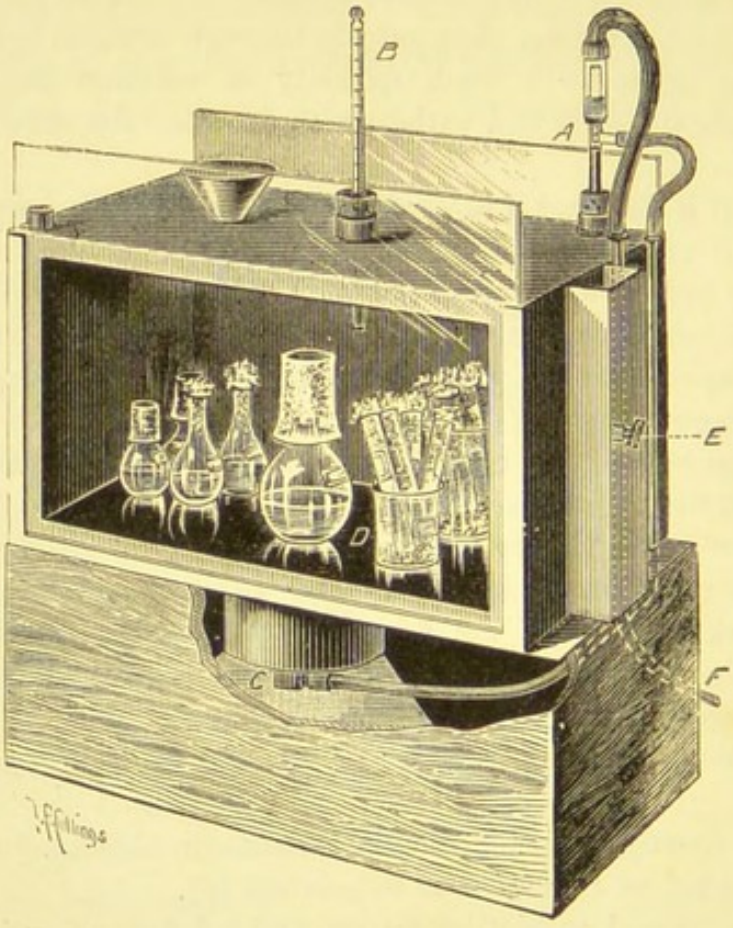

FIG. 1.—INCUBATOR, WITH PAGE'S REGULATOR.

A. Page's Regulator.—This consists of a tube filled with mercury, and immersed in the water surrounding the chamber of the incubator. In the upper part of the tube, above the mercurial column, is a fine open glass tube, having near the lower

end a fine hole. When the temperature of the water rises, the mercurial column rises, and at a certain temperature rises above the lower open end of the small inner glass tube just mentioned. If this point is reached, then the burner at *C* receives only the amount of gas that passes through the fine lateral hole of that inner glass tube. If the temperature of the water falls, the mercury falls, and the lower end of the inner glass tube becomes again free, and now the burner at *C* receives a much greater supply of gas. If so, the temperature of the water again rises, the mercury rises, obstructs the lower end of the inner glass tube, the supply of gas is reduced to what can pass through the fine lateral hole, and consequently the temperature again falls, and so on. To adjust the regulator it is necessary when the thermometer indicates the required degree of temperature to push the outer large glass tube, and with it the inner tube, of the regulator so far down that the top of the mercurial column just obstructs the free end of the inner glass. The temperature then regulates itself for the reasons stated previously. These regulators are sufficient for all practical purposes when it is not a question of small differences in temperature, since they are tolerably constant within one or two centigrades. The trouble one experiences in the working of these and other similar regulators arises from the inconstancy of the main gas supply, this, as is well known, varying within wide limits. The stopcock, *E*, obviates this to a limited extent; when this is put at an angle of 45° only a limited amount of gas passes from the main supply tube to the regulator, and therefore the variations in pressure of the gas are not felt to their full extent. A Sugg's regulator interposed between *E* and the main supply tap is very useful.

B. Thermometer to indicate the temperature in the chamber.

C. Gas burner.

D. Chamber of incubator. The front and back of the incubator is either a movable tin plate or glass covered with black paper.

E. Stopcock to regulate, when required, the supply of gas.

F. Main supply.—The upper, lower, right and left walls of the incubator are made of a double layer of tin; between the two is water. The front and back of the chamber are closed by a movable plate.

An excellent incubator for constant temperature is made by the Cambridge Scientific Instrument Company. It has a double gas supply: one small permanent flame, and a second one subject to the regulator.

peratures varying between 20° and 38° C., are necessary in order to study more accurately the life-history of the septic as well as the pathogenic organisms. Moreover, large numbers of them become available in a short time, and their relation to disease can be tested more conveniently. For if it should be found that, having carried on outside the animal body successive cultivations of a particular organism, the re-introduction of this cultivated organism into the animal body is again productive of the same disorder as before, then the conclusion becomes inevitable that this organism is intimately related to the causation of the disease. It must be conceded that after several successive cultivations in fluids any hypothetical substance supposed to be the *materies morbi*, and introduced at first from the blood or tissues, being in a very diluted condition in the

first cultivation, would after several cultivations be practically lost. But if this last cultivation should be found to act in the same manner pathogenically—*i.e.* if a small quantity of it, charged with the new brood of the organism, nevertheless

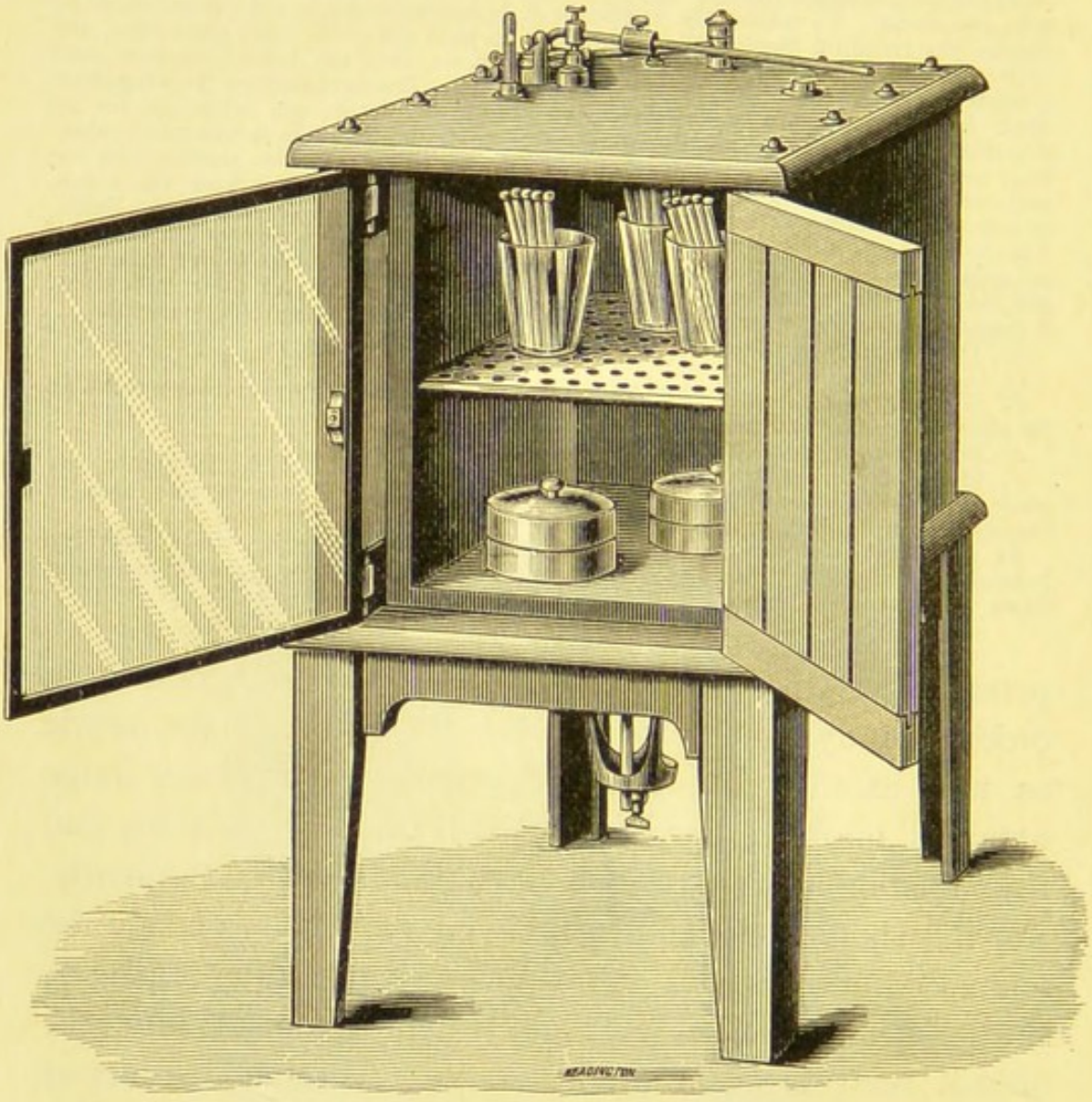

FIG. 2.—HEARSON'S INCUBATOR.
An excellent incubator for higher temperatures, as it possesses a very sensitive regulator.

possesses full pathogenic power, then it is logical to say that this pathogenic property rests with the organism. For this and other reasons it is of essential importance to be

able to carry on successive cultivations of one and the same organism without any accidental contamination or admixture—*i.e.* it is necessary to carry on *pure cultivations*.

ARTIFICIAL CULTIVATION MEDIA.

A.—Fluids.

As fluid nourishing material the following are used with preference :—

1. *Broth made from Meat—pork, beef, rabbit, chicken.*—The connective tissue and fat are first cut out from the fresh meat —in the case of rabbit or chicken the whole animal without head or viscera is used—and then placed in water and boiled. Generally for each pound half an hour's good boiling is allowed. With regard to the quantity of water, each pound of meat ought to yield ultimately at least one pint of broth. When boiled, the broth is allowed to stand, the fat is skimmed off, and the broth well neutralised, or even made faintly alkaline by adding liquor potassæ, or, better still, carbonate of sodium.

The fresher the meat the less acid (sarcolactic acid) is in the broth before neutralisation. The broth is then filtered through a filter [1] into flasks previously sterilised (see below). As a rule beef broth is clear, but if not it is filtered again. If not clear then, it is allowed to stand for several hours. A fine sediment is found at the bottom of the vessel, and from this the clear supernatant fluid is decanted into a sterilised vessel. The broth, if not clear after the first filtering, can be cleared by boiling it with the broken shell

[1] Unless otherwise stated all filtration is carried out by means of folded Swedish filter paper.

and white of egg. The now clear fluid is filtered again. The flasks which receive the broth are well plugged with sterilised cotton-wool (see below). In this state the flask is placed over a Bunsen burner on a wire netting, and boiled for half an hour or more; during the boiling the cotton-wool plug is lifted out for half its length. The flask ought not to contain more broth than about one-half or one-third of its volume, to prevent the broth from rising too much and wetting the plug. When turning off the flame the plug

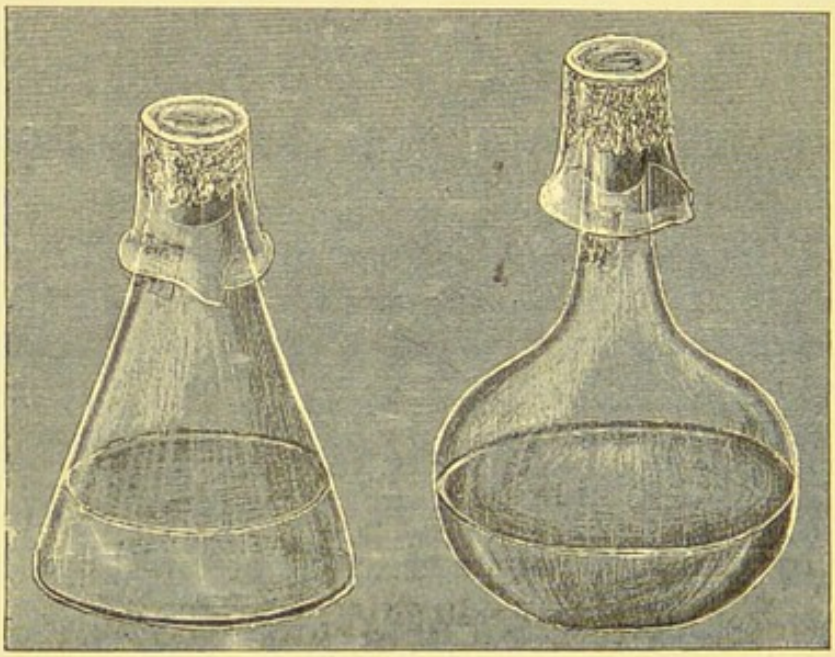

FIG. 3.—TWO FORMS OF FLASKS CONTAINING FLUID NUTRITIVE MEDIA.

is pushed down so as fully to plug the neck and mouth of the flask; a beaker with sterile cotton-wool cap is placed over the mouth of the flask, and this is allowed to stand for one night. Next day the boiling is repeated for half an hour or more in the same manner as before. If the meat has been fresh and the vessels and cotton-wool have been sterile, twice boiling is found sufficient to destroy every impurity. But to make sure, the broth is placed in the incubator and kept there for twenty-four hours at a tem-

perature of 32° to 38° C., and then boiled on the next day for half an hour in the usual way. The supposition is made, that if by any chance after twice boiling the broth it should contain unchanged spores of bacilli—the only organisms that will resist boiling, although they do not resist boiling for more than several minutes—the spores would germinate into bacilli when kept for twenty-four hours in the incubator at 32° to 38°, and these would then be killed by the third boiling. As a matter of fact I have not as a rule found any contaminating germs survive the second boiling. It is of course to be borne in mind that during the first as well as second and subsequent boiling the cotton-wool plug is not removed from the mouth of the flask, but is only raised out half its length from the neck. The cotton wool and the cotton-wool cap and beaker are replaced immediately or simultaneously with the turning off of the burner.

2. *Peptone and Salt Solution.*—Beef peptone (Savory and Moore's) is dissolved in distilled water, over a burner, to the amount of 1 per cent.; to the solution is added common salt to the amount of 0·5 per cent.; so that every 100 ccm. of the fluid contains one gramme of peptone and ½ gramme of salt. When dissolved it is made faintly alkaline, and then filtered (the vessels being of course also in this, as in all other cases, sterilised by heat).

A 10 per cent. peptone, 5 per cent. salt solution, represents a useful stock, because it can be kept as a smaller bulk, and used by dilution if large quantities of 1 per cent. peptone be required.

For general use where broth is required as culture fluid, the above stock-broth, plus 1 per cent. peptone and 0·5 per cent. salt, represents an excellent fluid, the *nutrient broth*; it is of course made faintly alkaline after the peptone is added

and dissolved, then boiled, and either kept in the flask as stock or decanted into sterile test-tubes to the amount of 5–10 cc., plugged with sterile cotton-wool. These are steamed (see below) on two successive days, each time for twenty minutes.

This nutrient broth, with 5–8 per cent. pure glycerin represents *glycerin broth.*

3. *Buchner's Fluid.*—10 parts of Liebig's extract, and 8 parts of peptone, in 1,000 parts of water.

4. *Hydrocele Fluid* (Koch).—A new or well sterilised trocar and cannula are used for the tapping; to the cannula is fixed an india-rubber tube that has been soaking in strong carbolic acid solution for forty-eight hours. The distal end of the tube is introduced carefully and rapidly into the neck of a sterilised flask plugged with sterile cotton-wool, and the fluid thus allowed to flow into the flask to about two-thirds of its volume. This fluid is then decanted into sterile test-tubes (plugged with sterile cotton-wool), each tube receiving about 5 to 10 ccm. The tubes are then exposed in the incubator to a temperature of from 55° to 60° C. for two to three hours on two or three consecutive days.

Ascites fluid is obtained in the same way.

5. *Blood Serum* (Koch).—A glass cannula and india-rubber tubing are soaked for forty-eight hours in strong carbolic acid; the cannula is tied into the carotid artery of a healthy horse, and the arterial blood, after opening the clip at the proximal end of the artery, is allowed to flow into sterile flasks, or cylinders with stoppers. After letting the blood stand for 24 to 48 hours in a refrigerator or in an ice-box, the serum is taken off by means of large sterile glass pipettes and introduced into sterile test-tubes, each receiving about 5 to 10 ccm. The test-tubes, plugged with sterile cotton-wool, are then exposed in the incubator to a

temperature of 58° to 62° C. in the same manner and for the same time as the hydrocele fluid was.

Blood of ox or sheep obtained in the slaughter-house is the blood from which generally "serum" is obtained; it is received into sterile glass vessels, and treated in the same way as just described.

6. *Urine* is neutralised and sterilised by boiling for 20 to 30 minutes like broth.

7. *Milk* (pure or better separated) is sterilised by gentle and careful steaming for 20 to 30 minutes on three successive days.

8. *Whey* is now also used as such, or better as an admixture to gelatine or agar; in either case it can be easily sterilised by steaming.

Of less common use are :—

9. *Pasteur's Fluid.*—In 100 parts of distilled water are dissolved 10 parts of pure cane-sugar, 1 part of ammonium tartrate, and the ash of 1 part of yeast.

10. *Cohn's Fluid.*—100 ccm. of distilled water, 1 gramme of ammonium tartrate, no sugar, and instead of the ash of yeast are substituted (A. Mayer) 0·5 gramme of potassium phosphate, or 0·5 gramme of crystallised magnesium sulphate, 0·05 gramme of (tribasic) calcium phosphate. These two fluids are sterilised in the same manner as the broth and peptone solutions. Pathogenic organisms do not thrive in either of these two fluids.

B.—Solids.

The solid media have the great advantage over the fluids that in the former artificial cultures can be carried out more easily; as, owing to the resistance the solid basis offers to

the growth of the organisms, they remain more limited to the spot or spots on which they are sown, and therefore can be watched more easily; besides, an accidental contamination—*i.e.* a growth appearing at a spot at which no sowing was made, can be recognised at once. These advantages are perhaps of the greatest use when it is intended to grow the organisms on a surface exposed to the influence of air—of course protected from contamination with other organisms.

These advantages of solid media have been very minutely pointed out by Koch in his researches on pathogenic bacteria.[1]

As solid media are used :—

1. *Slices of Boiled Potato or Boiled White of Egg or Paste* (Fokker, Schröter, Cohn, Wernich).—A boiled potato or a boiled unshelled egg is cut in half with sterile scalpel, and the cut surface is inoculated. Immediately after, it is placed on a clean glass plate and covered with a bell-glass, the edges of the latter being fixed on the former by vaseline or grease, the chamber is kept moist by a piece of wet blotting-paper being placed inside the bell-glass, or a glass capsule covered with another, both sterile, receive the potato. The progress of growth of a particular organism or of different organisms sown at a particular spot or line on the surface of these substances can be easily watched with the unaided eye.

Blocks of potato cut with a sterile cork-borer from a clean-cut potato are placed into test-tubes over a cushion of sterile cotton-wool, the test-tubes are then plugged and steamed on two successive occasions for 20 minutes each time.

2. *Gelatine* (Brefeld, Grawitz, Koch).—This is used advantageously as a mixture with broth, peptone, beef-extract,

[1] *Mittheilungen d. k. Gesundheitsamtes*, i. 1881.

blood serum, or hydrocele fluid. Koch, who introduced this mixture, used it for the cultivation of bacteria on solids, to be exposed to the air; the proportion of gelatine in the mixture was 2 to 3 per cent. But this mixture, although solid at ordinary temperature, does not keep solid in the incubator, not even at 20° C. I have found that at least 7·5 per cent. of gelatine must be contained in the mixture to keep it solid at 20° to 25° C. Above this last temperature not even 11 per cent. gelatine will keep solid.

Nutrient Gelatine, most useful for the growth of all kinds of bacteria, is prepared in this way: —

One pound of lean beef is cut up, to it is added one pint of water, and is kept boiling in the digester or any other vessel for from half to three-quarters of an hour. After having been strained through fine calico it is filtered through paper into a beaker; bring up by adding water to 600 ccm.; add to this 60 grams of the finest gold label gelatine cut up in small pieces, 6 grams of peptone, and 6 grams of common salt. Dissolve on waterbath, but do not let the water boil; neutralise with carbonate of soda or, better, liquor potassae till faintly alkaline; steam for half an hour, filter by hot filter (see Fig. 6) into a sterile flask plugged with sterile cotton-wool, and bring it up to boiling point, at which it is kept for a few minutes. This can be kept as stock gelatine, or can be decanted at once into sterile test-tubes plugged with sterile cotton-wool. These are steamed on two successive days for 20 minutes each time. Keeps solid up to about 25° C.

Prepared in this manner the nutrient gelatine passes easily and comparatively rapidly through filter paper on hot filter.

The same 10 per cent. nutrient gelatine can be of course obtained if broth is already made—*e.g.* broth in a stock flask, by adding the above-named quantities of gelatine,

peptone, and salt to 600 ccm. of the broth; further process is as above.

It is this gelatine which I generally use as "nutrient gelatine," not Koch's meat infusion gelatine, for I find that beef decoction gelatine as prepared above is conspicuously a better nutritive medium than the meat infusion gelatine prepared after Koch's method.

3. If it is necessary to expose the cultivation to higher temperatures than 25° C., the nutrient gelatine cannot be used as a solid medium. Solidified blood serum, or solidified hydrocele fluid, or solidified ascites fluid, or solid Agar-Agar mixture (Koch) must then be employed.

The first—*i.e.* the serum of blood, the hydrocele fluid, and ascites fluid—can be made solid by heating them in tubes (see page 51) *gradually* up to 68°, 70°, or 71° C. When this temperature is reached the material soon turns solid, losing slightly its limpidity, but when solidified with slanting surface (see serum inspissator) is sufficiently transparent for all practical purposes. By heating it rapidly, or heating it above 72°, it becomes solid, granular, and opaque. Of course, once thus made solid it cannot be liquefied again, and therefore must be already contained in the vessels (test-tubes and small flasks) in which the growth of organisms is to be carried on.

4. *Löffler's serum*, very useful for cultivation of the diphtheria bacillus on which this microbe grows with predilection, is composed of two parts of blood serum and one part of faintly alkaline beef broth; the fluid contained in sterile plugged test-tubes is sterilised and solidified with slanting surface in serum inspissator just like ordinary blood serum.

Serum solidified with slanting surface always shows on cooling a small amount of "condensation water"; but this

can be easily driven off by placing the test-tubes in a slanting position in the hot incubator (37°) and leaving them here for a few days. Owing to the large surface of evaporation the condensation water is soon got rid of.

5. *Kanthack's serum* is a mixture of solidified ascites fluid and Agar, which far surpasses all other media for the isolation of the diphtheria bacilli, even when in a given material these latter are almost swamped by other microbes : the cultivation of this material rubbed over the slanting surface of this serum (solidified) will at twenty-four to forty-eight hours' incubation show and pick out in a remarkable manner the colonies of the diphtheria bacilli in almost pure culture. It is prepared thus : Ascites fluid is received by sterile trocar and tubing into a sterile flask plugged with sterile cotton-wool. For each 100 cc. of ascitic fluid to be treated, take 2 grams of Agar-Agar and treat as follows : 2 to 3 cc. glacial acetic acid in 500 cc. of distilled water ; put Agar in this solution and allow to soak for thirty minutes, wash in several lots of tap-water and finish with distilled water, thoroughly drain. For each 100 cc. of ascitic fluid add 2 cc. of a 10 per cent. solution of caustic potash, and very thoroughly mix. Add this to the Agar, now add 6 per cent. of glycerine, and place in steamer and steam at 100° C. for one and a half hours ; filter through Chardin's filter paper, decant into tubes and steam at 100° C. on three successive days for thirty minutes.

6. *Nutrient Agar-Agar.* — "Nutrient Agar" is Japan isinglass. This was first used for preparation of solid culture medium by Koch. The best and quickest mode of preparing nutrient Agar is the following modification of Tischutkin's method described in Schenk's *Elements of Bacteriology*, English translation, p. 44. It is the nutrient Agar which I now use, being easily and quickly pre-

pared and of considerable transparency. The method is this :—

Twenty grams of *Agar strips* are placed in 500 cc. of distilled water in a flask to which are added 2 cc. glacial acetic acid ; in this the Agar is allowed to soak and swell up for fifteen to twenty minutes ; then, after pouring off, it is well washed in tap-water, and finally distilled water ; the fluid is well drained off. After this process the Agar is easily soluble ; to it (in the flask) are now added of (*a*) the ordinary beef broth (above stock broth) 600 cc. ; this is boiled for thirty minutes, in which time all the Agar has dissolved. To this solution is added the following mixture (*b*) consisting of 400 cc. of broth, 10 grams of solid peptone and 10 grams of solid salt ; the whole is now made slightly alkaline with liquor potassae and clarified with white of egg. After mixing well up the flask is put into the autoclave and kept therein at 120° C. for fifteen minutes ; it is then filtered through (folded) Chardin filter on the "hot filter." This Agar broth mixture is beautifully clear and limpid and filters rapidly—one liter per hour, a great advantage over other Agar preparations. This nutrient Agar contains then 2 per cent. Agar, 1 per cent. peptone, 1 per cent. salt all dissolved in beef broth. It is decanted into sterile test tubes, steamed on two successive days each time for twenty minutes ; when allowed to cool becomes solid. It can be solidified with slanting surface by being placed on the special tray. When quite solid with slanting surface a small amount of "condensation water" accumulates at the bottom of the tube.

7. *Grape Sugar Gelatine* and *Grape Sugar Agar* are the media best suited as solid media for the growth of anaerobic microbes. The first is the nutritive gelatine as above described, *sub* 2, but containing 2 per cent. of grape sugar.

In order to avoid the brown colour, which the nutritive gelatine assumes when the grape sugar is added, and after heating it, it is necessary to prepare a watery solution of the required amount of grape sugar separately, to add this to the solution of gelatine, peptone, and salt in beef broth, then to make the whole alkaline and steam. The grape sugar Agar differs from nutrient Agar described *sub* 6, in containing 2 per cent. of grape sugar added in substance to the Agar mixture before putting into the autoclave.

8. *Glycerin Agar*, first used by Roux and Nocard for the cultivation of the tubercle bacillus. It is, however, not only useful for this, but also for other microbes. It is the nutrient Agar mixture, *sub* 6, to which 5 to 8 per cent. pure glycerin has been added before putting the mixture into the autoclave.

CHAPTER III

VESSELS AND INSTRUMENTS USED IN CULTIVATIONS

ALL instruments, *vessels* (flasks, test-tubes, beakers, cotton-wool, filters, calico) to be used are first thoroughly sterilised by heating. In the case of flasks and test-tubes, this can be done by exposing them thoroughly in *all parts* to the open flame of a large Fletcher's burner; *while thoroughly heated* the mouth is plugged with a good long plug (1 to 2 inches) of sterile cotton-wool, this being pushed in by means of sterile forceps. The plug in all cases must not be loose, but also not too firm—an error in the latter direction being of course preferable to one in the former. Or the flasks and test-tubes, instruments, cotton-wool, &c., are placed in an air-chamber (see Fig. 4) heated by a large Fletcher's burner for several hours, up to between 130° and 150° C. In the case of small flasks, test-tubes, and cotton-wool this process is of course much more convenient, since a large number can be heated simultaneously. Beakers and glass filters to be used merely for a temporary operation are placed over a wire net on a tripod and heated by the flame of a Bunsen's burner. In the case of test-tubes which are to receive cultivation-fluids, I generally expose them, after having been cleaned with strong acid, washed out with water and dried

in the air-chamber for several hours (three to six) to a temperature of from 130° to 150° C. : while hot they are taken out *seriatim*, plugged with the sterile cotton-wool, and replaced in the air-chamber, and heated again for several hours. All this, and other operations to be described below,

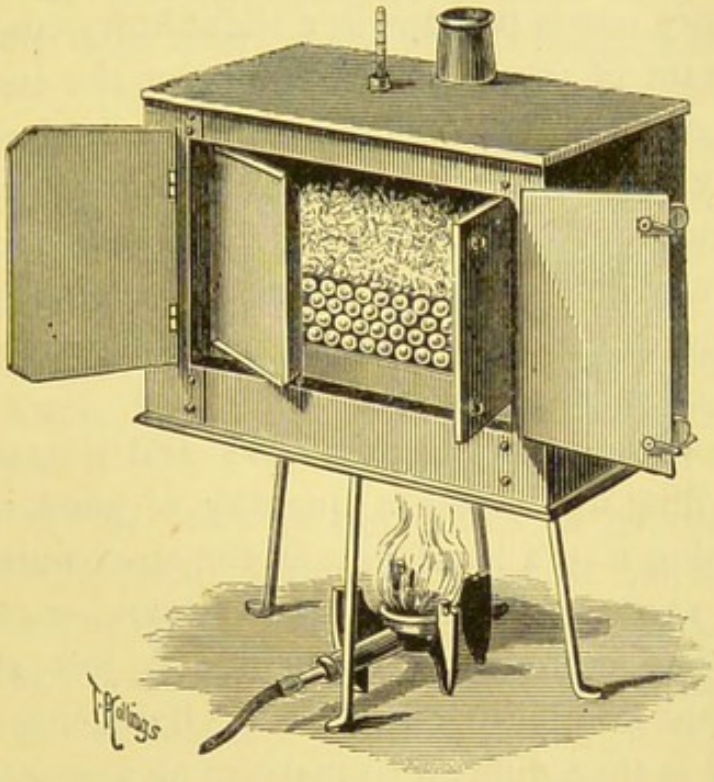

FIG. 4.—HOT AIR-CHAMBER FOR STERILISING TEST-TUBES, COTTON-WOOL, &C.

An iron chamber with double wall, the inner chamber having separate folding doors. In the inner chamber are placed the test-tubes, glasses, &c., and the cotton-wool, the latter in a loose condition. Both sets of doors are closed, and the apparatus heated by a large Fletcher's burner. A thermometer passing from the inner chamber through the upper wall indicates the temperature of the chamber. The hot-air apparatus can, according to the requirements of the laboratory, be constructed of larger size than the one here depicted. I use one that is made four or five times this size and is divided into several compartments.

may appear to some rather tedious and unnecessarily complicated, but it cannot be too strongly insisted on that in these matters one cannot be too scrupulous. A slight relaxation may, and occasionally is, followed by disastrous consequences in the shape of accidental contamination, and consequent loss of materials prepared at the cost of much

labour and time. Long experience in these matters has taught me that, although in some instances less scrupulous care has not been followed by bad results, still I have seen occasionally unpleasant failures owing to slight laxity in these matters.

Several weeks' work may be annihilated by a single omission. Sometimes one is perhaps in a slight hurry, and does not think the want of an additional heating of the test-tube or cotton-wool or an additional boiling of the fluid will be followed by any bad consequences. But, alas, nature does not take into account our convenience, and failure is our reward. If in any kind of experiments "overdoing" is an error in the right direction, it is in these very experiments in the cultivation of micro-organisms.

The *cotton-wool* used for plugging flasks and test-tubes is prepared by pulling up loosely a quantity of good cotton-wool and exposing it in a loose state in the air-chamber to a temperature of 130° to 150° C. *for several hours on two successive days.* The cotton-wool ought to be just slightly brownish—*i.e.* just faintly singed. Too much singeing makes it brittle, and it is then difficult to make of it a satisfactory plug. The plug used should not be too firm and not too loose : in the former case it is not easy to lift it up quickly, and in the latter it does not close sufficiently well. Cotton-wool that has been kept in the air-chamber for an hour or two is not absolutely sterile ; nor is cotton-wool that has been kept in a compressed state in the air-chamber for several days. The central portions remain under these conditions quite white and are not sterile. No cotton-wool that is not just brown—*i.e.* just faintly singed—is safe from risk of impurity. No cotton-wool steeped in absolute alcohol, strong carbolic acid, or any other disinfecting fluid, for ever so many days or weeks, can be absolutely relied on.

As stated above, a plug of sterile cotton-wool tolerably firm, of about one to two inches, is used for the plugging of the flasks and test-tubes. An assertion such as that made by Dr. Williams at the British Association (Biological Section, September 1883), that cotton-wool plugs are not reliable, because they do not protect the fluids in the vessels plugged with them from accidental air-contamination, is to be accepted only as applying to very loose plugs and to cotton-wool not properly sterilised. To good firm plugs of sterile cotton-wool it evidently cannot apply, since all the results of all workers in this field (Pasteur, Sanderson, Cohn, Koch, Klebs, Buchner, and many others) are against it.

FIG. 5.—A CAPILLARY PIPETTE, DRAWN OUT INTO FINE POINTS; LENGTH ABOUT 12 TO 14 INCHES.

Instruments, such as the points of needles, and forceps, used in the processes of cultivation, lifting up cotton-wool plugs, making cotton-wool plugs, inoculations, &c., must be heated in the open flame of a Bunsen burner, if they are to be absolutely relied on for cleanliness. Scissors and knives used for cutting tissues which are intended for inoculation, ought to be likewise scrupulously clean. One ought to keep a special set of instruments in a metal box, the whole capable of being sterilised in the hot air-chamber.

Syringes used for cutaneous, subcutaneous, or other inoculations, ought to be capable of being sterilised by heat. The ordinary Pravaz syringe of vulcanite not being capable of undergoing this process, Koch has devised a glass syringe similar to the Pravaz syringe. I do not use any syringe for inoculation

of small quantities, but prefer using each time a fresh *capillary glass pipette* made just before the inoculation. Into this pipette I draw the small quantity to be used for inoculation, and having made a very small puncture

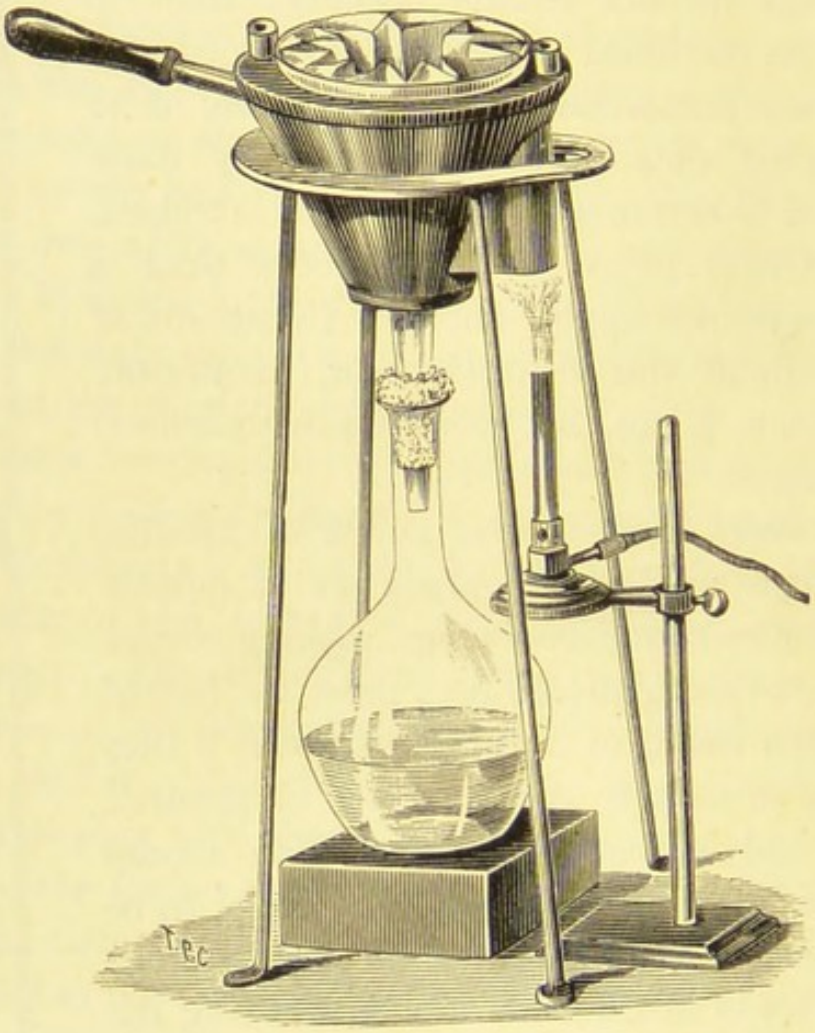

FIG. 6.—HOT-WATER FILTER FOR FILTERING NUTRITIVE GELATINE OR AGAR-AGAR MIXTURE.

through the skin, the pointed end of the pipette is pushed through it into the subcutaneous tissue for about half an inch or one inch and then the fluid is blown out into the tissue. In this way I am always absolutely safe from any contamination with a previously used virus, which might

possibly adhere to one or other part of a syringe not thoroughly sterilised.

The fine point of capillary pipettes (Fig. 5), used for in-

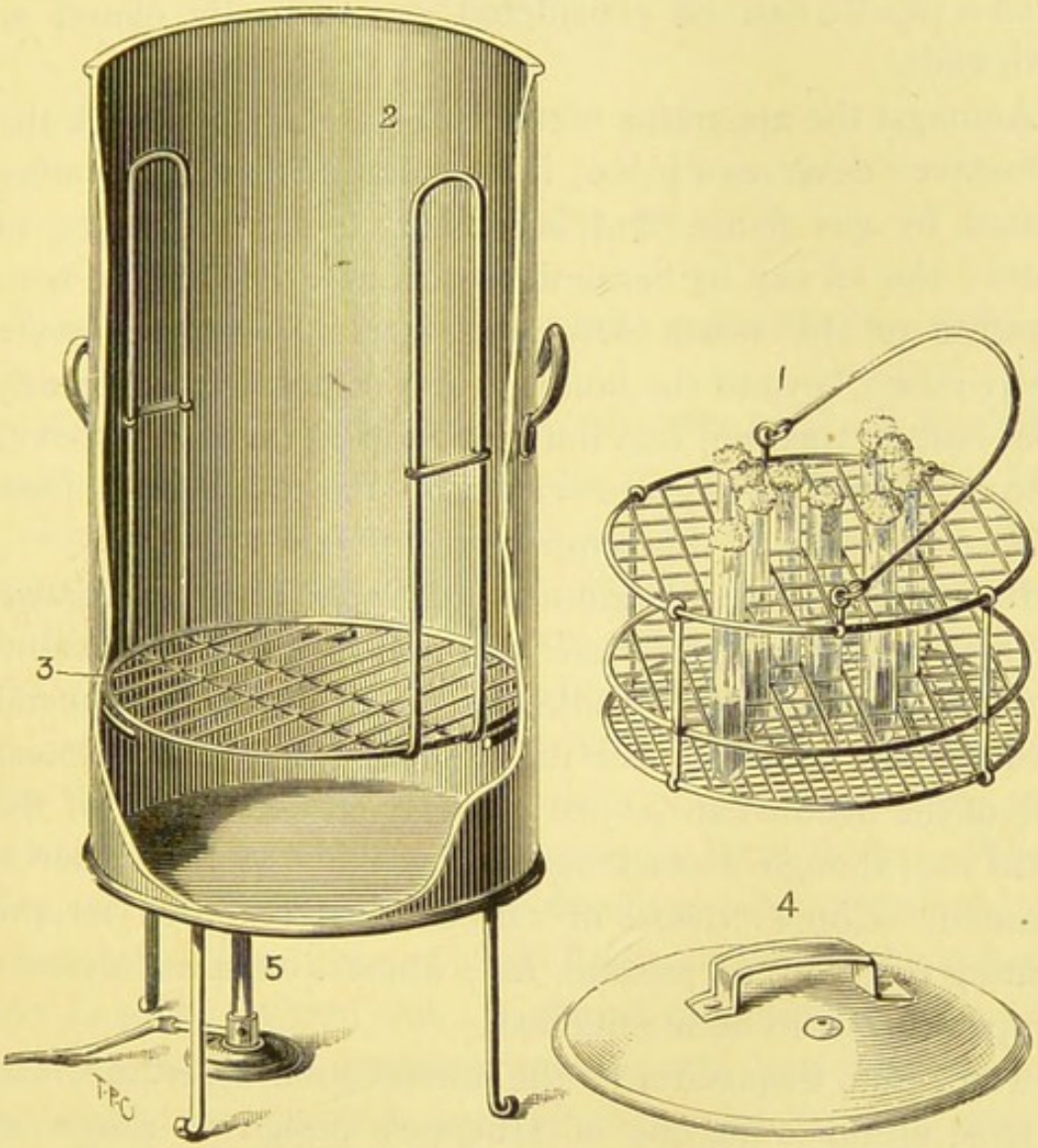

FIG. 7.—STEAMER FOR STERILISING CULTURE MATERIAL CONTAINED IN TEST-TUBES.

1. Wire-net to hold the test-tubes; 2. Tin vessel; 3. Wire diaphragm to hold 1; underneath it is water; 4. Lid; 5. Gas-burner.

oculation of animals, or for drawing out a drop of fluid of a cultivation in a flask or test-tube, or for inoculating material contained in a test-tube or flask, are thus made: while one

hand holds the bulb of the pipette, the other holds one end, and putting at some distance from this end the tube into an ordinary flame and quickly drawing it out, a point of extreme fineness can be made. The same is done with the other end. Such a pipette can be considered as practically closed at both ends.

Amongst the apparatus useful in bacteriological work the autoclave [1] deserves a place; it is a cylindrical metal chamber heated by gas flame, and containing a small amount of water; the lid can be hermetically screwed down; the temperature of the steam developed inside is under pressure easily raised beyond the boiling point of water, as when any fluid culture medium (*e.g.* nutrient Agar as described above) is to be heated to say 110–115° C.; one atmosphere pressure corresponds to this temperature of 110–115° C.

Platinum needles, platinum loops, or platinum lancets two to three inches long are fastened (melted) either in glass rod handles or in wooden handles by means of a long metal cylinder; in the latter case the sterilising by heat of the near end of the needle can be just as easily carried out as of the glass rod, though a cracking and breaking of this latter is avoided. Copper ovens of various sizes are used for the heating (melting) of paraffin, &c., where a constant definite temperature is to be maintained.

The serum inspissator for the solidification of serum which is most useful is the one of Hueppe's design as shown in Fig. 10.

Trays of wood or tin are useful for obtaining gelatine, or Agar tubes with large slanting surface during cooling (setting) are shown in Fig. 9.

[1] Sold by Wiesnegg in Paris.

CHAPTER IV

PREPARATION OF CULTURE-MEDIA FOR INOCULATION

We have on a former page described the methods to obtain sterile stock of nourishing media suitable for artificial cultivations. Those media which are to be used in a solid state must, before solidification, be contained in test-tubes and small flasks, sterilised and solidified in the manner above described, so as to be ready for establishing cultures—*i.e.* for inoculation. The Agar-Agar mixture however can, like broth, peptone mixture, beef extract solution, and gelatine mixtures, be kept as stock in large flasks. When thus sterile these latter can be decanted when required into a number of test-tubes or small flasks, in which the cultivation is to be carried out. Gelatine mixtures (gelatine and broth, gelatine and peptone, gelatine and beef extract) and the Agar-Agar mixtures, must of course be liquefied over a flame before being ready for decanting. The test-tubes most suitable are about six inches long, and should not be less than about $\frac{3}{8}$ to $\frac{3}{4}$ inch broad; the flasks are of the capacity of one, two or more ounces, and ought to have a neck of compararively good width. The test-tubes receive the fluids for about one and a half to two and a half inches in depth—more (up to four inches) for anaerobic cultures—

the flasks for about one-fourth to one-third of their bulk. All these test-tubes and flasks with their cotton-wool plugs, before receiving the material, should be thoroughly sterilised by heating. As I mentioned in the previous chapter, this ought to be well borne in mind, for starting with a sterile nourishing fluid—*i.e.* one that has been kept in the stock flask for several days to several weeks in the incubator at a temperature of from 32° to 38° C. and that has remained perfectly clear and limpid—and working with thoroughly sterilised test-tubes and cotton-wool plugs—very little care is required to obtain sterile material ready for inoculation. To start with a stock of nourishing material, however well sterilised, and to decant it into test-tubes with cotton-wool plugs not absolutely sterile must lead to failure. I have seen this happen over and over again, and all the material decanted became consequently contaminated and thereby useless for inoculations. The test-tubes, glass dishes, and flasks must be well cleaned with strong acid, then well washed with water, then dried, placed in the hot air-chamber, and kept there exposed for several hours to a temperature of from 130° to 150° C., or they may be thoroughly heated in all parts over the open flame of a gas-burner. The test-tubes and flasks are plugged by means of sterile forceps with the cotton-wool which is just faintly brown, and then replaced in the air-chamber and again heated up to a temperature of 130° to 150° C. To decant sterile stock fluid into these test-tubes and flasks I proceed thus: A clean beaker with spout, covered with a clean glass plate, is placed inverted on a net on a tripod over the flame of a Bunsen burner, and thoroughly heated for half an hour or so; then it is allowed to cool, and when cool the plug of the stock flask is lifted with forceps, and some of the sterile fluid quickly poured from the flask into the beaker. The plug is replaced in the

neck of the stock flask and the beaker covered with the glass plate. Of course the quantity poured into the beaker should be large enough to supply the required number of test-tubes or small flasks. The stock flask containing still some fluid, having been opened for however short a time, has of course been exposed to air-contamination, and therefore must be treated accordingly, if the fluid left in it is to serve as sterile nourishing material on a future occasion. Consequently it is subjected to boiling for from fifteen to thirty minutes.

Next, the fluid that has been poured into the beaker (covered with the glass plate) is poured as quickly as possible into the test-tubes, one after the other, by lifting with sterile forceps the plug and pouring in the fluid to a depth of one and a half to two and a half inches, and the plug replaced.

During this procedure contamination with air-organisms, if there be any about, becomes inevitable. To lessen this chance as much as possible, it is necessary to lift the plug with sterile forceps, to pour the fluid as rapidly as is practicable into the test-tube or flask, and to replace immediately the cotton-wool plug. Further, it is necessary to bear in mind that the atmosphere is not at all times and everywhere equally contaminated (see Prof. Tyndall's observations). I generally avoid undertaking this process on windy days, and when I do it, I generally close windows and doors and keep the air in the room as still as possible. I do not do it in a room in which recently (say an hour or two previously) the floor, walls, or tables have been swept.

I have opened under these conditions the plugs of test-tubes containing sterile material, and purposely exposed them for a time varying from one to ten seconds, and I have not seen more than from 1 to 2 per cent. contaminated.

Now, having filled the required number of test-tubes and flasks with the required quantity of fluid, I subject these *seriatim* to boiling. By means of an ordinary test-tube

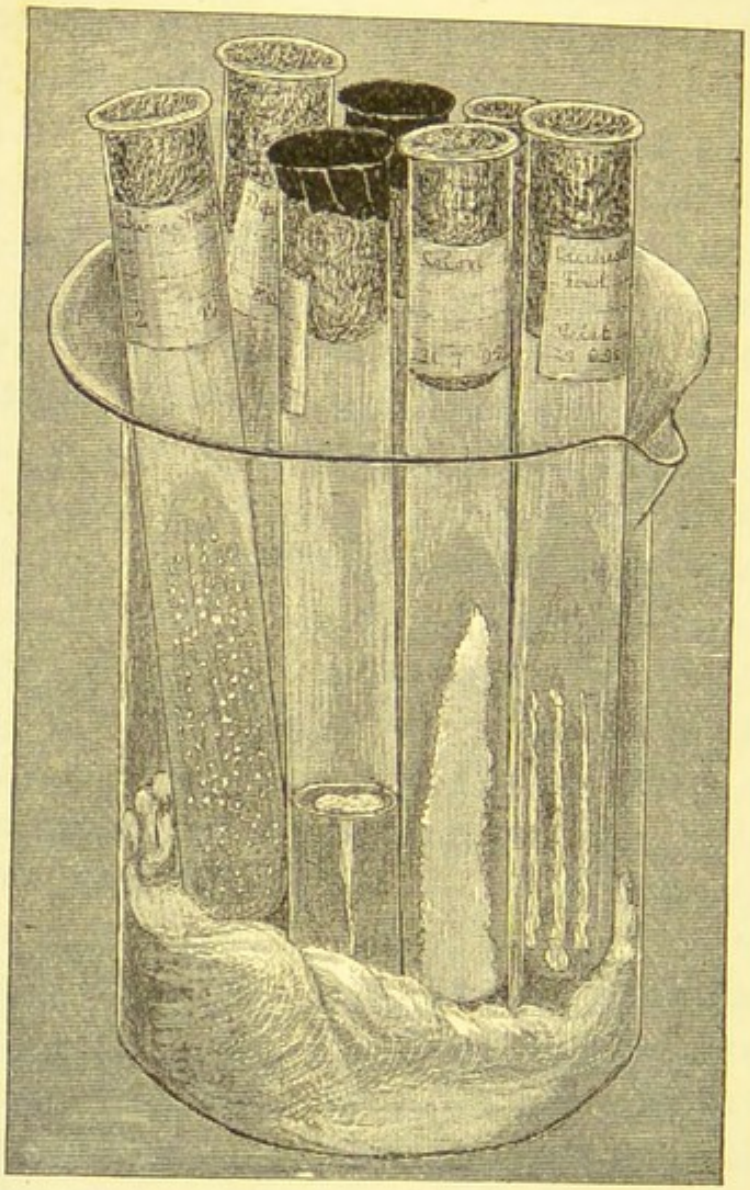

FIG. 8.—A BEAKER CONTAINING A NUMBER OF CULTURE-TUBES PLUGGED WITH COTTON-WOOL.

holder I hold them above a very small flame until the fluid boils, and keep it so boiling for from half to one minute. During this process of boiling the cotton-wool is only slightly pulled up, and immediately before ceasing to boil the plug

is again replaced, and pushed down with sterile forceps. Then the test-tube is placed (of course upright) in a beaker at the bottom of which a layer of cotton-wool—a sort of cushion—has been placed. When finished, the test-tubes in the beaker are all transferred to the incubator and kept there for from one to three days, and all those in which the fluid has remained limpid and clear are considered sterile and ready for use. As a rule, starting with sterile stock fluid, and using thoroughly sterile test-tubes and cotton-wool plugs, after once or twice boiling after decanting there ought to be no loss of tubes through accidental contamination with air-organisms (during decanting). Sometimes, however, I have had loss to the amount of 5 per cent. or more, but then there was always a hitch of some kind traceable. To decant under carbolic acid spray is not necessary or practicable, and possesses many unpleasant drawbacks, besides, in some instances when I used it there was really a greater percentage of contaminated tubes than without it.

A simple method and now generally used is to subject the whole number of test-tubes or flasks into which the nutritive material had been decanted (broth, peptone broth, potato, milk, alkaline serum agar, nutritive gelatine, Agar-Agar mixture) to a steamer (see Fig. 7). The test-tubes are placed into the wire net (see figure), the top of the group of test-tubes is covered with tinfoil, so as to protect the plugs from becoming wet, and then the wire net is placed into the steamer—the water at the bottom of which has been previously heated to boiling,—the lid is put on and the steaming is kept up for from fifteen to twenty minutes. This is repeated on one or two successive days. I have not seen any tubes go bad, after they have thus been steamed on three successive days each time for twenty minutes. Placed in the incubator and kept at a temperature of 35°

to 38° C., from some days to one or two weeks, they remain free of any growth.

Test-tubes containing solid nourishing material are for the purpose of large culture surface, kept sufficiently

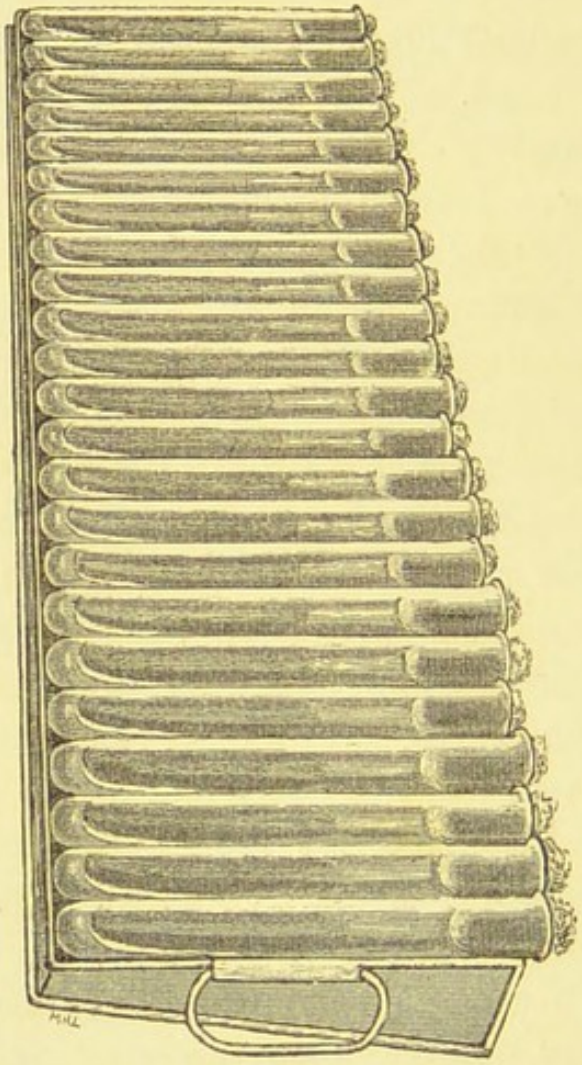

FIG. 9.—TRAY OF TIN USED FOR SOLIDIFYING GELATINES OR AGAR WITH SLANTING SURFACE.

inclined during solidification of the material to allow the material to spread into a layer of large area.

When test-tubes with sterile fluid blood-serum are to be subjected to the process of solidification, it is advisable to

keep the tubes in a slanting position, so as to allow the serum to spread out into a layer which is sufficiently

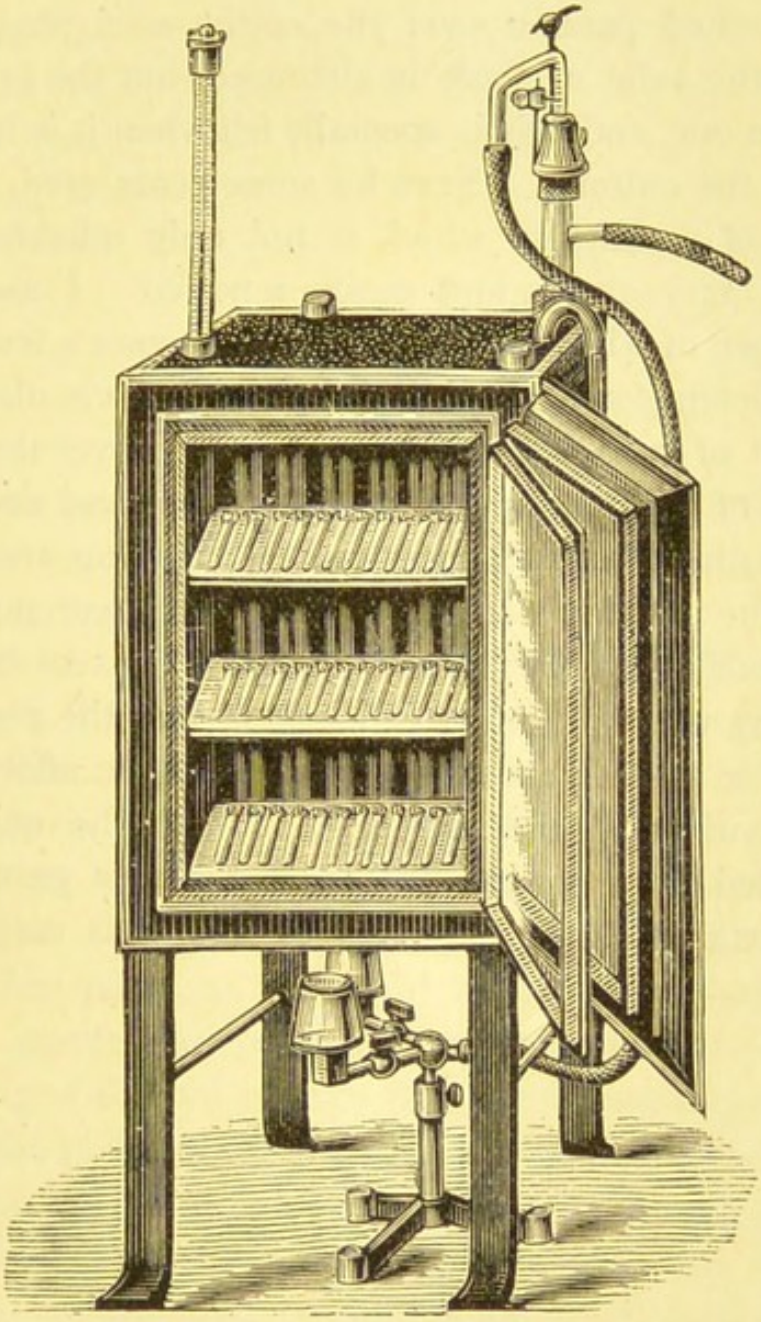

FIG. 10.—HUEPPE'S SERUM INSPISSATOR. (Made by Lautenschläger in Berlin.)

transparent even after solidification. For this purpose Hueppe's serum inspissator is generally used.

In order to keep and protect cultures in tubes or flasks

from drying up, it is necessary to seal them up or to cap them. The well-known indiarubber caps fulfil this purpose; unfortunately they are relatively expensive. Pouring a layer of melted paraffin over the cotton-wool plug of the mouth of the tube or flask is also used, but the process is not a clean one, and this is specially felt when it is intended to re-open the culture. I have for some years used, instead, a method of sealing up which is not only reliable, but is clean and very cheap, and easily renewed. I use gutta-percha paper, of which a whole sheet only costs a few pence, and is sufficient for several dozens of tubes; circular pieces are cut out of the sheet sufficiently large to cover the mouth and neck of the culture-tubes; the mouth and neck, after burning in the flame the upper part of the plug, are slightly warmed, the circular piece is neatly placed over it and the outer part of the piece pressed on to the glass of the neck, if necessary warmed so as to stick well, with the pressure of the finger a complete air-tight closure can be effected. It can again, when it is required to re-open the culture, be easily pulled off and then replaced by a new gutta-percha cap. As stated above this mode of closure is easy, cheap, and neat.

CHAPTER V

METHODS OF INOCULATION

HAVING now in test-tubes and small flasks sterile material ready for inoculation, it is necessary to describe the mode of inoculating the same.

1. *Inoculations from Artificial Cultures.*—The first and simplest is the case where it is required to inoculate a new tube or flask with a definite organism that has been growing previously in a culture-tube; that is to say, where it is required to establish from an artificial cultivation a new subculture. Take a freshly drawn-out capillary pipette, with a fine point, as described in a former chapter; draw up with sterile forceps slightly the top part of the cotton-wool plug of the old tube or flask, push carefully and gently one of the pointed ends of the capillary pipette—the other can be broken off blunt—through the remaining part of the cotton-wool plug, and push it downwards till it emerges into the culture-fluid, or, if this be solid material, till it reaches the spot or place where the organism is growing; allow a small droplet to ascend into the capillary pipette, which it readily does by capillarity; or if a larger quantity is required draw it up by gently sucking at the outer end of the capillary pipette. Then draw the capillary pipette

altogether out of the tube and cotton-wool plug, and push this latter down with the forceps into its former position. Immediately after this proceed to inoculate the new culture-tube by doing exactly the same as before—viz., draw up slightly with the forceps the top part of its cotton-wool plug, push through the remainder of this plug the pointed end of the capillary pipette, *i.e.* the one containing the droplet of the material to be sown, and push it into the material at the bottom of the test-tube or flask. A trace of the sowing material flows out by itself, or, if a large quantity is required, it is carefully blown from the pipette, but, of course, not so that the tube is emptied by the blowing. If the sowing is to be carried out on the surface of solid material, the seed is deposited on the surface ; if in the depth, the end of the pipette is pushed down into the depth of the material and the seed there deposited. The pipette is then altogether withdrawn and the plug replaced as before. The new tube is then placed in a beaker on a cushion of cotton-wool, and exposed to the required temperature in the incubator.

The simple and now universally adopted method, particularly in a place kept ordinarily clean, is this : the culture-tube or flask is held slantingly, the plug is withdrawn with forceps, and with a sterile platinum needle or loop a trace of the old culture is transferred to culture-medium in a new tube or flask, also kept slantingly, and of which the plug has also been withdrawn. After the transference, both the old culture and new subculture are plugged, both plugs before insertion having been passed through the flame. If the new subculture is made on solid medium, the inoculation is made either as *stab-culture—i.e.*, by dipping the end of the sterile platinum needle into the old culture material, then stabbing (piercing) about the central part the new solid medium ; *streak-culture*, by drawing the charged needle or

loop in one, two, three or more lines along the slanting surface of the solid medium, or rubbing it all over this surface.

If we have, however, a culture-fluid or any material that contains, as the microscopical examination proves, various species of organisms, which we wish to isolate, then the method of Klebs of "fractional cultivation," or the method of Lister and v. Nägeli of "dilution," or better still, the now universally-adopted method of Koch's "plate-cultivation," is resorted to.

The "fractional cultivation" consists in the attempt to isolate by successive cultivations the different organisms that have been growing previously in the same culture. If we take up by means of a capillary pipette or the point of a platinum needle a trace of the culture-material, and inoculate with it in the manner above described a successive series of new culture-tubes containing various nourishing materials, and expose these tubes in the incubator to a definite temperature, say 37° C., then the chances are that in the first twelve or twenty-four hours not all the different species of organisms sown out will have increased equally in numbers in all tubes; most probably only one or two species in each tube—*i.e.*, the ones that grow best in this particular medium and at this particular temperature—will be found to have increased to an enormous extent, while the others have made little or no progress as yet. The nourishing fluid appears turbid, and filled chiefly with the one or two kinds of organisms. Now take out with a fresh capillary pipette or a platinum needle a minute droplet of this new culture and inoculate with a trace of it a new culture-tube. The chances are that you inoculate only one kind, that is, the one which is most abundant or perhaps is solely present. After twelve or twenty-four hours' incubation this new tube

contains now probably only one kind of organism. To make it quite certain, inoculate from this a new culture-tube in the same manner, and now you probably have sown only a single species. In this manner by continued transference it is possible to obtain cultures of only one species of organisms. Many conditions, such as naked-eye appearances of a particular kind, coloration of the culture-medium, formation of a pellicle, the quantity of growth in a given time, soon indicate whether we have the desired single species ; in some instances it is, however, extremely difficult to isolate after this method.

The method of "dilution" means diluting the material containing the mixture of the various species to a very large extent with some sterile indifferent fluid, such as well-boiled saline solution of 0·6 per cent., and then inoculating new tubes with a droplet of this greatly diluted material. For this purpose take up with a platinum needle or loop a droplet of the mixture, then transfer it in to a test-tube or flask containing well-boiled saline solution, so as to greatly dilute (1000-fold or more) the particle or droplet of old culture-material, and from this dilution inoculate then a series of new culture-tubes containing different nourishing material, using always only a trace for inoculation. In this way it is probable that, owing to the great dilution, the trace of a droplet of this mixture used for the new inoculation contains only one species. Using a series of new culture-tubes and inoculating them thus, after twenty-four hours of incubation it will be found that some tubes have not received any seed, others only one species. If it be required to dilute the original fluid greatly, say if it teems with and is turbid by different organisms, then a droplet of this is placed into a large flask containing the well-boiled saline solution, so that a dilution of 1 in 1,000,000 or more can be effected.

The two methods—*i.e.*, that of fractional culture and of dilution—may be successfully combined in this way: from the first or second new culture, established after the method of fractional cultivation, in which after twenty-four or thirty-six hours one species greatly predominates, draw out with a large capillary pipette a droplet, and dilute this to a great extent with the saline solution, as described above, and now inoculate with a trace of this mixture a new culture-tube. Or, if after twenty-four hours' incubation the microscope reveals in this further culture more than one species, continue the process of dilution and inoculation for a further generation. Thus it is possible to obtain cultures of only one species, although the original fluid contained several species of organisms.

One of the best and universally adopted methods for isolation is that of the *plate-cultivation* introduced by Koch in connection with the isolation of the choleraic comma bacilli. A test-tube containing sterile nutritive gelatine as above prepared is liquefied by gentle heat, best by being kept in water of about 40° C., then the plug is lifted with sterile forceps and the gelatine inoculated with a mere trace of the bacterial mixture, either by means of the point of the capillary pipette or of the heated and cooled point of a platinum needle; the plug is replaced and the gelatine shaken so as to distribute uniformly the bacteria that had been introduced. A shallow glass dish with flat bottom and ground edge, and covered with a similar but slightly larger dish,[1] has previously been sterilised in the oven and then allowed to cool; the liquefied nutrient gelatine inoculated with the trace of the bacterial material is then poured out

[1] These plate-dishes are known as Petri's dishes, but it ought to be stated that several years before Petri I have described and figured this dish—viz., in fig. 9 (present fig. 11) of the third edition of this work.

into the lower dish so as to form a thin layer at its bottom; the lifting off of the dish-cover, the pouring in of the gelatine, and the replacing of the cover, ought to occupy only a moment. In order to allow the gelatine to set rapidly the dish is placed on moist blotting-paper; in hot weather a few bits of ice are placed on the paper.

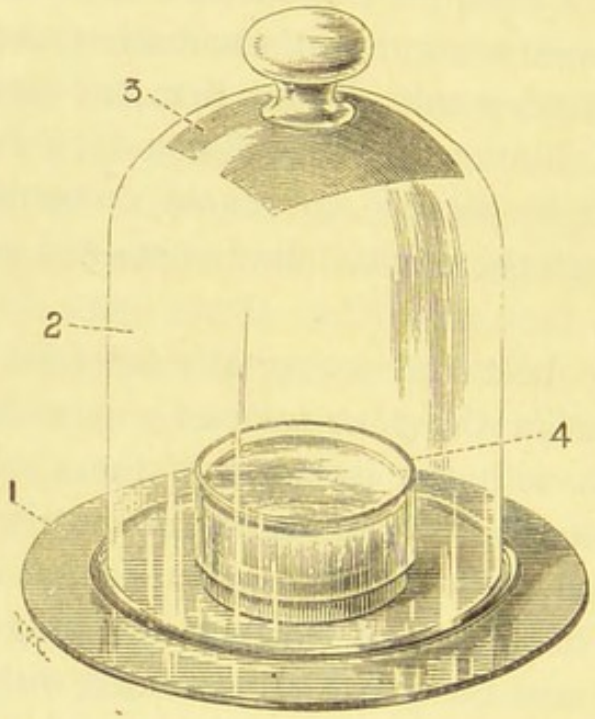

FIG. 11.—PLATE-CULTIVATION.

1. Glass plate.
2. Bell-glass.
3. Wet filter paper.
4. Glass dish containing the plate cultivation on a thin layer of nutritive gelatine. This glass dish is covered by a second glass dish.

This plate is then placed in the incubator as such or on a glass plate, to which by means of greased edge, a bell-glass can be fixed, on the interior of which is a piece of wet blotting-paper. In this way a closed moist chamber is established. But this is only of use if the plate is to be kept in the incubator for a long time; for ordinary work the plate is placed in the incubator without further addition.

The whole is then put into an incubator, the temperature of which does not reach above 21° or 22° C. (or the temperature of the room in the warm months), in order to insure the gelatine setting and remaining so. If a trace of material containing various species of bacteria is thus distributed into several cc. of gelatine, each microbe fixed by the gelatine on setting will start a separate colony after a few days' growth, and the individual colonies, if different, will be appparent by different characters, according to shape, colour, size of the colonies, and according to whether they liquefy the gelatine or not during their growth. In order to insure

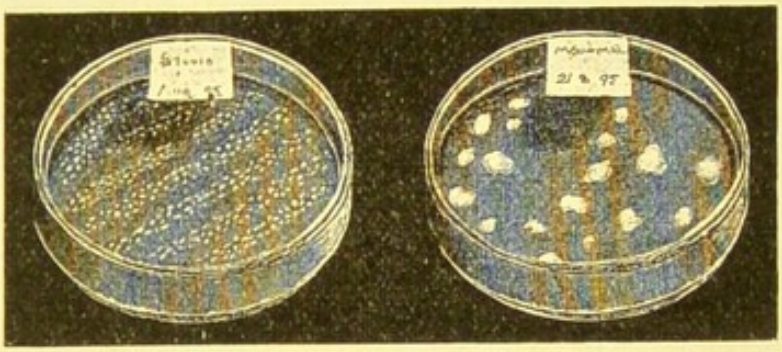

FIG. 12.—TWO GELATINE PLATE-CULTURES CONTAINING GROWING COLONIES ON THE SURFACE OF GELATINE.

success, it is necessary to infect the original gelatine in the test-tubes with only a trace of the bacterial mixture; if too many bacteria are introduced, their colonies sprouting up are too numerous and soon become confluent. But if the experiment is successful, the colonies are well separated from one another, and from the individual and separate colonies it is then easy by re-inoculation of gelatine tubes, or other nutritive material, to start pure subcultures of the different species. It must be borne in mind that not all bacteria can be isolated by this method, for some species of pathogenic organisms require for their growth a higher temperature

than the one at which the nutrient gelatine remains solid, while others refuse altogether to grow in gelatine, or grow only too slow. In the latter case no success can be looked for, if those bacteria which form their colonies much faster and are present in large numbers crowd out the others that require a long time to come up.

In such cases, particularly when one has to deal with bacteria that do not grow in gelatine at the temperature at which this latter remains solid, the same method of plate-cultivation can be used, but substituting the gelatine by the Agar-Agar peptone mixture above mentioned, previously liquefied by heating, care must be taken not to proceed with the inoculation of the Agar-Agar mixture before the temperature has fallen to about 42° to 50° C. All other manipulations remain the same.

It is perhaps not unnecessary to state that if the Agar mixture is of recent date—contains, therefore, while in the tube condensation fluid, after pouring it out in a plate dish, cooling this, letting the Agar set, and placing the cultivation in the incubator at 37° C., in all probability there will again appear condensation water in the plate; as the colonies begin to develop on the surface they will be swamped by that water and the whole surface will become covered with an indiscriminate film of growth. It is therefore advisable to keep the plate-dish in the incubator inverted and in slanting position. If, however, the Agar mixture used for the plate-culture is of some standing this is not necessary. But I keep also gelatine plates in an inverted condition in the incubator for the first day, in order to avoid too great a loss of water by evaporation; care must of course be taken that as soon as liquefying colonies appear and begin to spread the plate-cultivation must again be placed upright.

A method which I have found very useful for making

permanent plate-cultivations on gelatine or Agar, and which I first described in the Reports of the Medical Officer of the Local Government Board for 1886–1887, is the test-tube plate-cultivation. It is this: in ordinary plate-cultivations such as were described above it is obvious that only a certain proportion of the colonies appearing on subsequent incubation are situated on the surface, another proportion are in the depth, and these latter are either not characteristic for differential purposes or cannot easily be used for further operations. It is therefore advantageous to have all colonies appearing on the surface. This can be done in two ways: (*a*) By pouring out into the plate-dish the gelatine or Agar before inoculation, letting it set, and then smear or rub over the surface of the set gelatine or Agar by means of the platinum loop the infective material (see Fig. 12), or (*b*) by rubbing it over the surface of gelatine or Agar, set with slanting surface in test-tubes. In both cases all colonies make their appearance on the surface only. The latter method allows of the test-tube plate-cultivation to be preserved uncontaminated, as the test-tube on opening for the object of making subcultures can be held mouth downwards and safe against accidental contamination. When we are dealing with microbes liquefying gelatine the test-tube cultivations in gelatine are not practicable. The test-tube gelatine-cultivations yield excellent impression-preparations (see Chapter I.); for this purpose the interior of the test-tube can be easily cast out on to a glass plate by dipping the lower part of the test-tube for a few seconds in hot water; too long exposure would melt the gelatine and spoil the gelatine block for further operations.

2. *Inoculations with Blood, Juices, and Tissues.*—To establish a cultivation from blood of a dead animal, cut open the thorax by removing the sternum with clean scissors, cut

open the pericardial sac, pierce with the pointed end of a fresh capillary pipette the wall of the right ventricle or right auricle, and allow a drop or two of blood to ascend into the pipette, or if a larger quantity is required suck it up. Withdraw the pipette and inoculate new culture-tubes as above. Or, if blood of a large vein is required, separate the vessel with sterile instruments, and make a small incision with sterile scissors and push the pointed end of the capillary pipette well forward. If juice of a lymphatic gland, or spleen, or other parenchymatous organ be required, pierce the organ after having washed its surface with strong solution of perchloride of mercury (Koch), with the pointed end of a capillary pipette, then push it into the part required for a little distance, and squeezing the organ press a drop or two of the juice into it. The same procedure is adopted when the pus of an abscess is required, the wall of which can be pierced with the pointed end of the capillary pipette. If not, a slight incision is made and the pipette introduced through this into the abscess. If blood of a living animal is required, expose a vessel with sterile instruments, make a small incision with sterile scissors, push through this incision the pointed end of the capillary pipette well forward, and allow the blood to rise into the capillary tube. If blood of a living human being is required, clean well with soap and water and then with strong carbolic acid or perchloride of mercury solution the tip of a finger, make a venous congestion in the last phalanx by compressing it with a corner of a handkerchief, prick the volar skin of the phalanx with a clean (heated and cooled) needle, and plunging the pointed end of the pipette into the drop of blood, allow a droplet to ascend into the capillary tube of the pipette. But all these inoculations can also be practised by means of the platinum loop, only in this case contamination

with extraneous organisms is more possible than by the other method.

If solid tissues or parts of tissues are required—*e.g.* the base of an ulcer, a tubercle of the liver, spleen, or lung—it is possible to squeeze into the capillary tube of a pipette, after pushing its pointed end into the part, a small droplet of juice of the part required; but if this be not practicable —*i.e.* if a solid particle be required—or if it be preferred because simpler, then follow Koch's method, now generally used. This is as follows: Cut with clean sterile scissors or scalpel into the part, take up rapidly with the point of a needle or platinum wire previously heated in the flame of a burner a small particle, a drop of blood, pus, juice, or solid material, and quickly introduce this into the culture-tube to the place required—*e.g.* surface or depth of a solid or fluid nourishing material. Of course in this case the cotton-wool must be altogether lifted, and therefore contamination with air-organisms is possible. But inoculating several tubes at once and performing the operation quickly, and working in an ordinarily clean place, one always succeeds in getting most of the tubes without any air-contamination. I have made numerous inoculations with solid particles of different morbid tissues and products in this manner, and, like Koch and others, have seen only a very small percentage of tubes becoming contaminated with air-organisms, chiefly moulds.

The same plan—*i.e.* of using the clean point of a sterile needle or platinum wire for taking up the material to be used for inoculation—is resorted to if one has to deal with the culture in solid nourishing material, on or in which the organisms are growing that we want to transplant either for inoculation of a new tube or of an animal. A useful method, which does not require the lifting out of the plug

at all, and which can easily be employed in the last case, is this ; deposit from the pointed end of a capillary pipette a droplet of some sterile fluid (broth or thoroughly-boiled saline solution) on the spot of the solid medium on which the organisms are growing, then scratch this spot with the end of the capillary pipette in order to get the organisms off from the solid basis and mixed with the drop of fluid deposited there, then let this drop again ascend into the end of the capillary pipette, and withdraw this altogether. All this can be done without lifting out the cotton-wool plug of the test-tube or flask in which the growth is proceeding.

If one has to use a particle of tissue the surrounding portions of which are probably contaminated by putrefactive organisms—*e.g.* a tubercle in the lung or a tubercle in the spleen—it is well to follow Koch, and to disinfect the surrounding parts by just washing them with a dilute solution of corrosive sublimate, and then to remove these parts with clean scissors so as to obtain the central particle which one wishes to use for inoculation : of course one must not steep the organ too long in sublimate solution, since this would naturally destroy all organisms.

All these methods can be easily modified according to the requirements of the special cases, and it is not necessary here to give more than what has already been described in the preceding.[1]

3. *Fixing of cultures.*—In connection with this a method must be mentioned for the permanent fixing of plate- and tube-cultures. The growth in these can be at any moment arrested, and all further contamination and growth in

[1] Compare also Koch, *Untersuchungen über pathogene Bacterien*, in *Berichte aus dem k. Gesundheitsamte*, Berlin, 1881 : and *Die Aetiologie d. Tuberculose, Berlin. klin. Wochenschrift*, No. 15, 1882.

them prevented, by devitalising the microbes, and by sterilising the medium on and in which the growth has been taking place. This is done by the fumes of formalin ($\frac{1}{2}$ strength); commercial formalin is a 40 per cent. solution of formaldehide. A tube, or a number of culture-tubes, in which the further growth of the microbe is to be arrested, are placed best without their cotton-wool plugs into a wide-mouthed bottle or glass cylinder, into which a small quantity of formalin ($\frac{1}{2}$ strength) has been poured, then close the cylinder air-tight and let it stand. The vapours of formalin penetrating the tubes even through the wool plugs do their work in a day or two. If a plate-cultivation is to be fixed, a few drops of formalin are placed on the middle of the cover-dish, the plate-dish is now inverted, and allowed to stand for from some hours to a day or two. The formalin vapours fix thereby permanently and kill the colonies, and no further growth either of the colonies already formed or of new contaminating colonies occurs.

4. *Hanging drop cultures.*—In order to study microbes in the living state as for motility, growth, multiplication, and spore formation, the methods used are practically those known as "hanging drop preparations" first used by Koch. An object-glass slide, possessing a shallow circular pit, is covered, over the pit, with a cover-glass in the centre of which a drop of the fluid suspension of bacteria, or of serum, blood, &c., is deposited, the drop facing the pit; the edge of the cover-glass can be fixed around the pit by paraffin or oil or cement, and the observation can be carried out either at the temperature of the laboratory or by placing the object on a warm stage at any desired temperature. The droplet being small the examination can be carried out even with high powers as easy as an ordinary fresh preparation. Motility, the elongation of bacilli, their division, germination of spores,

of bacilli, and of fungi, and other life processes can be watched and noted. I have made very extensive observations on the growth and division of bacilli and of mycelial fungi, extending for hours, and noting the progress from day to day, by distributing a limited number of the microbes (by the aid of the point of a thin platinum needle) in a droplet of melted nutrient gelatine or Agar deposited in the centre of the cover-glass,[1] and then flattening the droplet by the platinum needle out into a film, of course limited to the centre of the cover-glass, and finally fixing this latter by means of sweet oil to the glass side film downwards. The gelatine, as also the Agar, sets at the ordinary temperature of the laboratory, and by a power up to 700 the bacteria or other microbes can be easily focussed and kept under observation, they being fixed in the set gelatine or Agar.

The glass cell (see Fig. 13) which I use is based on the same principle; it has the advantage of allowing the observation to be extended over longer periods as it is easier to keep the chamber of the cell moist by depositing a droplet of water on its floor.

These methods of watching and studying bacteria and fungi in the living state with high powers in a gelatine film or Agar film cannot be too strongly recommended; it can be carried out and extended over hours and days. A direct insight is obtained into the phenomena of growth, germination, and division, as also of spore formation which as a rule is only indirectly deduced. It is one of the most interesting experiments to make such a preparation from the blood of an animal dead of anthrax (care being taken to introduce only a limited number of bacilli), and to watch

[1] If a culture is used it is best first to make a distribution in sterile salt, or water, or broth, by shaking in it a small particle of the growth transferred by a platinum needle, and to inoculate the gelatine or Agar drop from this dilution.

the growth of the individual bacilli fixed in the set gelatine into threads and the formation of the characteristic colonies made up of curved and convoluted threads. Equally interesting is it to watch the formation of colonies by the proteus vulgaris or proteus Zenkeri, the "swarming" of them, and the manifold sprouting of threadlike outgrowths; or the gradual formation of bright globules and their enlargement into the characteristic oval spores in the threads of bacillus anthracis or of hay bacillus, and their ultimate

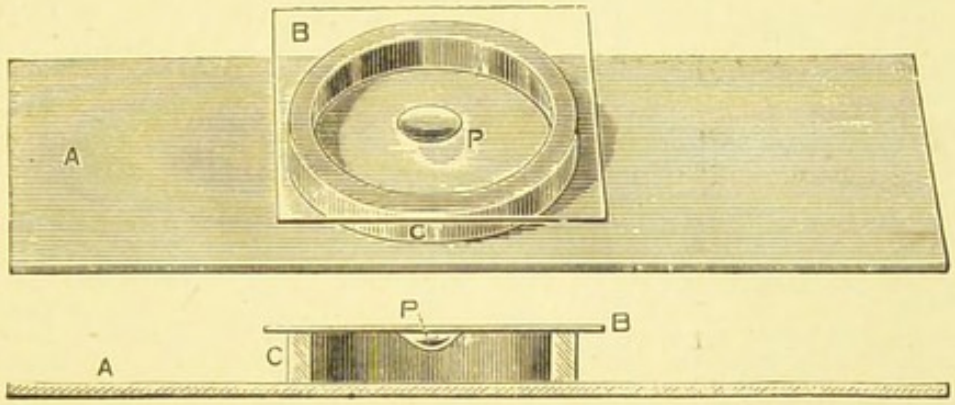

FIG. 13.—A GLASS CELL, FOR OBSERVING UNDER THE MICROSCOPE THE PROGRESS OF GROWTH OF MICRO-ORGANISMS.

The upper figure shows the cell in perspective; the lower figure in profile or cross section.

A. Glass slide.
B. Cover-glass.
C. Glass ring forming the wall of the chamber.
P. Drop of nourishing material in which the micro-organisms grow.

discharge and disintegration of the bacilli themselves. All these points can be directly studied by intermittent observation in the above preparations extending over several days.

5. *Bacterioscopic Examination of Water.*—Most waters contain bacteria of some kind, sometimes in great numbers without altering the limpidity of the water, at any rate not for the unaided eye or the ordinary tests of transparency.

In order to directly demonstrate the bacteria, the water

is allowed to stand ; from the bottom layer a small quantity is withdrawn and of this a drop or two are deposited in the centre of a clean cover-glass and evaporated by heating. This represents a film specimen, which is then stained, washed, and mounted in the usual way. On microscopic examination, according to the source from which the water is derived, there will be found particles of amorphous débris, cotton-wool threads, spores, and bits of mycelial threads and stained bacteria in small or great numbers according to the amount of pollution that the water has been exposed to. In a so prepared specimen of the water that the different London water companies, drawing their water from the Thames and Lea, distribute to their consumers, as a rule besides numerous bacteria, cotton fibres and amorphous débris, there will be found various infusoria (see below). In order to accurately study the number and character of the bacteria, present in water, cultivations must be made. These are of two kinds :

A.—To Determine the Number and General Character of the Bacteria.

Plate-cultivations are used for this purpose, generally gelatine plate-cultivations. But it must be remembered that by determining the number of microbes in a given small quantity of the water, added to the gelatine, by means of gelatine plate-cultivation, we are determining only the relative number of bacteria, that is to say, only those that do and can grow at the temperature at which the gelatine keeps solid, but there may be and sometimes are some species present which only grow well at higher temperatures ; in such cases their numbers must be determined by Agar plates. But as a general rule in practice it is sufficient to

determine the number of bacteria by means of gelatine plates. For this purpose a definite—*i.e.* measured—small quantity,[1] $\frac{1}{20}$, $\frac{1}{10}$ to 1 cc. (according to the turbidity) of the water after shaking, is added to a gelatine tube; this is melted in warm water and then poured out into a sterile plate-dish. This gelatine is allowed to set, and after incubation at 20° C. for three or four days the number of colonies that have sprung up are counted, and according to the quantity of the water that has been added to the gelatine for plate-cultivation the number is calculated per 1 cc. Several points have to be remembered in making an estimate that is to be approximately correct. (1) There ought to be always two plates made, and the number ought to be determined by the average. (2) The sample of water to be tested ought to be well shaken up before withdrawing the required quantity for the plates, in order to make as uniform a distribution of the bacteria in the water as possible. But notwithstanding this sometimes enormous differences will be found in two plates made from different portions; this is probably due to the fact that in some

[1] For measuring definite small quantities of water or any other fluids, I use a series of glass pipettes on the plan of those added to a hæmocytometer: 5 cmm. ($\frac{1}{200}$), 10 cmm. ($\frac{1}{100}$), 20 cmm. ($\frac{1}{50}$), 50 cmm. ($\frac{1}{20}$), 100 cmm. ($\frac{1}{10}$), and 250 cmm. ($\frac{1}{4}$), $\frac{1}{2}$ cc. and 1 cc.; each of these pipettes can be fitted with an india-rubber tube with porcelain or glass mouthpiece; these latter receive a plug of sterile cotton wool. The pipettes are sterilised, and when ready for use the tube is fitted on; of the fluid a little is poured into a sterile watchglass, and of this the required quantity is withdrawn and blown out into the test-tube containing the culture-medium. If a quantity smaller than $\frac{1}{200}$ cc. is required a dilution is previously made with a definite quantity of sterile distilled water; for instance, if $\frac{1}{1000}$ cc. of a given fluid is required, I take 5 cmm. —*i.e.* $\frac{1}{200}$ of the fluid—and add this to 5 cc. of sterile distilled water, each 1 cc. of this would contain 1 cmm. or $\frac{1}{1000}$ cc.

The pipettes can be sterilised either in the hot air-chamber, which is best, or by letting them lie for an hour or so in disinfecting fluid, then empty them, and wash them thoroughly out twice or more times in distilled water.

waters—*e.g.* the London waters—there are suspended in the water microscopic masses of organic débris loaded with bacteria. If such a mass happens to be in the particular quantity of the water that is added to the gelatine, and after shaking this up the bacterial mass is broken up the resulting number of colonies in the plate may be greatly in excess.

(3) It ought to be remembered that the number of bacteria in water is liable to considerably increase as time goes on, for some bacteria—special water bacteria—are capable of living and multiplying in water; they are capable of thriving even on very small amounts of organic matter present in the water. It is therefore necessary to make the plate-cultivations as early as possible after the water is taken from its stock. It is not necessary to do this on the spot, if the place of work is within a comparatively short distance say if the water can be delivered in the laboratory within a few hours after; but if water is sent from long distances, when it has to travel many hours by rail in the summer months, then the actual number of bacteria present at starting is not to be measured by that found in plates made say twenty-four hours after. In the cold weather and if the sample of water sent is kept in a cool place, the multiplication in twenty-four hours is not very great. To obviate these errors when water is sent from long distances, it is advisable to keep the sample packed in ice or in cotton wool in a cool place. It is obvious that the bottles in which the water is received and sent should be sterile; glass stoppered, narrow necked, about two- to four-ounce bottles, sterilised in the hot chamber, are best for this purpose—it does not require to fill the water in vacuum tubes. From a large experience I found that glass-stoppered bottles first well washed out with nitric acid or methylated spirit, then twice

successively with the water with which they are to be filled, are quite satisfactory.

(4) In order to determine the number of bacteria present per 1 cc. in a given sample of water by means of gelatine plates, a sufficient quantity of the water ought to be used to yield a fair number of colonies, such as can be counted fairly accurately.[1] If the water has a large number of bacteria—this can be easily determined in a few minutes by making a film preparation—*i.e.* by depositing one or two drops of the water, after shaking, in the centre of the cover-glass, drying and heating, staining and mounting and subjecting it to microscopic examination; after some practice it is quite possible to say from such examination whether the water has comparatively few or many bacteria, and accordingly to make the plate-cultivation with a small quantity, say $\frac{1}{10}$ or less or more up to 1 cc.

It ought further to be remembered that the rapidity with which the colonies appear in the plates depends in the first place on the temperature at which the plates are kept. If the plates are kept in a cool place—*e.g.* in a cupboard at the temperature of the room in the cold months—the growth is extremely slow, and the colonies appear only after many days. I have made comparisons in this respect. I have seen it stated by the water analysts of the London water companies that during particular months of the cold weather the number of bacteria in London waters as determined by gelatine plates kept in a dark cupboard at the temperature of the laboratory, which was certainly under 17° C., and the counting being done forty-eight hours after, was between 7 and 70 per 1 cc.; whereas in my experiments under similar conditions the plates were counted not after forty-eight hours

[1] What is here stated of water refers to all other fluids of which the number of microbes are to be determined by plate-cultivation.

only but after seven days, and the number of colonies in the plates was over twelve hundred per 1 cc. The plates ought to be kept in the incubator always at a temperature of 20 to 21° C., if counted after two days it will be found that even under these conditions not all colonies have sufficiently developed as yet, and for these reasons the counting ought to be repeated after three or four days. It seems to me that the low figures of bacteria present in London waters as published by the public analyst of the Local Government Board as also by the analysts of the London water companies are not to be accepted without hesitation, for the reason that in neither case were the conditions for obtaining a full and correct number of the bacteria fulfilled: the plates were not kept at the most favourable temperature, or the counting was done too early.

Koch has devised a plate in equal squares of known area which enables one to count the number of colonies easily. But it is just as easy and just as accurate to count the colonies in a plate-cultivation, by drawing in ink lines on the outside of the plate-dish (not the cover) by which the area is divided in two, four, eight, sixteen, and so on, and to count under a magnifying glass on a black ground the actual number of colonies in each division. If the number of colonies in the plate-cultivation is small or moderately large counting is easily and soon done, but if the number is excessively large, say several thousand, then only an approximate estimate can be made, by selecting two, three, or four small subdivisions, say $\frac{1}{16}$ or $\frac{1}{32}$, representing a fair average distribution of the colonies, to count them patiently in these divisions, and then by calculation give the total.

Messrs. Washbourne and Pakes have designed a print which embodies the same principle, only is much simpler; it is a printed circular area in black of the size of a plate

dish ; in this circular area are white radii subdividing the area into sectores $\frac{1}{16}$ each, and cross circles subdividing these sectores into three.

Under these circumstances the estimate can only be an approximate one, but as stated above if the number of colonies is not excessively large, the counting can be done accurately.

A question that is constantly being asked is as to the number of bacteria that ought not to be exceeded in water if this is to be regarded as of good quality. It is quite clear that water in which there is an average amount of vegetable matter ought, and as a rule does, contain large numbers of bacteria—*e.g.* moorland water, lake water supplied from moorland, water in lakes and ponds in meadows surrounded by reeds, &c.—yet this number of bacteria need not in the least interfere with or deteriorate the good quality of the water, whereas water even if taken from deep wells, in the chalk or other formations, may contain a small number of bacteria yet be wholly unfit for drinking purposes if at any point percolation of sewage into this water takes place. Koch's standard which is accepted now generally is : that wherever pollution of water with animal refuse potentially or actually takes place, the number of bacteria should not exceed 100 per 1 cc. But this, for the above-named reasons, does not apply to waters which are not and cannot be so polluted. Taking, for instance, the water which the London water companies distribute to the London inhabitants, we find that with the exception of the Kent Company—which nominally, at any rate, draws its whole stock from the chalk —all other companies draw their raw water from the Thames or Lea, that is to say, from sources which are notoriously open to pollution, and as a matter of fact are constantly actually polluted with animal refuse—human ex-

crements included—such water should, on Koch's standard, not contain above 100 bacteria per 1 cubic centimetre. I had the opportunity of examining these waters (eight companies) for eight consecutive weeks, and found that out of sixty-four samples thus examined only in eight were the number one hundred or below, in the others above, in a majority as numerous as in the unfiltered raw water.

The plate-cultivations thus made for ascertaining the number of bacteria can be used for a superficial estimation and the study of the character of the microbes, but it must be understood that having used for each plate only a very small quantity of the water, a fraction, say, of one to two cubic centimetres, only those microbes will be met with in these plates which occur in large numbers; as to those that are distributed in the water as a whole in limited numbers, there is little chance of meeting them in a couple of plates inoculated with only 1–2 cc. of the water. The bacteria almost constantly present in small quantities, leaving out yeast and fungi, are :—(*a*) bacillus fluorescens liquescens, easily recognised by the rapid liquefaction and greenish tint of the liquefied parts ; (*b*) bacillus sulcatus, in several varieties, not liquefying, white, rounded, flat moist colonies ; (*c*) micrococcus, liquefying and non-liquefying ; (*d*) not infrequently one or the other variety of bacillus mesentericus, motile large bacilli, liquefying slower than fluorescens. The most important part of the examination refers to the detection of microbes which are present in putrefying animal matter, notably in sewage or animal excrements, or are derived from the diseased bowels of man. Amongst these are bacillus coli, proteus vulgaris, proteus Zenkeri, or a variety of it, and above all bacillus of typhoid and vibrio of cholera.

B.—Detection of Special Microbes by Special Methods.

Bacillus coli, being chiefly derived from the intestinal contents of man and animals, would in the nature of things occur in all matters: dust, earth, food-stuffs, mucous membranes which have been exposed to pollution with matter tainted with dejecta. Thus in large towns almost everything is liable to become so polluted owing to the almost ubiquitous presence of dust tainted with animal dejecta. The same applies to any place and any material to which such dejecta find access. This bacillus coli can, therefore, under particular conditions of locality, be regarded as almost ubiquitous. The same applies to proteus vulgaris, but in a somewhat more limited degree, since this organism, although present in the alimentary canal, is nevertheless not so common in this; but being the chief organism producing the putrid decomposition of albuminous substances, it will be found wherever such substances undergo this change. These two organisms or either, notably bacillus coli, if present in *large numbers* in any water, would indicate that that water had been subject to excremental pollution or that there exist in the water putrid animal matter. A very limited number of bacillus coli need not be and is not sufficient to condemn such water, because the accession to it of a little dust, carried there by air currents, originally impregnated with animal excreta, would produce such a result, but in this case the bacillus coli would in a large bulk of water be very scantily distributed. It is different if the bacillus coli or proteus vulgaris, particularly the former, be present in large numbers, for then pollution with excremental matter has probably taken place. Take, for instance, water derived from deep

wells; if there be no soakage of sewage, practically no bacillus coli will be found in it, but if there be such soakage bacillus coli is easily found in moderate numbers. It must be obvious that where sewage pollution does take place, the facility of discovering the bacillus coli in the water will depend *cæteris paribus* on the relative amount of pollution and water. If, for instance, it is a case of a small water-course to which sewage has continually access, numbers of colonies of bacillus coli would be found in a gelatine plate made with even small quantities of the water, say $\frac{1}{4}$—1 or 2 cc. But if it is a question of a water-course like the river Thames, even after a moderate sewage pollution, the volume of water is so great that bacillus coli can be demonstrated only by subjecting large quantities of the water to the culture test. To expect to find the bacillus coli in a few drops or even a few cc. of water taken from the Thames at Hampton, above the intake of the water companies' water, is very strange, and stranger still to say that not finding it in so small a quantity, it is absent from the unfiltered Thames water at Hampton; such statements are liable to throw doubt on bacteriological examinations in the eyes of sanitarians, for it is notorious that apart from the surface flushing of streets in many places on the upper Thames, there is obvious pollution of the Thames with human excrements, along the shore, from barges, house-boats, &c. And the same applies to the examination of water for typhoid bacillus or cholera vibrio—viz., it is essential that large volumes of water should be subjected to bacterioscopic examination, and even then a negative result should be put forward for what it is worth. Statements such as one occasionally sees—viz., a few drops or a few cc. of the water had been examined and no bacillus coli, or no bacillus of typhoid, or no cholera vibrio was found, therefore such

and such water does not contain any of these organisms, are absurd; the latter statement would be unjustified even if large quantities of the water had been subjected to examination.

For the detection of bacillus coli, bacillus of typhoid, and a certain variety of proteus Zenkeri, a normal inhabitant of sewage, the following method[1] will be found to answer well: Through a Berkefeld or Pasteur pressure filter a large volume of the water is pumped. The filter I generally use for the purpose is a Berkefeld large bougie, which by a screw can be well and tightly fastened into one end of a cylindrical glass; the metal tube projecting from the candle is fixed through an indiarubber stopper into a large bottle holding about 1,000—1,200 cc.; this bottle has at the neck a lateral glass tube, which by means of a stout indiarubber tube is connected with an exhaustion pump, a good size hand-pump. Before using the filter the candle, screw, indiarubber stopper, glass cylinder, and glass bottle are all sterilised, the glass and screw in the hot air-chamber, the candle and indiarubber stopper in boiling water, in which the candle is kept from half to one hour.

The water to be examined is poured by means of a sterile glass beaker into the glass cylinder, and exhausting the air in the bottle the water filters easily and rapidly; 1,200—1,500 or 2,000 cc. are thus easily filtered in a moderate space of time; 1,200 cc. pass through in about 15–20 minutes. Then the candle is unscrewed carefully, taken out with clean hands, and 10 cc. of sterile water (or of the filtered water from the bottle) are measured into a sterile glass dish; the whole surface of the candle is well brushed into these 10 cc.

[1] I have practised this method since 1892; when with Dr. Theodore Thomson the outbreak of typhoid fever in Worthing, 1893, was investigated, it was this method by which the typhoid bacillus in the suspected water was demonstrated.

of sterile water by means of a thoroughly clean nail-brush. In this way the whole or practically the whole of the particulate matter of the bulk of water that had been filtered is distributed in the 10 cc. of sterile water. By brushing the surface some of the soft filter material is also brushed off, but since the bulk of this easily settles down it is of no material consequence. This distribution is used for cultivation; every cubic centimetre of it contains the amount of the particulate matter of a definite bulk of the original water. If, for instance, 1,200 cc. had been driven through the filter, each 1 cc. would contain the particulate matter of 120 cc. of the original water; if 2,000 cc., each cc. of the distribution contains the particulate matter of 200 cc. of the original water. There is no difficulty in subjecting to analysis if necessary the whole 10 cc.—*i.e.* the whole particulate matter of the whole of the original 2,000 cc. of the water.

The cultivations of the distribution are made after Parietti's method in phenolated gelatine, or in phenolated broth, or in both. Parietti first pointed out that by adding a solution of phenol to the broth or the nutrient gelatine previously melted, these media while remaining favourable for the growth and development of the bacillus coli and typhoid, are not so well suited to that of the ordinary water bacteria, some of the latter being either altogether suppressed while others thrive only slowly, and therefore bacillus coli or typhoid have in the meantime an opportunity to develop. Of a 5 per cent. solution of absolute phenol 0·1 cc. (or 100 cmm.) are added to 10 cc. of broth or gelatine; this represents the phenolated gelatine or phenolated broth respectively. The addition of hydrochloric acid, as recommended by some, is according to my experience not required.

Of the above distribution then, to each phenol gelatine or phenol broth tube $\frac{1}{2}$ or $\frac{1}{1}$ cc. is added, the gelatine is shaken and poured out into a plate, and after setting kept at 20° C., the phenol broth is incubated at 37° C. From the phenol broth incubated for twenty-four hours, plates in phenolated gelatine are then made. If in the original water the bacillus coli or the sewage variety of proteus Zenkeri or the typhoid bacillus be present, the phenol broth-cultivation will be found uniformly turbid after twenty-four hours' incubation; by placing a droplet of this culture into 10 cc. of sterile salt solution and making with a platinum loop of this dilution a phenol gelatine plate, this after incubation for two to three days will show either of the above organisms in numerous colonies.

The phenol gelatine plates when ready—after two to four days' incubation at 20° C.—must be carefully examined, and all the surface colonies which resemble in aspect the above organisms have to be tested by fresh preparation, by flagella staining, and by subcultures in different media. Of this more when we come to deal with the differential characters of the bacillus coli and typhoid bacillus. The sewage variety of the proteus Zenkeri, as will be also described later, is in its surface colonies so characteristic and conspicuous that this and the microscopic examination are sufficient for diagnosis.

Another method is this: mix in a sterile flask equal volumes—50, 100 or 200 cc.—of the water and broth, having added to the latter the required quantity of phenol, then incubate the flask at 37° C. for twenty-four hours, and make phenol gelatine plates as before.

By these methods I was enabled to demonstrate the presence of an abundance of the typical bacillus coli not only in Thames water above Hampton, that is the intake of the

London water companies, but also on several occasions during the examination of samples of the water taken once a week during January and February, 1895, in the filtered waters distributed by the London water companies. This is shown in the following table :—

—	2nd week.	3rd week.	4th week.	5th week.	6th week.	7th week.	8th week.
Southwark	—	—	—	+	—	+	—
East London . . .	+	—	—	—	+	—	—
Kent Water . . .	—	—	—	—	—	—	—
Lambeth	+	—	—	—	+	—	—
New River	—	—	—	—	—	—	—
West Middlesex .	—	+	—	—	—	—	—
Chelsea	—	—	—	+	+	—	—
Grand Junction . .	—	—	—	—	+	—	—

+ = Bacillus coli in London waters.

The third test to which drinking waters ought to be subjected, is the microscopic examination of its suspended matter, particularly as to the presence of protozoa.

The water remaining after the quantity required for above filtration had been withdrawn is put away and allowed to stand in a cool place for twenty to twenty-four hours.

By means of a pipette drawn out into a long capillary tube 5–8 cc. or more are drawn up from the bottom layer, the end of the capillary tube is sealed and the pipette fixed in an upright position so as to allow the suspended matter to settle in the capillary tube. When this has taken place, the capillary tube is broken off and its contents subjected to microscopic examination.

I will give here a table showing what kind of living animalculi were found by me in examining the "filtered" water as distributed by the various London water companies during six weeks of examination, and from this it will be

Animalculi found in London Waters during January and February 1895.

Name of Co.	3rd week.	4th week.	5th week.	6th week.	7th week.	8th week.
Southwark . .	Cercomonas and trichomonas.	Trichomonas.	—	Paramaecium coli and trichomonas.	Paramaecium coli, trichomonas and cercomonas.	Trichomonas.
East London .	—	Gregarina, trichomonas, chlamydomonas.	Diatome, rotifer, trichomonas and cercomonas.	Rotifer, anguillula and trichomonas.	Anguillula and numerous trichomonas.	Very numerous trichomonas and cercomonas.
Kent	—	—	—	Anguillula.	—	—
Lambeth . . .	Paramaecium coli.	Paramaecium coli and trichomonas.	Cercomonas, trichomonas and anguillula.	Paramaecium coli.	Trichomonas very numerous.	—
New River . .	—	—	Trichomonas.	Trichomonas and cercomonas.	—	Cercomonas.
West Middlesex	Trichomonas.	—	Cercomanas.	—	—	Trichomonas.
Chelsea . . .	—	Anguillula and trichomonas.	Anguillula.	—	—	Rotifer, anguillula, trichomonas, cercomonas and diatome.
Grand Junction	—	Paramaecium coli, amœba, and numerous trichomonas and cercomonas.	Anguillula.	Trichomonas.	—	—

seen that the London waters must have been considerably polluted, and at the same time imperfectly filtered.

For the detection of the cholera vibrio in water the peptone salt method is the simplest and best. A stock solution of 10 per cent. best peptone and 5 per cent. common salt in distilled water is made; this is made faintly alkaline and sterilised by boiling. To each 90 cc. of the water to be examined, contained in a sterile flask, 10 cc. of the above peptone solution are added, so as to make the mixture in reality a 1 per cent. peptone 0·5 per cent. salt solution. The flask is then incubated at 37° C. for twelve to twenty-four hours.

The cholera vibrio grows well and rapidly in a 1 per cent. peptone, ½ per cent. salt solution (Dunham), and is undoubtedly for this reason the best means of detecting the vibrio. Such a peptone solution shows already, provided cholera vibrios had been present, after twelve hours' distinct turbidity, and if of the top layer a droplet is removed and examined fresh, briskly moving (revolving) comma bacilli will be found; they are easily recognised as commas if a drop of the top layer of the cultivation fluid is deposited in the centre of the cover-glass and without spreading it out is dried, stained, and mounted. After twenty-four hours the turbidity is much more pronounced, and cholera vibrios, whether in pure or impure condition—*i.e.*, without or with admixture of other microbes, notably bacillus coli or proteus vulgaris—can be isolated by gelatine or Agar plates in the usual way, and then subjected to the various tests for cholera vibrios (see cholera).

The two microbes which next to the cholera vibrio grow fairly well in the above peptone-salt solution, are the bacillus coli and the proteus vulgaris; for the detection of the latter in water the peptone method is excellent, since a large quantity

of water can hereby be subjected to examination, otherwise ordinary gelatine plate-cultivations must be relied upon. But since for these only small quantities of the water can be used, the former method is far preferable, as by the ordinary gelatine plate method the proteus could be detected only if present very freely.

Examination of Air. — Miquel, Hesse, P. Frankland, Carnelly, Robertson, and others have investigated the number of microbes present in various samples of air, under various conditions (town air, country air, mountain air, air of schools, dwelling-rooms, hospitals, &c.). The method is always in principle this: by means of an ordinary gas clock or gasometer a measured quantity of air is drawn at a moderate rate by means of an aspirator—fall of water or mercury—through a cylindrical tube (Hesse), or through a flask (Frankland, Carnelly) containing a thin layer of solidified nutrient gelatine. Hesse's tubes are cylindrical, in which nutrient gelatine while still liquid has, by slightly rolling the tubes, set at one side in a thin film; they are plugged at each end with a sterile india-rubber stopper containing a sterile glass tube; to each is fixed a sterile tube, one is connected during the experiment with the gas clock, the other with the aspirator; the time during, or the rapidity with which the air is drawn through and the amount of air so drawn through are accurately noticed; after the experiment the glass tubes are plugged with cotton wool so as to serve as a filter against the entrance of further microbes. In Dr. P. Frankland's experiments, the air is passed through a sterile plug (asbestos or glass wool) contained in a tube or flask, and this having retained all microbes is then thrown into the gelatine; this is liquefied and well shaken so as to wash out of the plug all microbes and to uniformly distribute them in the gelatine.

The gelatine is then used for making plate-cultivations in the ordinary plates, or is set as a thin film on the inside of the flask. The writer uses a glass tube four to six inches long, half an inch wide, containing in the middle a cotton-wool or glass-wool plug about one and a half to two inches long; at each end the tube is plugged with a small cotton-wool plug. One end is drawn out in the shape of a large

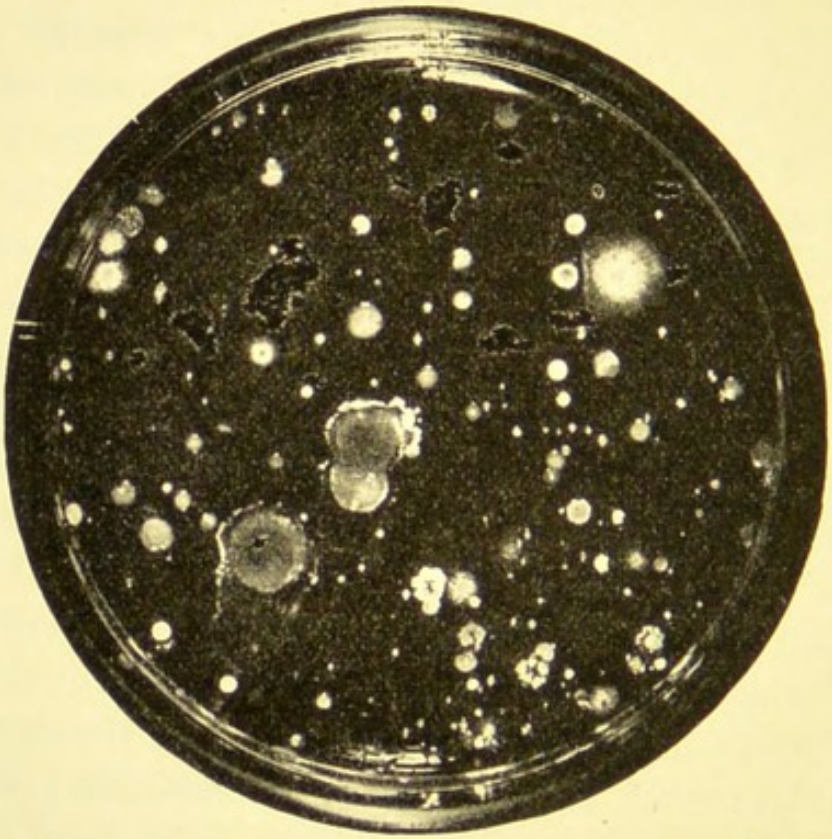

FIG. 14.—PLATE-CULTIVATION IN WHICH THE SURFACE OF THE GELATINE SET IN A PLATE-DISH HAD BEEN EXPOSED FOR THREE MINUTES TO AIR IN OXFORD STREET. Natural size of the colonies.

canula; the whole is sterilised. When used the plugs of the ends are removed, the canula end is joined to an aspirator and air is drawn through; at the end of the experiment the ends are again plugged. In order to use it afterwards for plate-cultivations, the plugs of the ends are removed, the central plug is then pushed out by means of a thin glass rod, placed in liquefied nutrient gelatine

or Agar, well shaken, and then plate-cultivations are made.

In Carnelly's experiments the nutrient gelatine is allowed to set at the bottom of a large sterile flask, the mouth of which is closed by a sterile indiarubber stopper, through which two glass tubes are passed—one long one through which the air passes from the gas meter, the other a short one connected with the aspirator: as the air passes into and out of the flask the microbes are deposited on the surface of the set gelatine.

Examination of Ice.—A piece of ice is dug out from a block, the surface of this having been previously well washed with sterile water; the piece is placed into a sterile test-tube and after it has melted is treated like water.

Milk, for the detection of the number and general character of bacteria, is treated like water—viz., a small quantity, $\frac{1}{10}$ to 1 cc., is used for ordinary plate-culture. If bacillus coli, bacillus of typhoid, sewage bacillus, or cholera vibrio are searched for, the best method is this:—

The whole or half of the quantity of milk sent for examination is put into a sterile flask or flasks, then of a 5 per cent. phenol solution is added, so as to make the whole contain 0·05 per cent. phenol; then it is incubated at 37° C. Next day phenol gelatine plates are made as in the case of water. For cholera vibrios the same method is used as for water.

Examination of soil, mud, earth, food-stuffs, or any other solid material. Here, as in the examination of water, the determination is (*a*) of the number and (*b*) of the character of the organisms, and it is followed on exactly the lines described of water examination.

(*a*) To determine the number: a definite weighed amount of the solid material is distributed in a definite quantity of

sterile fluid, salt solution, or distilled water, and then plate-cultivations in gelatine or Agar are made with definite quantities of the distribution.

(*b*) To determine the general character of the microbes, the colonies in the gelatine or Agar plates are subjected to a close study in microscopic specimens and in subculture. For determining the presence of bacillus coli, bacillus of typhoid, the sewage variety of proteus Zenkeri, particles of the solid matter are inoculated into melted phenol gelatine or phenol broth and then proceeded with as in the case of water. For detection of the diphtheria bacilli see the chapter on diagnosis of diphtheria.

For the diagnosis of the cholera vibrio, particles of the material are inoculated into tubes containing 1 per cent. of peptone and $\frac{1}{2}$ per cent. salt, incubated at 37° C. for twelve to twenty-four hours and examined in the same way as mentioned of water.

Methods of Anaerobic Cultivation.—If it is required to grow and isolate bacteria which cannot grow or only very slowly and feebly in free air, it is necessary to make anaerobic cultures. Various methods and modifications have been designed for this purpose, but I have found in actual practice that all those species which have hitherto been described can be grown in the depth of grape sugar gelatine (at 20° C.) or in grape sugar broth or grape sugar Agar (at 37° C.), without any difficulty. A test-tube containing to two-thirds its height the solid sugar gelatine or solid sugar Agar, or fluid sugar broth, is easily inoculated in the deeper parts—*i.e.*, that nearer the bottom than the surface of the tube—by means of a capillary pipette containing the bacteria in fluid suspension, the pipette being well pushed down into the medium and a droplet pressed out by blowing. The tube is then sealed up with tissue paper or paraffin or indiarubber.

Buchner places the culture-tubes, after inoculation, plugged simply with cotton wool but not sealed, into a glass bottle, and then adds into this carefully and liberally pyrogallic acid and liquor potassæ (for each gramme of pyrogallic acid 1 cc. of liquor potassæ) and hermetically closes the bottle. I have not found any advantage in using other methods over the two just described, and I use the second or Buchner's method to grow anaerobic microbes on the slanting surface of solid media.

CHAPTER VI

GENERAL CHARACTERS OF BACTERIA

Bacteria are minute organisms not containing chlorophyll, and multiplying by fision—hence the term *schizomycetes* (v. Nägeli). They are composed of a kind of protoplasm, the mycoprotein of Nencki, and are invested with a membrane, which is composed chiefly of cellulose and a certain amount of mycoprotein (Nencki).

Their contents are transparent and clear, but sometimes contain minute bright granules of sulphur (Beggiatoa). Owing to the cellulose membrane they resist the action of acids and alkalies. Under favourable conditions of growth bacteria are able by rapid multiplication to form colonies; the individuals are then embedded in a hyaline gelatinous matrix produced by them; this is also mycoprotein. Some species are possessed of one, two or more straight or wavy or spiral cilia or flagella, and thereby they are capable of locomotion; some darting through or spinning round in the fluid in which they are suspended. Such is the case with some kinds of bacilli and spirilla as will be described later.

Bacteria grow best when left undisturbed in the dark; movement of the vessel in which they grow is not advantageous. Light and electricity do not appear to have a

decided influence on some bacteria, since they grow well in the light, while on others diffuse daylight, and still more decidedly direct sunlight has a strongly deleterious effect. According to Cohn and Mendelssohn,[1] strong electric currents have a noxious influence on the growth of micrococci.

Engelmann [2] describes a bacterium photometricum, the motility of which directly depends on light; it ceases in the dark. Duclaux found that exposure to direct sunlight injures the life and growth of some bacteria, both septic and pathogenic.

The powerful inhibitory influence which insolation has on the growth and life of aerobic bacteria has been first investigated by Duclaux, then by Downes and Lunt, and more recently by Buchner, Marshall Ward and others. This latter observer was the first to demonstrate the important differences of action that exist in the red and blue end of the spectrum, the latter acting more decidedly bactericidal than the former. Dr. Westbrooke made the important contribution to this subject by showing that this germicidal action of sunlight depends on, or rather comes into play during free supply of oxygen, that this action is absent when oxygen is absent (or for instance in the case of anaerobic microbes which grow only in absence of oxygen); he further suggests that in the case of aerobic bacteria the germicidal influence of light may be due to oxydising or ozonising influences.

Bacteria may be roughly divided after Pasteur into two great groups, according to whether they grow under, and require free access of oxygen—*aerobic*, or whether they can do and grow better without it—*anaerobic*. On more

[1] Cohn's *Beitr. z. Biol. d. Pfl.* Bd. iii. 1.
[2] *Unters. aus. d. physiol. Labor.* Utrecht, 1882.

careful examination, however, it is found that while some bacteria cannot at all or only very feebly grow in air (or oxygen), there are others which cannot at all or only very feebly grow without it: the first are obligatory anaerobic, the second obligatory aerobic. Further it is found that some bacteria can grow fairly well without oxygen, but grow very much faster and more copiously under free access of oxygen (air); these are facultative anaerobic; while other bacteria can grow fairly well under free access of air but grow much better without it; these are facultative aerobic.

Thus the bacillus of malignant oedema, quarter evil or symptomatic anthrax, of tetanus, clostridium butyricum are obligatory anaerobic, though also these are capable of becoming more or less accustomed by subcultures to grow on the surface under access of air.

The bacillus of anthrax, bacillus mesentericus, bacillus prodigiosus, bacillus tuberculosis, bacillus coli and typhosus, bacillus diphtheriae, vibrio of cholera and many others grow best aerobically and show either no growth or only very feebly so in the absence of free oxygen.

For a large number of bacteria it is difficult to assign a correct place amongst the facultative anaerobic or facultative aerobic bacteria, because the boundary line between these is somewhat ill defined.

The growth and multiplication of bacteria is *cæteris paribus* principally influenced by the nature of the nutritive medium. Since the substance of bacteria contains proteid, all bacteria obviously require for their growth and multiplication nitrogenous matter which in most instances of pathogenic bacteria must be of the nature of albumin. While there are bacteria which can exist on extremely simple nitrogenous matter—*e.g.* ammonium carbonate—as is the case with the nitrifying microbes, there are others

which can obtain this nitrogen from air and from nitrates, —*e.g.* bacillus radicicola—a bacterium which forms part of the substance of the nodules on the roots of leguminosae; other bacteria can exist on organic nitrogen in low composition —*e.g.* ammonium tartrate (in Pasteur's and Cohn's fluid); or urea (micrococcus ureae and other bacteria that thrive

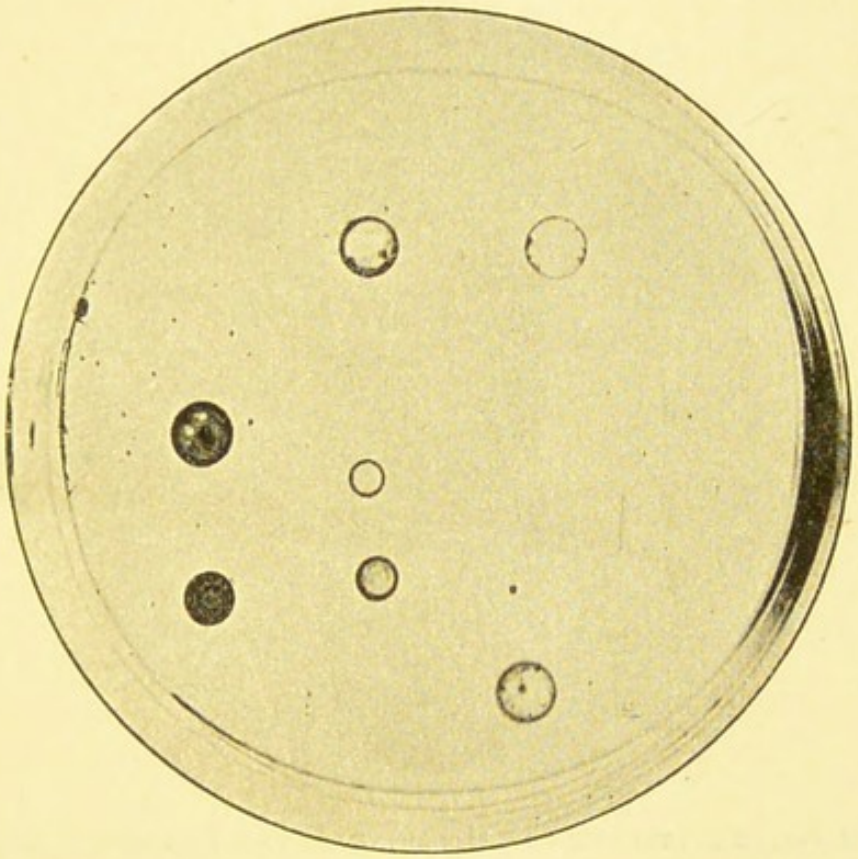

FIG. 15.—GELATINE PLATE-CULTIVATION OF BACILLUS RADICICOLA, THE COLONIES ARE LIQUEFYING.
Natural Size.

in urine). Most bacteria thrive well in media like the usual culture media containing albuminous substances. But also in this latter case great differences exist; while for instance, the bacteria occurring in water (bacillus fluorescens liquescens, bacillus sulcatus and others) can even when the water contains only traces of albuminous matter, well thrive therein and under

favourable conditions of temperature can rapidly and strikingly multiply, there are bacteria which under ordinary conditions live on rich albuminous food and for their multiplication require a comparatively large amount of albuminous matter. Of this nature are most pathogenic bacteria, for their natural breeding-ground are the animal

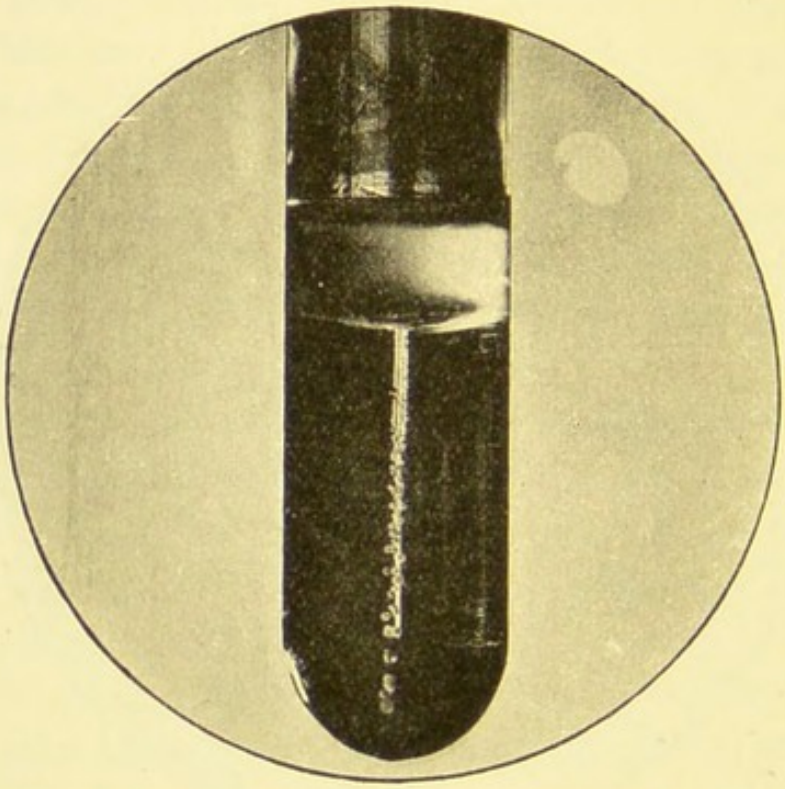

FIG. 16.—STAB-CULTIVATION IN GELATINE OF THE LIQUEFYING BACILLUS RADICICOLA.
Natural Size.

tissues, and the preparation of all our culture media previously described is based on this fact.

All nutritive media must contain salts (sodium or potassium salts), in some cases the addition of particular salts (nitrates, phosphates) enhances the growth. Some bacteria require other special additions—*e.g.* grape sugar in the case of bacillus of Koch's malignant oedema, bacillus of

symptomatic anthrax, bacillus of tetanus; asparagin and sodium salt in the case of phosphorescent bacteria (Beyrinck), milk sugar in the case of bacterium lactis.

The nature of the nutritive medium has in many cases an important effect not only on the morphology but also on the physiological action of bacteria in general, and of pathogenic

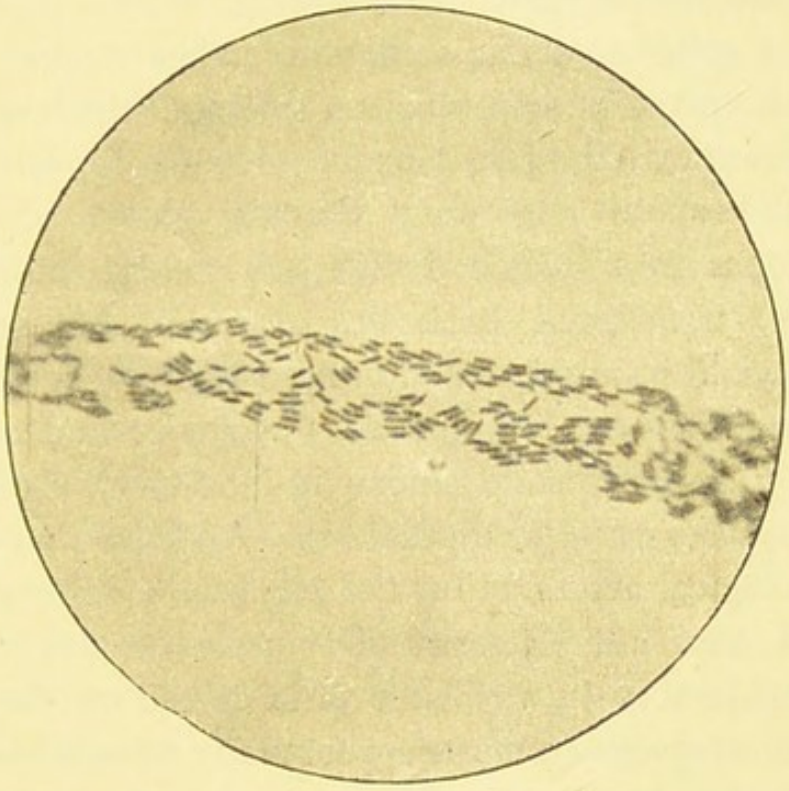

FIG. 17.—IMPRESSION OF A VERY YOUNG GROWTH OF BACILLUS RADICICOLA.
× 1000

bacteria especially. Thus, for instance, the addition of excess of salt to nutritive gelatine affects considerably the morphology of a series of bacteria—*e.g.* bacillus coli and its varieties. This in ordinary gelatine are mostly short oval bacteria, some are cylindrical, and few even threadlike, but if an excess of chloride of sodium is added most of these bacteria grow out into threads, some of great length. Or

take bacillus anthracis, this bacillus in nutritive gelatine (beef broth, peptone, gelatine) often forms already during the first twenty-four to forty-eight hours torula-like chains and filaments, of which the elements are spindle-shaped or pear-shaped. Bacillus diphtheriae grown on nutritive Agar, forms already after forty-eight hours longish jointed filaments, whereas in gelatine most of the bacilli are short cylindrical. The cholera vibrios forms in fluid media in a few days longish spirals, on solid media it sometimes takes weeks. I have isolated from the human tonsils a microbe which, grown on blood serum or Agar forms exquisite commas, semicircular forms, and spiral and wavy threads, grown on gelatine the microbes are rod-shaped with just a faint indication of curvature, transferred back on to serum or Agar they promptly yield commas, semicircles, and spirals. Some species of streptococci—*e.g.* streptococcus pyogenes and erysipelas form long and exquisite chains in fluid, very short chains and diplococci on solid media, &c. And the same applies to physiological action; thus the tubercle bacillus grown on glycerine Agar after a series of transfers on glycerine Agar loses considerably in virulence when tested on the guinea-pig, while when grown on serum for many generations retains its full virulence. When it is kept growing on glycerine Agar for a considerable series of generations it loses almost entirely its virulence, but when so weakened it is replanted in glycerine serum it soon regains it.

Many instances can be mentioned when similar alterations in physiological functions of bacteria take place differing with the medium in which they are grown.

The temperatures at which bacteria best grow show considerable differences: (*a*) while some grow best at temperatures at or below 20 or 21° C., and do not grow at all, or only very feebly, above these—*e.g.* bacillus prodigiosus, vibrio

Finkler, certain water bacteria (bacillus sulcatus); (*b*) the majority grow well not only at low temperatures but grow best at temperatures above 22° C.; and a still further group (*c*) comprises bacteria which do not grow at all or only very feebly at temperatures below 22° C. To the latter class belong the pneumococcus of Fraenkel, the bacillus tuberculosis. Amongst groups *b* and *c* the optimum temperatures lie between 28 and 38° C. Higher temperatures than 38° C. have on these two groups a retarding influence, which is feeble one or two degrees above this figure, but becomes in many cases pronounced above 40° C. But this is not the case with all, since, for instance, bacillus anthracis and bacillus tuberculosis at 42·5 or even 43° C. show still copious growth.

Miquel described one species of spore-forming bacillus, which, strange to say, has its natural habitat in soil, the bacillus termophilus; it grows well at temperatures at which other bacteria are prevented from growing, injured and even killed; this bacillus termophilus grows well at 65° C., and forms spores at 70–75° C.

In all cases of bacteria that do not form spores—and the majority of species are of this kind—an exposure to a temperature of 60–70° C. for ten to thirty minutes devitalises them, but there are slight differences to be noticed—*e.g.* whereas the bacillus coli is not killed by exposure for five minutes to a temperature of 62° C., the typhoid bacillus is killed under these conditions. All non-sporing bacteria (also those that are capable of forming spores but do not contain them, or had not formed them yet in particular cultures) are invariably killed when exposed for five to ten minutes to a temperature of 70° C.

Spores of bacilli are not killed even by an exposure to 98° C. for a minute or two; there exist differences in this, for while the spores of bacillus anthracis are killed at 100° C.

in half a minute, those of bacillus subtilis (hay bacillus) are not killed at this temperature in less than five to seven minutes, and some spores (in some species of bacillus mesentericus) require ten and even fifteen minutes' exposure.

Owing to the great resistance of spores to heat it is possible and is easy in a mixture of non-sporing and sporing bacilli to separate the former from the latter, by subjecting the fluid containing the mixture to a temperature of 70° C. for five or ten minutes, here by all non-sporing forms are killed whereas the spores remain unharmed, and cultures made from the so heated mixture produce growths of the spores only.

I will take a case in point. If a guinea-pig be injected subcutaneously in the groin with a fair quantity of recently manured garden earth, it dies as a rule in twenty-four to thirty-six hours from Koch's malignant oedema; the malodorous sanguineous fluid in the subcutaneous tissue of the groin, thigh, abdomen, and chest contains large numbers of motile and non-motile bacilli, some of these latter containing bright oval spores; the fluid is collected in a sterile test-tube and this is kept exposed for ten minutes to a temperature of 70° C., then anaerobic cultures are made in grape sugar gelatine by deep inoculation. The tubes are then sealed and incubated at 20° C. The growth that makes its appearance now is a pure growth of the anaerobic, liquefying bacillus of Koch's malignant oedema. But if a culture be made with the subcutaneous fluid not heated, generally only a copious growth of a bacillus similar to the bacillus coli is produced, at best an abundance of the latter and a very scarce growth of the former (Koch's malignant oedema) is produced. And the same method of separating the spores of any other microbe: anthrax, symptomatic anthrax, tetanus bacillus, butyricus from a non-sporing microbe that happened to be coexistent in a given material:

solids and fluids, as exudations, water, cultures, tissues, &c., can be successfully employed.

Growth and Division.[1]—The rapidity with which bacteria grow and multiply is subject to very great variations, and *cæteris paribus* constitutes definite and characteristic peculiarities; that is to say, some species under the same conditions of soil, temperature, &c., show a more rapid growth and multiplication than others, these bearing no relation to any known condition. Thus of the staphylococcus aureus and the streptococcus pyogenes, growing under exactly the same conditions, and on good nutritive media, the former shows incomparably greater rapidity in multiplication, and produces much more copious growth in a given time than the latter; or if the bacillus of swine erysipelas and the bacillus of swine fever be taken, the latter is found to grow much more rapidly than the former; and, again, bacillus subtilis and Finkler's spirillum grow very much faster than bacillus anthracis and cholera spirillum respectively.

Comparative experiments which the writer has made with a number of microbes as to the rapidity of multiplication, by way of observing them directly under the microscope in a drop of solidified nutrient gelatine at 22° C. ("suspended solid drop") show as the average of several observations—

(*a*) The streptococcus pyogenes. Complete division of the cocci took place in thirty minutes.

(*b*) The staphylococcus aureus liquescens in twenty minutes.

(*c*) The streptococcus of erysipelas in forty-five minutes.

(*d*) An orange-coloured non-liquefying micrococcus in forty minutes.

[1] From Klein's article "Infectious Diseases" in Stevenson and Murphy's *Treatise on Hygiene*, &c., vol. ii., pp. 19–23.

(*e*) The bacillus anthracis in thirty minutes.

(*f*) The bacillus subtilis of hay infusion in twenty minutes.

(*g*) A filamentous bacillus liquefying gelatine, not mobile and isolated from sewage, in eighteen minutes.

(*h*) A mobile bacillus (bacillus fluorescens liquescens), rapidly liquefying gelatine and common in ordinary London drinking water, in eighteen minutes.

(*i*) A bacillus, non-mobile, non-liquefying, rapidly forming spores, and slightly filamentous, isolated from London sewage, in forty minutes.

(*j*) The bacillus of the Middlesbrough pneumonia in eighteen minutes.

(*k*) The bacillus of fowl enteritis in twenty-four minutes.

(*l*) The bacillus of typhoid fever in thirty minutes.

(*m*) The bacillus diptheriæ in forty-five minutes.

In all these instances a single organism lying isolated was focussed and watched, and, after a distinct division had been noticed, the time was marked, and the interval it took for one of these to again completely divide was taken as the time for a division. In these observations, which do not claim more than approximate accuracy, it was remarked that the division of the two members of the dumb-bell cocci or dumb-bell rods does not proceed at the same rate, the difference being as much as a quarter to a third of the whole time. The above numbers indicate the average of three successive divisions, and therefore they only represent approximately the main periods that these several microbes require for dividing under the above conditions. Buchner (*Centralbl. für Bact. und Parasit.* II. No. 1) calculated the time required for the cholera vibrio for a division at 37° C., and found it to amount to twenty minutes on an average.

Observations were made on the common staphylococcus pyogenes aureus, the bacillus of swine fever, the bacillus of grouse disease, the bacillus of fowl enteritis, and the bacillus of diphtheria, as to the amount of multiplication these several microbes undergo when a definite number of them is introduced into faintly alkaline beef broth (eight to ten cubic centimetres), and kept in the incubator at about 37° C. All these different organisms grow with great rapidity, and after twenty-four hours the broth is uniformly turbid, provided the number introduced at starting be comparatively large. By making gelatine plate-cultivations with a given small quantity of the broth previously diluted to a definite degree, and then counting the number of colonies that make their appearance on incubation, it is easy to calculate the number of microbes present per cubic centimetre in the broth. In some experiments made with the staphylococcus pyogenes aureus it was found that on introducing 248 microbes per cubic centimetre, they increased in the first twenty-four hours to 20,000,000 per cubic centimetre; in another experiment 640,000 per cubic centimetre were counted after the first twenty-four hours' growth, 248,000,000 per cubic centimetre after the second twenty-four hours—*i.e.* after forty-eight hours' incubation, and 1,184,000,000 per cubic centimetre after the third twenty-four hours—*i.e.* after seventy-two hours' incubation. From a number of experiments it was calculated that for each microbe introduced, the multiplication during the first twenty-four hours is 80,000-fold, during the second twenty-four hours 400-fold, and during the third twenty-four hours 5-fold.

The rapidity of the growth and multiplication of the bacillus of fowl cholera in the living blood was ascertained in an experiment made on a rabbit. Of the microbes

20,000 were subcutaneously injected into a rabbit. The animal died in about twenty hours. The bacilli in the heart's blood were then counted by the ordinary method of gelatine plate-cultivation, and it was found that their number per cubic centimetre of heart's blood amounted to 14,150,000. The weight of the rabbit was 1,250 grammes, and taking eighty-three grammes ($\frac{1}{15}$) as the amount of blood present in the animal's body, and assuming that the bacilli were more or less uniformly distributed through the blood, it follows that the total blood contained about 1,200,000,000 of the bacilli. This would mean that each one of the 20,000 bacilli injected had given origin to a host of 60,000 bacilli in twenty hours.

The manner in which the individuals of the same species divide varies considerably; thus in the streptococcus scarlatinae and str. pyogenes the writer has observed that in gelatine some of the elements of a colony increase rapidly to five, six, and more times the size of a typical coccus, grow, in fact, into a ball of great size, then a cleft appears by which the organism splits up into two demilunes, then each of these again divides under a right angle to the former line of division, so that the original ball is divided into four quarters, each of which separates gradually from its neighbour and becomes more or less spherical, and a further division into two, and even into four, cocci of the average size takes place. But the above mode of division does not take place everywhere in the preparation, for many of the typical cocci only slightly enlarge and then divide into two, thus forming a diplococcus; each of these divides again transversely, and thus a chain of four minute cocci is the result.

In broth cultures the writer has observed, as a rule, the latter mode of division, though also here occasionally an

element is noticed in a chain which is much larger than the rest, and this larger element divides into two and four cocci successively. So also those large elements described above as occurring in the chains of streptococci show the successive fission into two and four cocci. And it is this which prompts him to say that these large elements found occasionally in the chains or in the diplococci are not involution forms, but are active elements which before successively dividing grow up to large size. In staphylococcus aureus liquescens, growing in gelatine, he has also observed some of these large elements, though on the whole they are not so numerous as in the streptococcus growing in the same kind of medium. The normal mode of division of a coccus is then (1) a slight enlargement and division into two by transverse fissure, or (2) a coccus enlarged to considerable size (four to six and more times) and then successively divided into two and four and further eight cocci of the normal size.

As regards bacilli all observations hitherto recorded agree that a rod before dividing elongates sometimes more sometimes less, and then a transverse indentation appears about midway, which ultimately becomes a fissure by which the originally single rod divides into two; according as the rod was short or long, the resulting offsprings are more coccus-like or more cylindrical. Now, in the observations which the writer has carried out as to the time of the division of the different microbes mentioned above the writer has repeatedly noticed that a single cylindrical bacillus not infrequently divides almost simultaneously into three and even four short rods. The writer has observed cylindrical bacilli in preparations of bacillus anthracis, made directly from the blood of guinea-pigs, which were uniform, and there was no indication in the fresh specimen that they

were other than single elements. These elements he has seen to give origin almost simultaneously to as many as four short slightly rod-shaped elements; and these same elements were, on continued observation, seen to elongate and the terminals within several minutes seen to have increased almost to twice their length, then each of them again to have divided, one into three, the other into two distinct rods.

Observations were carried out on a filamentous bacillus isolated from sewage liquefying gelatine as a clear fluid; it was non-motile, rapidly growing into threads, and in the filaments copious spore formation took place; this bacillus resembled morphologically the bacillus anthracis, but it grows on the surface of gelatine more as a continuous membrane of threads arranged parallel and coming off at right angle from a central stalk; it grows much more rapidly than the bacillus anthracis.

Now, directly observing under the microscope the growth and multiplication of this bacillus in solidified gelatine, threads of bacilli are seen shooting out with considerable rapidity from a short cylindrical bacillus measuring 0·5μ, to 1μ, a thread more or less wavy is formed in the course of two hours and a half, which extends across the whole field of the microscope under a magnifying power of 500. On such a growing thread the simultaneous division after elongation of cylindrical elements into three and four rods is also distinctly and repeatedly noticed.

Also on the rods of the bacillus of diphtheria the same simultaneous fission of elementary cylindrical cells into two, three, and four elements was noticed. We conclude then that in the division of bacilli the elements increase in length and then by transverse fission divide into two, three, or four elements, and according to the length of the cell before

division the elements resulting from the division differ in length.

Spores.—One of the most important and interesting phenomena in the life-history of bacteria is the power of some species to form *permanent seeds or spores*, by which the species can preserve itself and can withstand a variety of adverse circumstances. Various conditions in nature are often at play, in consequence of which weaker species are less liable to survive in the severe struggle for existence. There is first the adverse circumstance of competition, such as constantly obtains under the general conditions of growth in soil, in water, and in various organic materials exposed to contamination from air, water, and soil. Here numerous species find access and multiply, some more, others less easily, till all the available nutriment is exhausted. Some species, capable of forming spores, when this stage of the exhaustion of the nutriment has been reached, remain as spores and, till they are transferred by some means or other to new material, or till new nutriment is added, retain their power of again germinating and giving rise to a new crop of the same species, and this survival occurs even under severe adverse circumstances—*e.g.*, the presence of various noxious chemicals, cold, heat, drying, &c.; but those species that do not form spores retain life only under exceptionally favourable conditions: as a rule, owing to the presence of acids or other chemicals, *e.g.*, products of the growth of bacteria, and owing to drying, &c., they are easily deprived of life. This question of the formation of spores for the above reasons plays a most prominent *rôle* as regards infectious diseases. A few illustrations will easily show this. Take, for instance, the bacillus anthracis. This organism, although present in enormous numbers in the blood and blood-vessels of

animals dead of the disease, does not at any time form spores when kept away from the air—*i.e.*, from a supply of oxygen; consequently in such an animal when left unopened all the bacilli, after having gone on increasing in numbers after death for some time, gradually degenerate and disappear, so that sometimes after five to eight days in the case of small animals like mice and guinea-pigs, living anthrax bacilli are no longer to be found in the tissues, they having been suppressed by putrefactive organisms. The spleen of such an animal after this distance of time produces no infection with anthrax after inoculating it in comparatively large doses into a fresh guinea-pig or mouse; whereas if a trace of a droplet of the splenic blood of an animal dead of anthrax is used for inoculation of a guinea-pig or mouse, say within three days after death, virulent anthrax follows. Or if the blood and tissues of an animal dead of virulent anthrax are by some means or other thoroughly dried, such blood loses all virulent power, since by thorough drying the bacilli anthracis are killed. But let either the blood or the nasal or other discharges of an animal dead from anthrax be exposed to air for a sufficient time to allow the bacilli to form spores, then neither putrefaction, nor drying, nor chemical agencies such as acids and alkalies, will affect the power of these spores to germinate again into bacilli and to produce virulent anthrax when finding access to a suitable animal body. This is actually the case when cattle and sheep are sojourning on and feeding in a field, where months or even years previously an animal having died from anthrax, the blood and discharges of such an animal found access to the surface of the soil, that is where the bacilli anthracis find opportunity to multiply and to form spores. It is these spores which afterwards are picked up by the animals grazing in such a field. The same thing occurs in wool-

sorters' and hide-sorters' disease, which is virulent anthrax in the human beings engaged in the sorting of wool or the handling of hides derived from animals—sheep, goats, and cattle respectively—which had succumbed to fatal anthrax. In these cases it is always spores of the bacillus anthracis which are the cause of infection of the human beings handling these articles.

Observing bacilli, which do form spores (*e.g.* bacillus subtilis, various species of "potato bacillus," bacillus mesentericus, bacillus anthracis, and the bacillus filamentosus above mentioned), it is noticed that the first sign of the appearances of spores is indicated by the presence of a bright, glistening globule in the protoplasm of the bacillus; at the same time the bacillus is distinctly broader and paler in its substance as compared with the other bacilli. This globule gradually enlarges in diameter, becoming at the same time slightly oval; this continues till the thickness of the globule often exceeds the breadth of the bacillus, this latter being now markedly pale and transparent. The writer has watched in bacillus anthracis and bacillus filamentosus the spores from their first appearance as bright globules till they had reached their full thickness and length; this took about three hours, and he has also noticed that after sowing on the surface of solidified agar the blood of the heart or spleen of a guinea-pig dead of anthrax and keeping it under observation at the temperature of 20° C. spores would be noticed in a few of the bacillary filaments after twelve hours; in the case of the bacillus subtilis, various potato bacilli, and bacillus filamentosus growing in broth, copious spore formation was noticed in a superficial pellicle after sixteen hours. Koch first observed that spore formation in bacillus anthracis occurred after six hours. But not all bright granules that

make their appearance in bacilli are spores; thus in the typhoid bacillus growing on potato, and in other species of bacilli growing on potato, there appear bright granules, either terminally or centrally, which are not spores; they do not show the reaction of spores, either to dyes, or on drying, or heating. Nor are all the bright granules that make their appearance in bacilli capable of forming spores to be at once taken as spores, since under certain conditions such granules do occur, but never reach the size of full spores; this is observed occasionally in anthrax bacilli when growing under conditions unfavourable for the formation and development of spores. The appearance of real spores in all bacillary species is very characteristic; the spores are of a bright, glistening aspect, are oval in shape, and generally thicker than the typical bacilli. The substance of these latter is at the same time pale and transparent, and broader than the bacilli not containing spores; the threads of the bacilli appear beaded by the spores, the beads being the glistening oval thick spores, while the rest of the thread is pale and appears thinner. In the single bacilli the spores are placed either centrally or terminally; in the latter case, if the bacillus is of some length, it looks not unlike a spermatozoon, the spore corresponding to the head, the bacillus to the tail. Sometimes in motile bacilli short chains are noticed, in which, in one terminal element, a spore has already made its appearance, while the other bacillus is still possessed of motility; and here, on account of the motility, the resemblance to a spermatozoon is still more striking. Under the most favourable conditions almost every element constituting a bacillary thread or chain forms a spore, in other threads only here and there a cell contains a spore; in the first case the thread is regularly and densely beaded, in the latter the beads are

relatively few and far between. The first phase is reached when the bacillus itself swells up into a gelatinous capsule enveloping the spore and ultimately [illegible] then the spore is free and has reached its full size and development. Examining in stained [illegible] spore-bearing bacilli, the spores appear unstained, whereas the rest of the bacillary substance takes readily the dye; under these conditions the spore looks like an oval clear space, not unlike the vacuoles above mentioned; but the spore has a sharp outline of its own, the vacuole has not. It is, however, not easy to distinguish in a given specimen, stained after the ordinary methods, the spores which are not stained from vacuoles, and in these cases other methods of staining must be resorted to.

In order, then, to decide whether or not spores are present in a bacillary species, the morphological investigation, fresh aspect, special methods of staining, drying, and heating have to be resorted to.

The spore formation is associated with supply of oxygen in all bacteria that generally live well under access of air; in some species this is more pronounced than in others, for in some species—*e.g.* bacillus anthracis, and bacillus filamentosus, spores are only formed—*cæteris paribus*—if oxygen has free access, and no spores are formed if there is no free supply of oxygen—*e.g.*, deep in the fluid; while in other species, though spore formation is greatly enhanced by the free access of oxygen, it nevertheless takes place to a certain limited extent deep in the fluids. Thus, in the case of bacillus subtilis and various other bacilli a pellicle soon makes its appearance on the surface of the fluid (broth, &c.). This pellicle is made up of filaments and bacilli matted together, and in them copious spore formation is going on, but also in the depth there are a few spore-containing bacilli

to be noticed. When such a pellicle is broken up by shaking, it in most instances falls to the bottom of the fluid, and then after another day's growth a new pellicle appears, and in this also copious spore formation is noticed; and this can be repeated for several days till the nutriment is exhausted. The same can be seen in hay infusion, in the case of bacillus subtilis. In neutral or faintly alkaline hay infusion kept at 37° C. spores of bacillus subtilis are present in the pellicle as early as the second day, and continue to be formed till the end of eight to ten days. The view has been expressed by some observers, amongst them Buchner, that the spore formation in bacilli occurs on exhaustion of the nutritive material, but it seems that the facts just mentioned as to the continuous and successive pellicle and spore formation occurring in broth are incompatible with that assertion; and, besides, the formation of spores in other bacilli can be shown to take place long before any exhaustion of the nutritive matter is noticeable—*e.g.*, in anthrax bacilli, in the tetanus bacillus, and others. In the bacillus filamentosus, growing on Agar or on potato, the spore formation is apparent even before the first day is over and long before the active growth and multiplication of bacilli all round is finished. In some species, however, that do not thrive under free access of air (*e.g.*, oedema bacillus, tetanus bacillus) spore formation does not take place if free oxygen is present. A temperature of at least 16° C. is required for the formation of spores, though spore formation occurs in all temperatures between that and 45°; at least spore formation has been seen to occur in bacillus anthracis even at 45° C. The mode of spore formation hitherto described is called that of endo-spores, and it ought to be here stated that many species of bacilli exist in which no spore formation can be demonstrated—in this statement we rely on the morpho-

logical as well as the experimental test—*e.g.*, typhoid fever bacillus, bacillus of glanders, of diphtheria, of fowl cholera, of fowl enteritis, and many others. So far as actual demonstration is concerned no other mode of spore formation can be accepted at present. A mode of formation of spores is described by Hueppe to occur in certain spirilla, according to whom the comma-shaped elements and the spirilla form special aggregations of protoplasm in the shape of terminal granules, to which the value of spores is ascribed, and which are called arthro-spores. But the evidence and proof for this is quite unsatisfactory, and, judging these appearances in the light of the character of well-ascertained spores of other bacilli, they are contrary to the assumption of spores. These arthro-spores of Hueppe do not look like spores, do not behave in staining like spores, and do not behave in drying and heating experiments like spores. In the first place they do not differ in aspect from ordinary protoplasmic granules observable in some of these bacilli under all conditions; they stain in the ordinary dyes and after the ordinary methods like the ordinary protoplasmic contents of bacteria; and they are killed by drying and exposure to 60° C. for five minutes.

It can be easily shown that artificial cultures of these comma bacilli growing under conditions very favourable for the formation of real spores in other bacilli—*e.g.*, a good supply of oxygen, temperature, soil, and moisture—contain after some weeks and months those granules or supposed arthrospores in enormous numbers; in fact, there is almost nothing else left, and yet no subcultures can be established from such a culture, it being barren of all life. Structures have been described also in the typhoid bacilli as occurring in potato cultures, which can be, however, shown by special modes of staining to be different from real spores, and the

experimental test of drying and heating conclusively proves that they are not comparable to spores.

On the other hand the tubercle bacilli have been shown experimentally (by Koch and others) to possess spores, although it seems difficult to identify them under the microscope. True, there are present in microscopic specimens made of fresh material—*e.g.*, tubercular sputum—bright granules within many of the bacilli which might be taken for spores, and in specimens stained after the customary method of staining for tubercle bacilli numerous stained granules occur in the bacilli—the bacilli appearing beaded—but, as has been stated above, most of them are merely elementary masses of protoplasm segregated in the bacilli. They occur in some tubercle bacilli more numerously than in others—*e.g.*, in the tubercle bacilli of the human subject they are common; in the tubercular material of the fowl and in artificial cultures they are sometimes seen with great regularity, but there is no means available of identifying these granules with spores. But by thorough drying of tubercular material it can be shown that the tubercle microbes remain uninjured, and that heating them up to 100° C. for a minute leaves the tubercle bacilli unharmed.

Spores have been described also of some micrococci, but here again certain differentiation of structure cannot be taken as proving the existence of spores. The writer has examined very numerous preparations of the most varied cultures of different species of micrococci, and the test of drying and heating to 70° C. proves them barren of anything comparable to the well-known spores present in some species of bacilli. He also examined experimentally various species of spirilla and agrees with Koch that no spore formation can be demonstrated in them. We arrive then at the conclusion that real spore formation, or the formation

of permanent seeds capable of retaining life under very adverse conditions, and under favourable conditions capable of germinating and of giving origin to a new brood of the same species, can be shown to exist only in certain limited species of bacilli, but not in micrococci or spirilla. These real spores are endo-spores, they are formed within the protoplasm of the bacillary elements under favourable conditions, and they have certain definite morphological and experimental characters of their own, at the same time representing as it were the last phase in the life-history of these bacilli. Bacilli or bacteria not capable of this power of producing spores, though they go on multiplying as long as the conditions of nutriment, temperature, chemical by-products, competition, &c., permit of it, ultimately degenerate, some sooner, some later. To propagate their species they must as living bacilli find access to new soil before the stage of degeneration is reached, whereas in the spore-bearing bacteria their spores can remain dormant, but possessing potential life for indefinite periods.

The statement has been occasionally made that spores are capable of dividing, and thus giving origin to two new spores. The writer has not been able to detect anything of this sort; he has never seen any appearances that would indicate such a division. True, in bacillus anthracis, in bacillus filamentosus, and in some species of "potato bacillus," spore formation may be going on so copiously that at some places every element constituting the threads contains a spore, some of the spores closely adjoining one another, sometimes so closely that it looked as if they were the two elements of a dumb-bell, and here a division of one spore into two could be thought of; but in these places the elements of the threads were extremely short and the spore occupied the main part of each element. It is also a fact

that spherical globules occurring in some bacilli and being in aspect and staining power comparable to young phases of spores, are occasionally met with as dumb-bells within the same element, but as regards the oval, bright, unmistakable spores so prominent in the bacilli and their threads (bacillus anthracis, bacillus subtilis, bacillus filamentosus, bacillus mesentericus, &c.) it is very doubtful whether they are capable of dividing or of undergoing any other change than that of germination into bacilli when they are transferred to new soil.

Spores when placed under suitable conditions germinate again into bacilli. This is easily observed if, for instance, of any culture-material containing spores a trace is placed on a cover-glass, then covered with a tiny droplet of gelatine which is made rapidly to set, or in a droplet of broth ("suspended drop") and is then observed under the microscope, particularly in the latter medium, which can be kept on the warm stage heated to 37° C.

Spores while fresh have a conspicuously sharp and dark outline, their general aspect is glistening, and it is supposed by Cohn that they are possessed of a double envelope, an inner one of a fatty and an outer one of a gelatinous nature: it is particularly the former which provides the spores with their great resistance to drying and to heat. The first indication that the spores are going to germinate is shown by their outline becoming less sharp at one point. This is generally at one of the poles, as in the case of the spores of bacillus anthracis, bacillus filamentosus, and bacillus subtilis; or it is at one point of the long side—*e.g.*, in bacillus amylobacter, and also in the spores of some of the species collectively spoken of as "potato bacillus"; the investment seems to become thinner at that point and a slight pale knob appears there; this knob gradually elongates

in the form of a pale rod thinner than the spore itself; as it elongates it protrudes more and more from the rest of the spore, its free end being rounded, while at the same time the rest of the spore outline becomes thinner and less dark; ultimately the whole spore has been consumed as it were in the formation of the rod, which now looks like a cylindrical bacillus of the same character and aspect as the bacilli from which originally the spore had been derived. The bacillus once formed divides and then continues to grow and multiply. The time required for the production of a bacillus from a spore varies with the different species. Koch observed the germination of the spore into a bacillus anthracis to be completed in about an hour; the writer has observed the time required for the complete formation of a bacillus from a spore of the bacillus filamentosus in broth, in the "suspended drop," at 37° C. to be certainly less than one hour; that of bacillus anthracis between one hour and a half and two hours; that of the bacillus subtilis of hay infusion to be more than one half but less than one hour. Occasionally one meets in these observations with motile bacilli to which a spore which has not yet commenced to germinate is attached and is dragged about by the former: this evidently indicates that of two spores originally joined by interstitial material (*see* spores in threads) only one has already changed into a motile bacillus—the other has not yet so changed.

Motility.[1]—One of the most interesting phenomena shown by bacteria is the power of active locomotion possessed by some species. When examined under the microscope in a fluid medium all bacteria show the kind of oscillation known as Brownian molecular movement; but in some species there is

[1] Copied from Klein's article in Stevenson and Murphy, vol. ii., p. 13 and *passim*.

an active locomotion, by which the individual bacteria are enabled to move actively and to change their place; this movement shows itself either by the bacteria darting with great rapidity across the field of the microscope in one or another direction, or spinning round with greater or lesser velocity, or briskly moving like a screw in one direction and then back again. Observing a single straight bacillus in its movement, either a darting or spinning movement in one direction is noticed; when two such bacilli are connected endwise, but bent one to another under an angle, then often, with a forward or backward movement of the one, a spinning movement of the other is noticed, the former not really actively moving but being simply propelled by the spinning movement of the latter bent under an angle. When comma-bacilli or spirilla move, the motion is always more or less spiral.

When longer chains or leptothrix of bacilli move, the movement is always more or less serpentine. The locomotion of bacilli is either rapid or slow; the latter may be a character of the species, that is to say, the individuals as a rule show only a relatively slow movement—*e.g.*, typhoid bacilli generally move comparatively slowly, and the longer bacilli move in a serpentine manner. The mobile individuals do not continue to move indefinitely, since often an individual which has been spinning round or darting about gradually comes to rest and remains so for some time; besides this, all motile bacilli during the phase of division are at rest, and when they form groups—*i.e.*, when they are in an active state of division—they do not move. But of such groups here and there an individual may be seen to separate itself from the margin and to move briskly away; on breaking up a group, crowds of motile bacilli sally forth. The writer has watched single bacilli of the human Middles-

brough pneumonia spinning round with great velocity without much changing their place. One and the same bacillus was noticed to spin round for five minutes without any diminution in its velocity; then this gradually lessened, and ultimately, after further five minutes, the organism came to rest. When a drop of broth was added the spinning round commenced again with great vigour. Some mobile bacilli show motility under a certain condition and not under others; others again show it under all conditions. Thus, for instance, many individuals of the bacillus of the Middlesbrough pneumonia show active locomotion in specimens made of gelatine and Agar cultures; made of broth cultures the motility of many individuals is observable only while the broth cultures are of recent date—24–48 hours old; later on only very few motile individuals are met with. The loss of motility may be and sometimes is due to chemical by-products in the cultivation (*see* bacillus of grouse disease and of pneumonia). Some species of motile bacilli when growing on a solid medium are capable by their locomotion of distributing themselves from a given point rapidly over and through the medium—*e.g.*, certain species of bacilli known as proteus of Hauser, certain species of the potato bacilli, &c.; this phenomenon is spoken of as "swarming," thus, when a colony of such bacilli appears on gelatine, Agar mixture, or potato, irregular streaks and lines and patches of the growth are soon seen extending in different directions, this being due to the swarming of the bacilli from the first colony and by the establishment of new colonies by the former. There exist great differences in this respect between different species of motile bacteria, for while some species do not swarm at all and their colonies on solid media remain localised and more or less well defined, though they

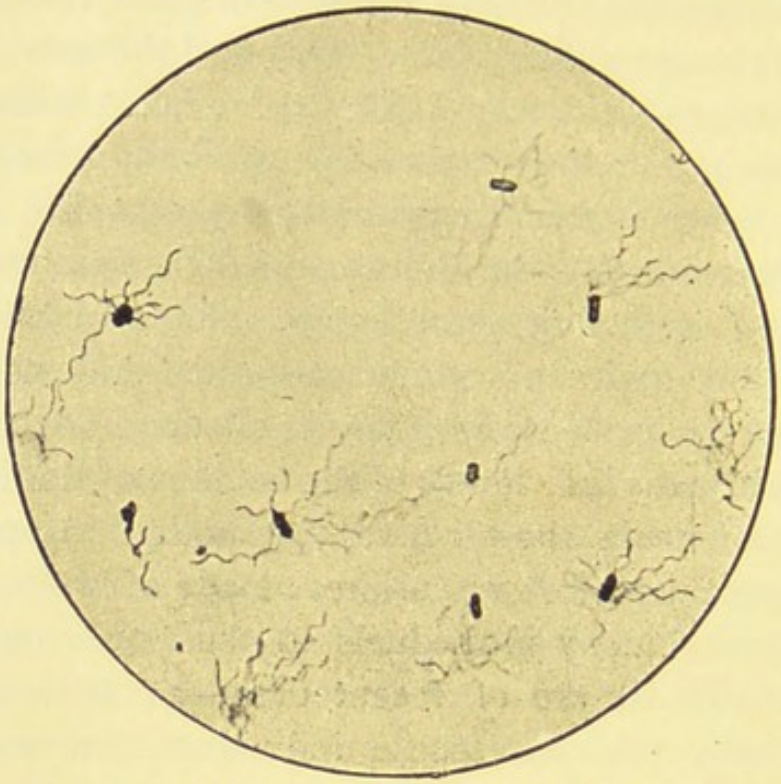

FIG. 18.—TYPHOID BACILLI, SHOWING FLAGELLA.
× 1000.

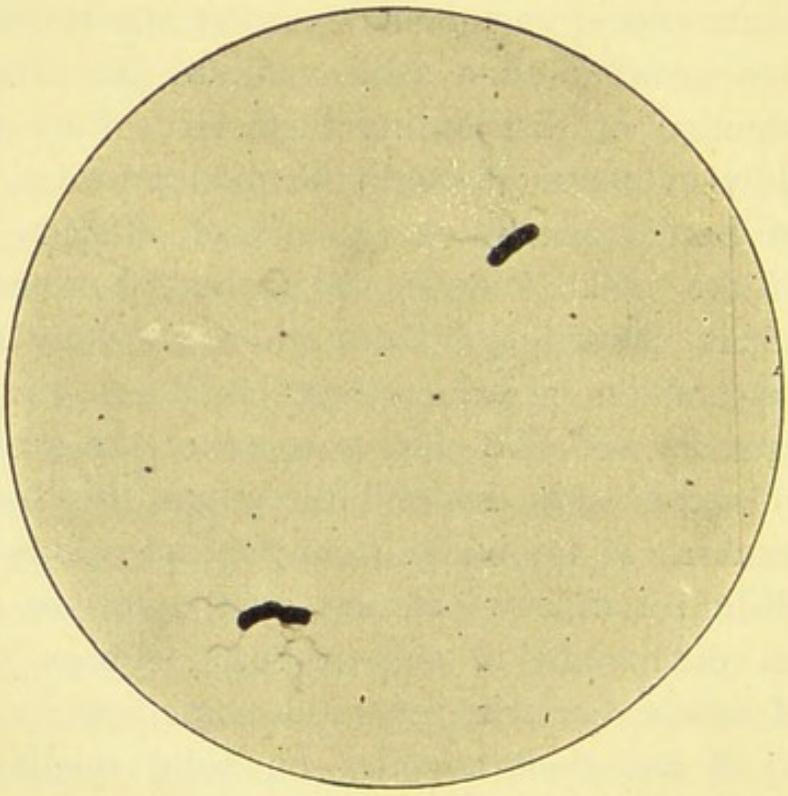

FIG. 19.—BACILLUS COLI, SHOWING FLAGELLA.
× 1000.

gradually enlarge—*e.g.*, bacillus subtilis, bacillus fluorescens, some species of proteus, and many spirilla; other species possess this swarming propensity and therefore the first colonies do not remain well defined, but gradually extend in lines and irregular streaks in different directions. But it is not correct to conclude that a bacillus is motile if its colonies do not remain defined, that is, if they extend in the shape of threads or irregular streaks on or through the medium, for there exist several well studied species which do this (*e.g.*, bacillus anthracis, bacillus filamentosus), though their bacilli are not motile, as will be more minutely described when speaking of the cultural characters of bacilli.

When certain bacilli show only slight motility it may be extremely difficult to distinguish this from Brownian molecular movement, but no locomotion can be ascribed to bacilli unless one or the other individual can be distinctly seen to show a darting or spinning movement. As mentioned above, the easiest and best way to see locomotion is to examine the fresh bacilli in a fluid, as sterile broth or sterile salt solution in the "suspended drop."

The motility of bacilli and spirilla is due to their possessing at one, and occasionally at both ends, or also over the general surface, fine flagella or cilia, the movement of which causes the motility of the microbe. Where two or more microbes are connected into a chain or thread, only the terminals have the flagella. Although the flagellum has not been stained and photographed hitherto in all bacilli and spirilla, there can be no doubt that all motile organisms do possess the flagellum, for without it motility would not be possible. Micrococci are not possessed of motility, but recently Ali-Cohen has isolated from drinking-water a species of micrococcus (*Micrococcus agilis*) which forms an

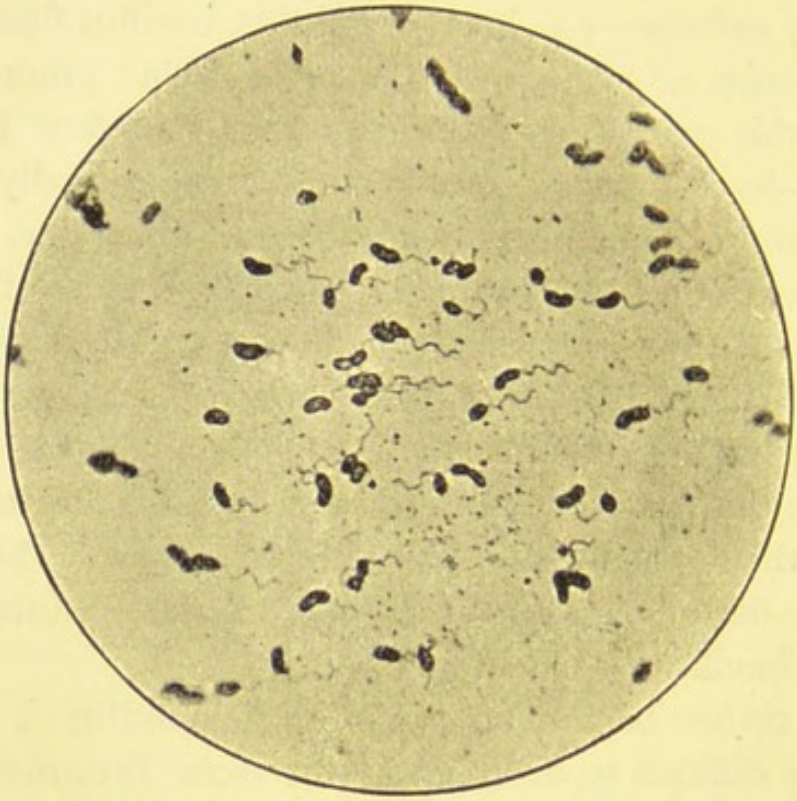

FIG. 20.—CHOLERA VIBRIO OF CULTIVATION, SHOWING FLAGELLA. × 1000.

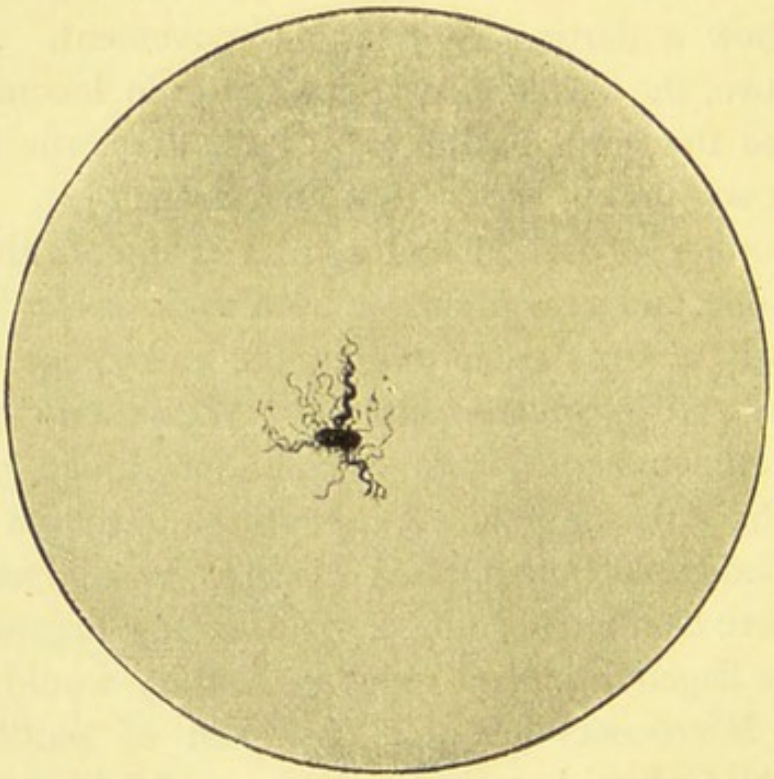

FIG. 21.—BACILLUS TETANI, WITH FLAGELLA. × 1000.

Figs. 20, 21, 22, 23 are from specimens prepared by Dr. Kanthack.

exception, since this species is motile (*Centralbl. für Bact.* VI. 2).

In aerobic bacilli and spirilla which are possessed of motility this is intimately connected with a supply of oxygen. Though some species seem to obtain this readily even when in deep fluids (*e.g.* bacillus of hay, certain species of proteus), many others cease to move when the supply of oxygen becomes insufficient. Engelmann has made some very interesting experiments with certain motile bacilli, showing the direct influence of oxygen on their motility. When motile bacilli, owing to insufficient oxygen or after the consumption of the oxygen previously present, come to rest, by adding to them new oxygen in a drop of fresh fluid containing air, the motility is resumed. On removing the oxygen and adding carbon dioxide or hydrogen gas, ammonia, chloroform, or ether, the movement ceases, but on removing these gases and replacing them again by oxygen (or air) the movement is again resumed.

Motile bacilli and spirilla when growing in a fluid medium have a great tendency to seek the surface of the fluid—*i.e.* move towards the part where they can obtain oxygen, and here form more or less coherent pellicles, in which they are in a resting state, and in which a rapid multiplication goes on; but it is quite incorrect to assume that an organism which in a fluid medium forms a pellicle is a motile organism, since some species which form a pellicle are not motile, and some species of motile organisms do not form a pellicle.

On making a comparative study of the presence of flagella best by v. Ermengem's method, two things will be found of interest: (1) that there are flagella present even in bacilli which in the fresh state show no locomotion or only a very feeble one; and (2) that the length and

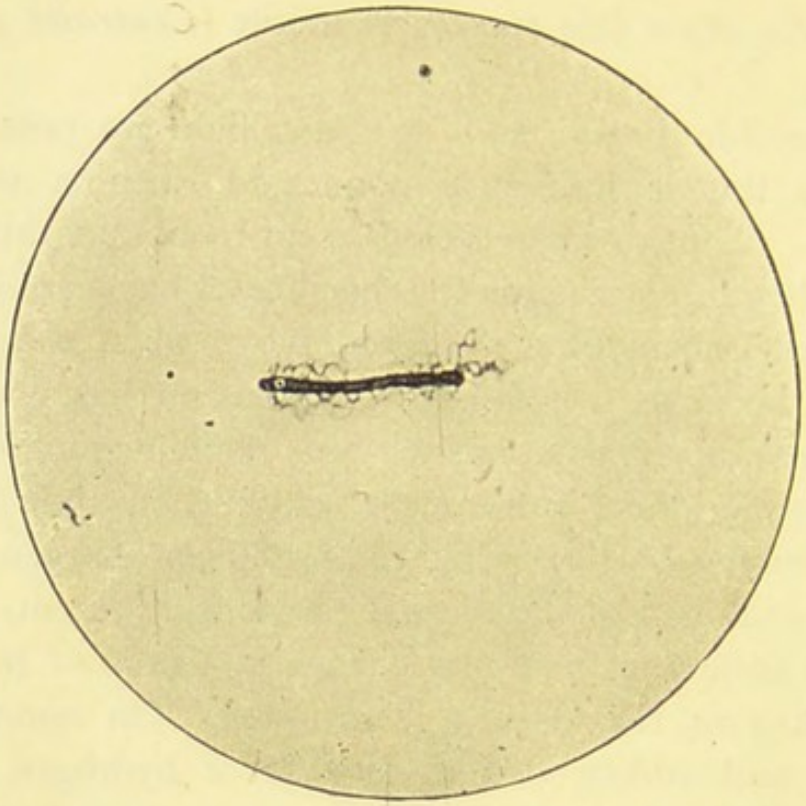

FIG. 22.—BACILLUS TETANI, SHOWING FLAGELLA. ×1000.

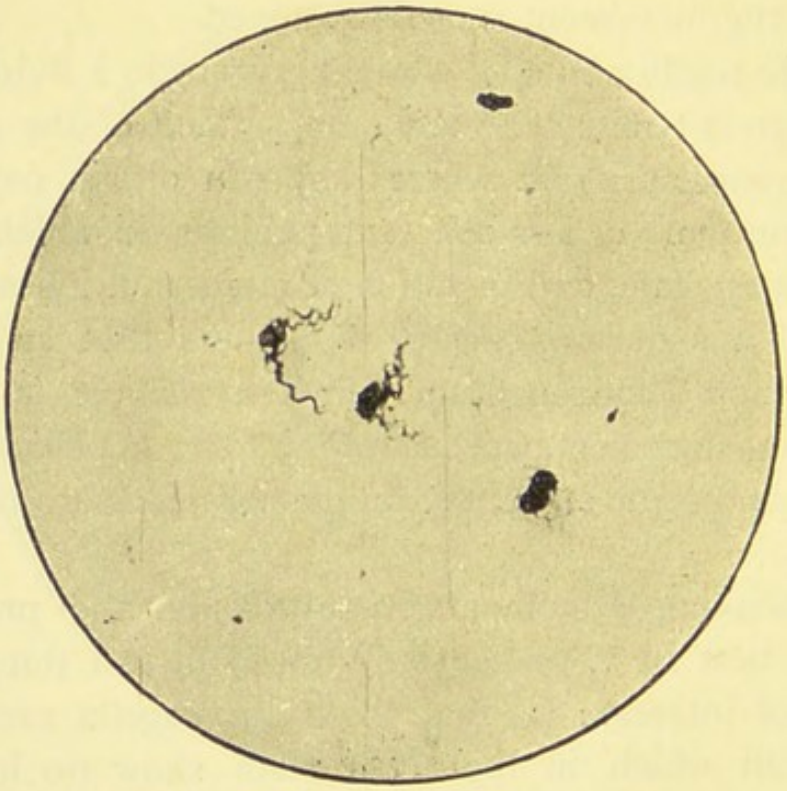

FIG. 23.—BACILLUS TETANI, SHOWING FLAGELLA. ×1000.

number of flagella stand in no definite relation to the intensity of the movement. Tetanus bacilli of a culture examined in the hanging drop show at best only sluggish motility, and yet on staining for flagella the astounding fact (see Kanthack's specimens) will appear that most of the bacilli possess at one or both ends, and on the sides, long flagella, these sometimes in bundles. I have isolated a spore-forming virulent anaerobic bacillus (bacillus enteritidis sporogenes) from the fluid evacuations of cases of epidemic diarrhœa, which is closely related to the bacillus butyricus of Botkin; it shows only feeble motility; in fact, in an ordinary fresh preparation made from a sugar gelatine culture amongst the many rod-shaped or cylindrical bacilli there is rarely one met with that shows motility. And yet when staining for flagella numerous bacilli possess flagella; one, two, three, or more, at one or both ends, some short individuals possess a bunch of flagella of extreme length (many times longer than the bacillus itself) at one end, and a few long cilia at the other. In fact, no greater misproportion between feeble motility of only a few bacilli and the frequency and number of flagella can be imagined. As to (2), from the intensity of the motility of the fresh microbes no conclusion can be drawn as to the number and length of the flagella. To mention a few examples: the cholera vibrio of a culture, though motile in a most extraordinary manner, possess only one short spiral flagellum; the very motile bacilli of proteus vulgaris possesses only one flagellum at one end; some varieties of bacillus coli extremely motile possess only two flagella, while other varieties less motile possess two, three, up to ten flagella; the tetanus bacillus and the bacillus enteritidis sporogenes are good cases in point.

CHAPTER VII

CHEMISTRY OF BACTERIA

Some of the most interesting and important manifestations of bacterial life are the chemical changes which are brought about by bacteria. They are so manifold, many of them of such a complicated character and so little understood, that it is at present impossible to arrange them in a system, or to classify them in any comprehensive scheme. All that is at present possible is to give an outline of the more obvious chemical manifestations observable during the growth of certain species or of groups of them.

1. One chemical change frequently exhibited is the power of bacteria to peptonise nutritive gelatine ; this exhibits itself as more or less rapid liquefaction of the nutritive gelatine in which growth is taking place, and as the growth proceeds liquefaction of the whole nutritive medium is effected. Many bacteria have this power : those occurring in water, in the air, in the soil : bacillus fluorescens liquescens, bacillus subtilis, bacillus mesentericus, micrococcus liquescens albus and aureus, several species of sarcina, bacillus prodigiosus, bacillus pyocyaneus, proteus vulgaris ; then many disease germs : bacillus anthracis, the (anaerobic) bacillus of symptomatic charbon, of Koch's malignant œdema (anaerobic), of

tetanus (anaerobic), bacillus enteritidis sporogenes (anaerobic), bacillus butyricus (anaerobic), Koch's cholera vibrio, vibrio of Finkler; actinomyces, aspergillus and penicillium, &c. Some liquefy the gelatine extremely slowly, the liquefied gelatine being more of the consistency of thick syrup, *e.g.* bacillus of swine-erysipelas and of Koch's mouse-septicæmia.

In the case of all aerobic microbes, which have the power to liquefy (peptonise) nutritive gelatine, this power is intimately bound up with a free supply of oxygen (air); it proceeds from, and is conspicuous on the surface, it is greatly retarded when air is excluded, and in some cases is only noticed where the growth occurs on the surface in contact with air.

But there are a good many microbes which do not peptonise, do not liquefy the gelatine: all the species forming the group of bacilli causing hæmorrhagic septicæmia in the rodents: bacillus of fowl cholera, of swine fever, of fowl enteritis, all varieties of bacillus coli, bacillus of typhoid fever, bacillus of "Wildseuche," &c.—all or nearly all (a few species excepted) species of streptococci, a number of chromogenic cocci, &c.

2. Another widespread manifestation is that of producing acid or alkali; when growing in a neutral medium, as in Petruschki's neutral whey, of turning this acid or alkaline, as the case may be, the latter being more often met with than the former (Petruschki, *Centralbl. f. Bakt. and Parasit.* 1889 and 1890.)

Buchner has first suggested a method which is very easy of employment, and which demonstrates conspicuously whether a microbe during its growth produces acid or alkali or is neutral—viz., by mixing with the nutritive medium, before steaming, a small amount of litmus tincture, sufficient to stain it bluish. The nutritive gelatine, slightly

alkaline (see a former chapter), is then inoculated with the microbe and incubated. During the growth, on inspection the gelatine next to the growth will be found to have become violet and then red if the microbe produces acid, and the more rapidly and conspicuously so, the more rapidly and more acid it produces. If the gelatine remains bluish, then no acid has been produced. In this case a neutral nutritive gelatine is prepared and mixed with neutral litmus and then inoculated with the microbe. On incubation, as the growth appears, if the violet colour of the gelatine has turned blue next to the growth then the microbe is an alkali-producer, if the gelatine remains neutral then the microbe does not produce either acid or alkali. As mentioned above, it is common to find that the microbe produces acid, some rapidly and distinctly (*e.g.* bacillus coli and typhoid), others only slowly and in small amount (*e.g.* some varieties of the vibrio of cholera). An interesting phenomenon is that many microbes—even highly specialised microbes like the glanders bacillus—grow well on potato (steamed), although the reaction of this is acid (mallic acid)—in some potatoes very pronounced, in others only very slight. Now the curious thing about it is that some of the bacteria that show rapid and good growth on potato show only very feeble or no growth if planted on an acid medium, *e.g.* acid broth or acid gelatine.

3. Some microbes have the power to liquefy and peptonise such resisting substances like solid agar and solid blood-serum, though this power is possessed only by few species. Most of the species that are capable of liquefying and peptonising gelatine leave the agar and blood-serum unaltered. The bacillus of Koch's malignant œdema, the vibrio of Finkler, the vibrio of cholera (Koch), rapidly liquefy blood-serum, but do not alter solid agar.

4. A further not uncommon phenomenon is the formation of gas (methan gas or marsh gas). This is best shown by making the inoculation into deep gelatine or by inoculating the gelatine, then melting it, shaking it, and letting it again solidify—"shake culture." On incubation every colony that appears in the depth of the gelatine is associated with a gas bubble. A shake culture of ordinary nutrient gelatine after inoculation with bacillus coli gives a very characteristic appearance, being in its deeper layers crowded with small gas bubbles. After some days they become fewer, most of them escaping to the surface. In the cultures in deep sugar-gelatine of bacillus of Koch's malignant œdema, of the bacillus of symptomatic anthrax, of tetanus, the formation of gas is a conspicuous feature. Some species of bacillus coli form copiously gas bubbles in deep nutrient agar cultures and even in broth cultures as the growth becomes conspicuous, *e.g.* after twenty-four to thirty-six hours at 37° C.; on watching the culture numerous small gas bubbles are seen to ascend to the surface.

5. A number of microbes have the power to produce in special materials specific chemical changes representing specific fermentations. The alcoholic fermentation of sugar by yeast is the best-known and longest-established instance; the acid fermentation (oxidation of alcohol) by bacterium aceti and mycoderma aceti, the change of lactic sugar into lactic acid by various species of bacterium lactis and other bacilli, is a widespread one; so also is the formation of butyric acid by bacillus butyricus (van Tighem). The hydration of urea and conversion into ammonium carbonate by micrococcus ureæ, the dextrose fermentation, the mannit fermentation, are further instances. In this category must be included the conversion of albumen into peptone, previously described. In all these instances a particular substance,

glycose or grape-sugar, alcohol, lactic sugar, urea or gum, &c., as the case may be, are by the growth of particular microbes changed in the manner of fermentation into other substances.

6 Many bacteria have the power to produce pigments: these appear either on all media on which their growth occurs, or only on particular media. In the first case the

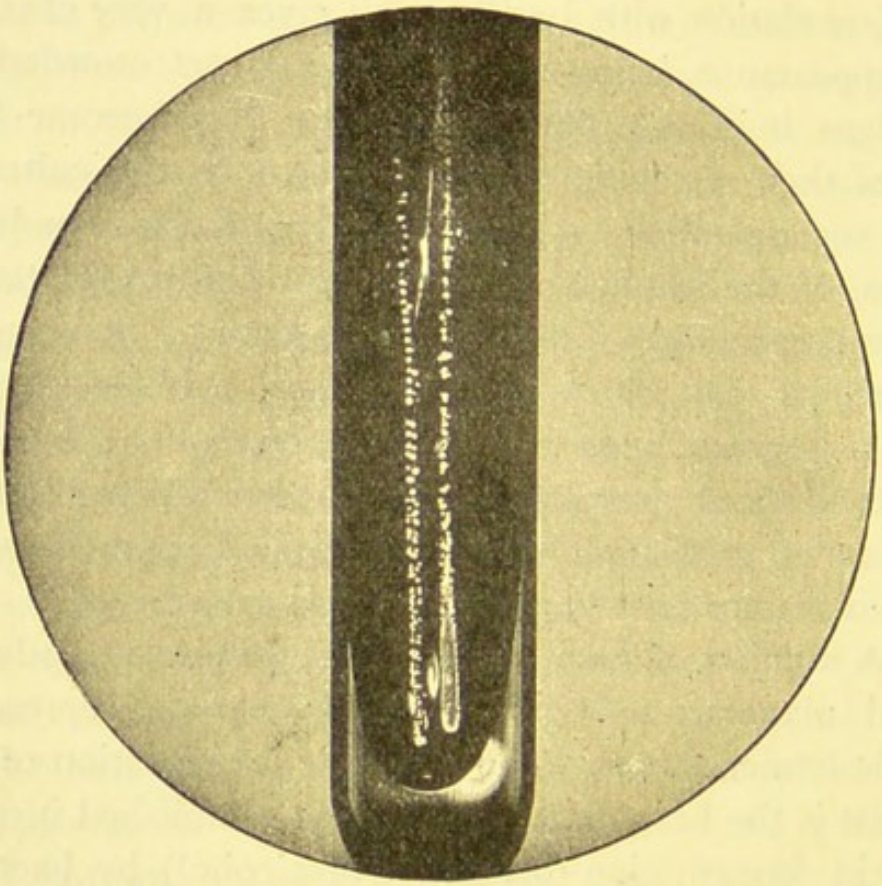

FIG 24.—SURFACE (STREAK) CULTURE ON GELATINE OF THE COMMON BACTERIUM LACTIS.

pigment formation is real, in the second only apparently so. Thus a variety of bacilli, *e.g.* bacillus subtilis, bacillus mesentericus, bacillus coli, bacillus of glanders, when growing on potato, form a brownish or yellowish-brown smeary layer, but do not produce any pigment on other media; the bacillus anthracis turns agar brownish after the growth has reached a certain long duration, &c.

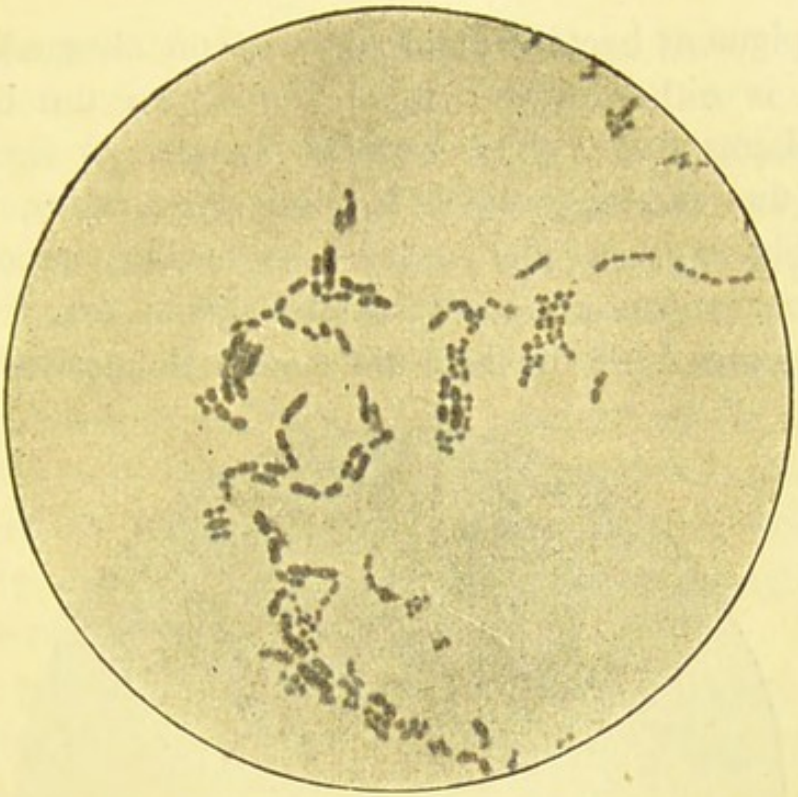

FIG. 25.—A STAINED FILM SPECIMEN OF BACTERIUM LACTIS.
× 1000.

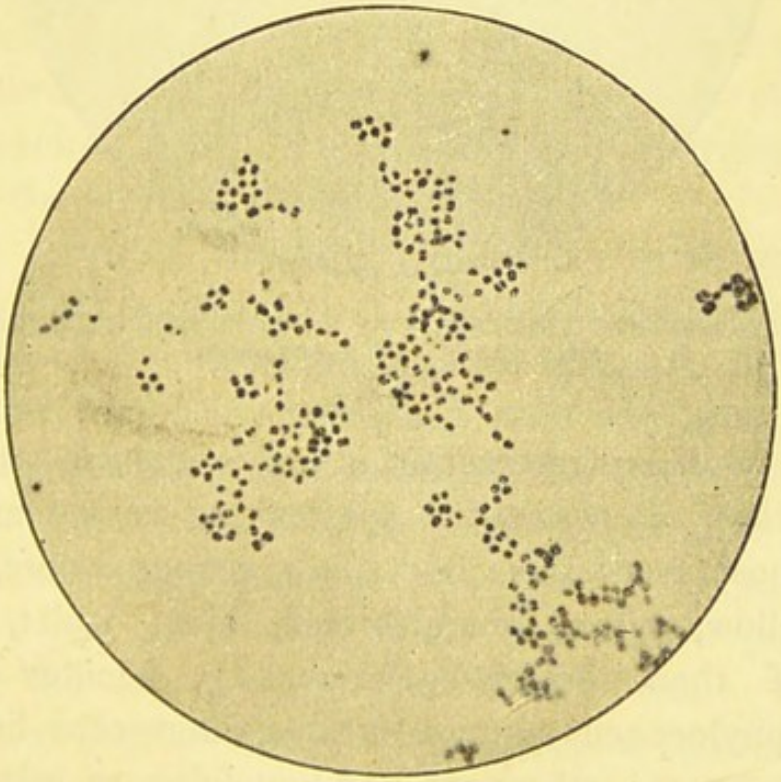

FIG. 26.—MICROCOCCUS UREÆ, FROM A GELATINE CULTURE.
× 1000.

True pigment bacteria form pigment on all media; this pigment is either diffuse or is limited to the bacterial bodies themselves; thus, bacillus fluorescens liquescens, bacillus fluorescens putidus, bacillus pyocyaneus, form a diffuse bluish-green pigment, while bacillus prodigiosus, staphylococcus aurantiacus, spirillum rubrum, &c., &c., form pigment limited to the bacterial bodies themselves. The

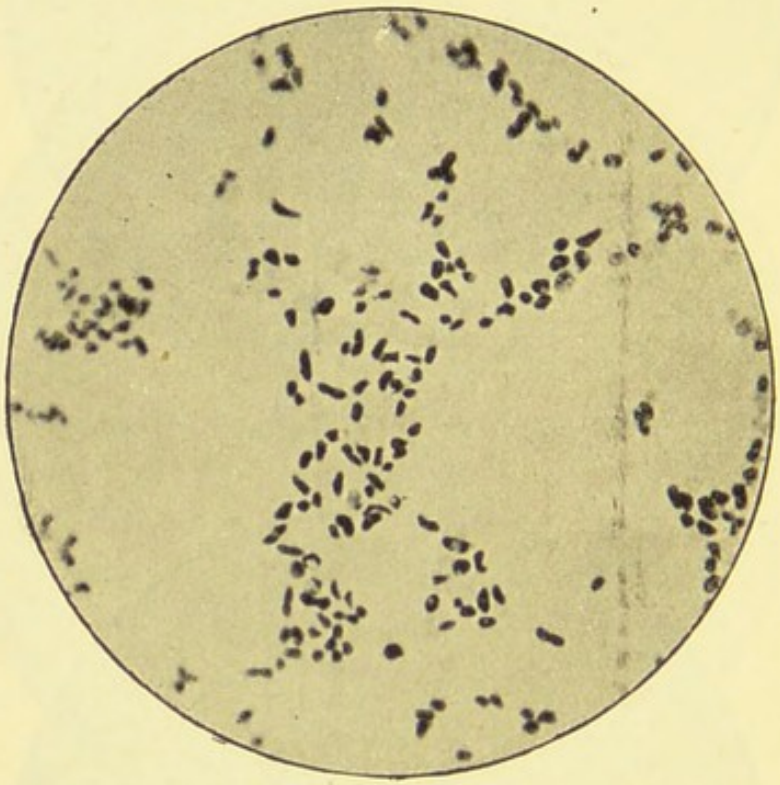

FIG. 27.—BACILLUS PHOSPHORESCENS, FILM SPECIMEN FROM A CULTURE ON GELATINE BROTH AND ASPARAGINE.
× 1000.

meaning of the pigmentation is not understood, though a large variety of pigmented species are known and comprise almost every tint: red, pink, orange, ochre, yellow, lemon-yellow, green, greenish-blue, blue, violet, purple. Some of them liquefy gelatine, *e.g.* bacillus prodigiosus, staphylococcus aureus, bacillus fluorescens liquescens and pyocyaneus; others are non-liquefying, as micrococcus aurantiacus, spirillum rubrum, bacillus fluorescens putidus.

7. Winogradski and Warrington have shown that by nitrification ammonium salts in the soil are converted into nitrites by one set of short bacilli, and these nitrites into nitrates by another set of bacilli; the two species differ from one another in their motility and general morphology. The nitrates thus produced are the forms of nitrogen which serve as nitrogenous food for plants. This proposition as to the necessity of intervention of special bacteria to nitrify ammonium salts was first enunciated and experimentally established by Schlösing and Müntz, and they were more accurately investigated by Winogradski, Warrington, and Percy Frankland.

8. The power of certain bacteria to become phosphorescent and to give the medium in which they grow the character of phosphorescence has been first noticed by Pflüger (phosphorescence of putrid fish, menthol wood). Katz, Fischer, and Beyrinck have described various species of phosphorescent bacteria; particularly the latter has studied them in pure culture (broth, salt, asparagin) and has described various species. Elwers and Dunbar have described vibrios that have the power of phosphorescence.

9. The series of changes produced by some species of bacteria, called putrefaction of albuminous substances, consist chiefly in the decomposition of albumin into lower nitrogenous principles associated with the evolution of sulphuretted hydrogen and ammonia, and the formation of alkaloidal bodies known as ptomaines of Selmi. Brieger, who has first isolated a number of alkaloids (cholin, neurin, cadaverin), has shown that, while some have poisonous action on the animal system, others have not. The fact that injection—either directly into a vein or indirectly into the subcutaneous tissues of animals—of putrid fluids in sufficient doses causes acute poisoning: rise of temperature

at first, vomiting, purging, spasms, great fall of temperature, collapse, and death, has been known since Panum, Schmidt, Billroth, and others; this constitutes what is now known as sapræmia, or septic or putrid intoxication caused by the ptomaines of Selmi and Brieger. And further, research has shown that all the pathogenic bacteria, that is those which when introduced into a suitable body multiply therein, produce infection and cause a series of symptoms characterising the particular infectious disease, do so by virtue of their producing specific chemical poisons, toxins, within the body. Not only in the animal body, but also in artificial cultures, do these specific bacteria elaborate these toxins, which, if injected into an animal, set up the same symptoms of disease as if produced by the multiplication of the microbes within the animal. These toxins have been investigated for a series of specific microbes: septicæmia (Roux and Chamberland), typhoid fever (Brieger), diphtheria (Roux and Yersin, Sidney Martin), tetanus (Behring and Kitasato), anthrax (Hankin, Sidney Martin), and others. These toxins are considered by Fraenkel and Brieger to be of the nature of proteids and are called tox-albumins, while Roux has given good evidence that some (particularly the diphtheria toxin and the tetanus toxin) are more of the nature of ferments. Hankin has shown that in anthrax a poisonous albumose is formed, while Sidney Martin has obtained, besides poisonous albumoses, certain alkaloidal bodies having poisonous action. In diphtheria Sidney Martin obtains alike from diphtheria cultures and the diphtheritic membrane and spleen in human diphtheria, besides a poisonous ferment (the toxin), also albumoses, alkaloidal and acid bodies acting poisonously. The fact is then established that the specific or pathogenic bacteria produce in artificial nutritive media,

as also in the body affected with the disease, specific toxins.

10. Many species of bacteria include in their protoplasmic bodies substances which when injected in sufficient doses into the subcutaneous tissue—or, better still, into the peritoneal cavity—of rodents, produce symptoms of disease and death. Bacteria of various kinds, and not having any connection with infectious disease—in fact, harmless and non-pathogenic—can, when injected in sufficient doses into the peritoneal cavity of guinea-pigs, set up acute intensive peritonitis and death in 16 to 20 hours. If, for instance, $\frac{1}{5}$ to $\frac{1}{6}$ of an Agar surface culture (6 cm. by 2 cm.)[1] of bacillus prodigiosus, bacillus subtilis, bacillus coli, bacillus proteus vulgaris, vibrio of Finkler—all microbes which have no connection with any infectious disease of man or animals—be injected into the peritoneal cavity of a healthy guinea-pig, the animal shows decided illness already after a few hours: first rise, then decided fall, of temperature; it is quiet, refuses food; later on, its movements become impaired, and it may be found dead in 18 to 24 hours. The rapidity with which death takes place depends on the size of the animal and on the quantity injected. After death extensive and intensive peritonitis is found: solid lymph on the peritoneum, pseudo-membranes on the liver, spleen, and omentum; the intestine is as a rule greatly congested, and there is more or less copious peritoneal exudation, either turbid or sanguineous. If the culture has been injected as living culture, the peritoneal exudation is crowded with

[1] The culture is made by rubbing over the whole slanting surface of the agar a platinum loop dipped previously into the active culture, then incubating at 37° C. for forty-eight hours. A definite quantity of broth (sterile) is then added, and the growth rubbed down with the platinum loop; the turbid emulsion is poured off and used for injection.

the microbes injected; occasionally also the blood yields, in culture, colonies of the microbes, but far less numerously than the peritoneal exudation; if the animal survives 36 to 48 hours it as a rule recovers. The same fatal acute peritonitis is produced by the bouillon mixture previously sterilised at 70° C. for 5 to 10 minutes, only in this case a larger dose is required than of the living mixture in order to produce a fatal result.

The same disease and the same fatal result are produced by other bacteria, as the vibrio of cholera, bacillus of typhoid fever, staphylococcus aureus, and bacillus pyocyaneus. Bacillus coli and bacillus prodigiosus act in this respect more virulently than the others, so that a smaller dose of the former is required to produce the fatal peritonitis than of the latter.[1]

Since all these microbes act in the same way and produce the same disease and *post-mortem* appearances, whether used as living culture or as sterile culture, and since in these experiments only the bacilli are used (the growth is scraped from the surface of solid Agar), it follows that the microbes above mentioned contain in their bodies similar or the same poisonous substances—*intracellular poisons*. The curious thing is that some noted pathogenic bacteria do not contain these intracellular poisons, *e.g.*, sporeless anthrax bacilli, bacillus of fowl cholera, and bacillus diphtheriæ can be introduced as sterile bacilli in large quantities—far larger than in the case of the above microbes—without producing poisonous effects. Moreover the living bacillus diphtheriæ from gelatine culture can be introduced in large quantities

[1] Subcutaneous injection of large doses produces a local swelling and œdema, which may lead to suppuration and necrosis; in the case of proteus vulgaris and bacillus coli it may lead to acute general infection and death.

($\frac{1}{3}$ to $\frac{1}{4}$ of a culture) into the peritoneal cavity of guinea-pigs—highly susceptible to this microbe when subcutaneously injected—without producing disease or death.

Injecting, then, the bacilli of a particular species, dead or living, in fair quantities into the peritoneal cavity, and producing thereby disease and death, does not prove in the least that this species is, strictly speaking, pathogenic, since some notoriously non-pathogenic bacteria (bacillus prodigiosus, vibrio of Finkler, bacillus subtilis) do the same, while some notoriously specific bacteria (bacillus of fowl cholera—sterile; bacillus diptheriæ—living or dead; and bacillus anthracis—dead) do not produce such a result. All that can be said in such cases is that the bacillary bodies do or do not contain the intracellular poison that causes fatal peritonitis, or contain it in small amount, or contain it very abundantly. Whether a given species is or is not pathogenic—can or cannot produce in the natural or artificial culture media specific toxins—is a question totally separate from the above. Voges separated by watery extract from growths of bacillus prodigiosus a substance which causes on injection a temporary rise of temperature in guinea-pigs; this is evidently a substance distinct from the intracellular poisons that cause the above-mentioned fall of temperature and fatal peritonitis.

The intracellular poisons present in many, absent in some, species of bacteria are thus of a distinctly different order from the specific toxins elaborated by pathogenic bacteria: the former are present in the bacillary bodies as such, no matter whether dead or living; the latter are the products of metabolism, *i.e.* results of chemical changes induced in the culture media by the growth and multiplication of the specific bacteria. Any specific change that the living body undergoes, any specific reaction that it is capable of acquiring after the growth in it of the living

bacilli, is, partly at least, a result of the specific toxins created by the bacteria in it; while the change that is produced in the peritoneal cavity, into which the intracellular poisons of dead bacilli had been previously introduced in less than fatal dose, may be, and as a matter of fact is, a local one and different from that produced by the previous growth of the living bacilli and elaboration of their specific toxins in the peritoneal cavity (see a later chapter).

CHAPTER VIII

MICROCOCCI

By the specific term micrococcus is understood a minute spherical or slightly oval organism (spherobacterium, Cohn) that, like other bacteria, divides by fission (schizomycetes). and that as a rule does not possess any special organ, cilium or flagellum, by which it would be capable of moving freely about. Excepted herefrom is the micrococcus agilis discovered by Ali-Cohen and mentioned in a previous chapter. Micrococci, like other granules when suspended in a fluid medium, show (Brownian) molecular movement. Micrococci propagate always by division; any other mode, *e.g.* gemmation and spores, is unknown. All assertions to the contrary must as far as present knowledge goes be considered as unproven. All micrococci, like other bacteria, possess a delicate membrane of cellulose, and, owing to this, resist the action of alkalies and acids. The contents are homogeneous and highly refractive while active, pale when inactive. They consist, like those of other bacteria, of mycoprotein (Nencki). The size of micrococci varies within considerable limits, say 0·5 to 2μ, or even a little more. Micrococci vary greatly as regards both size and mode of growth. All multiply by slightly elongating and then dividing by a transverse constriction into two: a dumb-bell; each of

these again divides into two, either transversely or in the same direction as before. The new elements of successive divisions may remain connected linearly, forming a chain ; or they separate into single organisms or dumb-bells or form smaller or larger connected masses. In some species there is a pre-eminent tendency to form chiefly dumb-bells or diplococcus of Billroth, in others to form shorter or longer chains generally more or less curved, streptococcus (Billroth), and in still others to form connected masses, staphylococcus (Ogston).

Such exquisite chains one meets with sometimes in serum of blood exposed to the air for some days, and in pleural and peritoneal exudations of animals dead for a few days. I have seen in an artificial culture made by my friend Mr. A. Lingard from a blister in a rabbit's ear the most exquisite convolutions of threads of micrococci. Similarly the streptococcus pyogenes and that of erysipelas form in fluid media long, twisted, and convoluted chains.

In the dividing cocci the single cells are generally more or less crescentic ; this is particularly noticed in staphylococcus aureus and albus and in gonococcus ; it is not marked in others, as in diplococcus pneumoniæ and in the streptococci.

Some species are specially characterised by this that, having divided into a dumb-bell, each of the elements divides again transversely into a dumb-bell, thus forming a group of four (tetrade or sarcinaform). Some species are occasionally met with, particularly in products of air-contamination, in which the four individuals are closely pressed against one another, and then each assumes more or less the shape of a cube, a true sarcina. But each of these cubes divides into four small micrococci arranged as a small sarcina, so that a sarcina-within-sarcina form results (sarcina lutea, sarcina ventriculi).

As has been pointed out in Chapter VI. under Growth and Division, in some species the cocci when growing on solid media enlarge many times the size of the typical unit before division commences, others only enlarge slightly and then at once divide.

In many instances the individual members resulting from division remain closely adherent without any definite arrange-

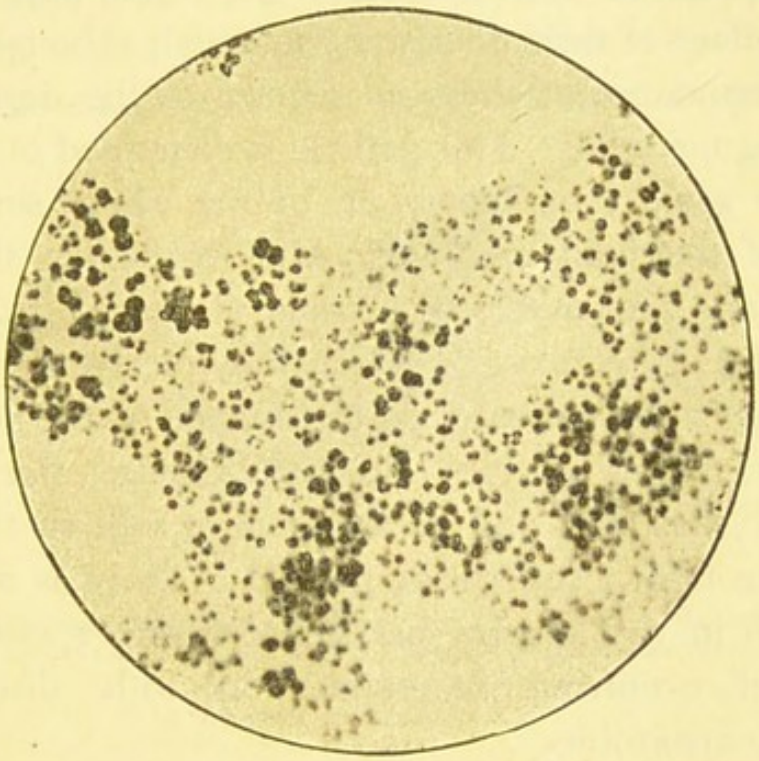

FIG. 28.—MICROCOCCUS FROM A GELATINE CULTURE, SHOWING VARIOUS PHASES OF GROWTH.

× 1000.

ment, and thus form smaller or larger clusters (staphylococcus), a kind of *zooglœa* or colonies, in which the individuals appear embedded in a hyaline gelatinous matrix ; the amount of this varies in the different species ; in some there is little of the matrix actually visible, the micrococci being in close juxtaposition, in others it is easily recognised, the interstices between the individuals being measurable.

In some of the pigmented species (see below) the interstitial

matrix contains the pigment. Zoogloœa masses always present themselves as uniformly granular, the granules or micrococci being either of the same size or differing considerably.

True micrococci never elongate to form rods, although in certain rod-like bacteria the individual elements owing to rapid division have the shape of spherical elements (see below).

Some species of micrococci form after some days a pellicle on the surface of fluid nourishing material, although there is also an abundance of these micrococci in the depth of the nourishing material. This pellicle is composed of zoogloœa, and after some time bits of it, or the whole, sink to the bottom of the fluid medium. Micrococci that thus form pellicles are pre-eminently aërobic (Pasteur), *i.e.* require a great deal of free oxygen, which they receive from the air to which they are exposed on the surface of the nourishing material. Other species do not require free oxygen (anaërobic, Pasteur), and therefore grow well in the depth and do not form a superficial pellicle. There is a marked distinction in this respect between different species. The micrococci occurring in connection with disease are facultative anaërobic.

When cultivated in suitable fluids they produce after a day or two general turbidity; growing in solid nutritive gelatine some produce liquefaction of the gelatine, others do not, and it is with micrococci as with other bacteria that identification of different species is possible by their mode of growth in and on solid media and in fluids, in plate cultivations, in their power of liquefying gelatine, and in their behaviour in the animal body.

Besides those mentioned in connection with certain special fermentative changes (micrococcus ureæ), and others to be mentioned in connection with disease, various species

of micrococci occur in air, in water, in dust, in soil, and in all organic materials in which decomposition occurs, differing from one another in size and in their cultural characters. To the same class belong many of the micrococci found in the normal fluid of the oral cavity and on the surface of the tongue and mucous membrane of the tonsils and pharynx—these are probably derived from the outer air; similarly in the bronchial and nasal secretions in catarrhal inflammation, on ulcerated surfaces, in the epidermis of the normal skin, in the contents of the large intestine in health and disease.

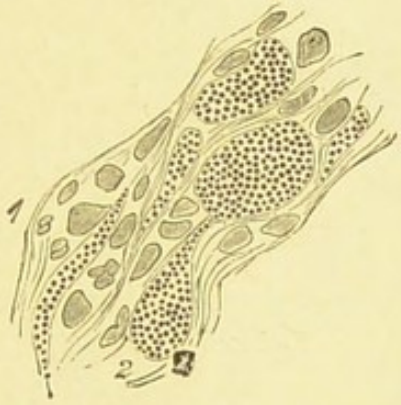

FIG. 29.—FROM THE BASE OF AN ULCER OF THE MUCOUS MEMBRANE OF THE LARYNX IN A CHILD THAT DIED OF ACUTE SCARLATINA.

1. Nuclei and fibres of the tissue.
2. Zooglœa of micrococci.

In all cases of diarrhœa the secretions of the bowels swarm with micrococci. In typhoid fever clumps of micrococci may be found very extensively on the ulcerations of the bowels and in the mucous membrane surrounding the ulcerations, and may be even traced into the mesenteric glands and the spleen.[1]

In dead tissues within the living body, such as occur after embolism, and in the case of various infectious maladies, micrococci may be found in colonies, *i.e.* as zooglœa.

[1] Klein, *Reports of the Medical Officer*, 1876. Letzerich, Sokoloff, Fischel, &c.

Ascococcus.—Billroth first described certain peculiar spherical, oval, or knobbed masses of minute micrococci, which he found in putrid meat infusion. Each of the masses is enveloped in a resistant, firm, hyaline capsule of about 0·010 to 0·015 mm. thickness. The masses are of various sizes, from 0·02 to 0·07 mm. in diameter, and are composed of small spherical micrococci. Cohn found them also in his (Cohn's) nourishing fluid (see Chapter II.), where they produce the peculiar smell of cheese. They are capable

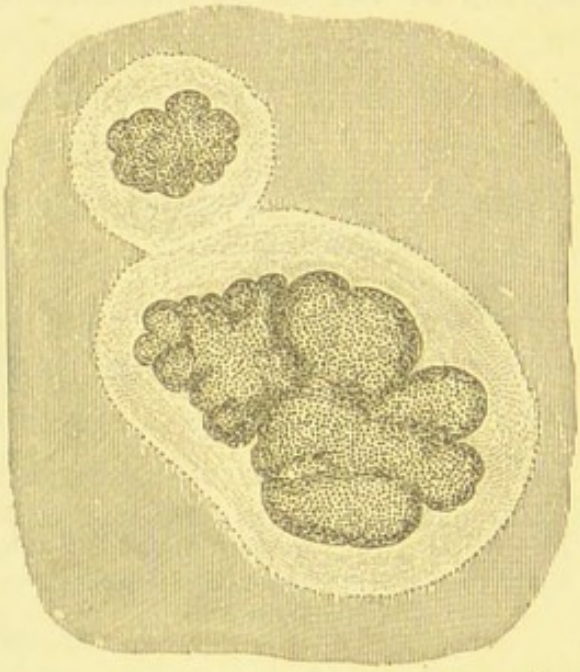

FIG. 30.—ASCOCOCCUS BILLROTHI (AFTER COHN).

of changing acid nourishing material into alkaline. Cohn called the organism *ascococcus Billrothi.*

Sarcina Ventriculi.—Goodsir was the first to describe in the vomit of some patients packets of four cubical cells, with rounded edges, and closely placed against one another. These *sarcinæ ventriculi* are of a greenish or reddish colour. The diameter of the individual cells is about 4 μ. They are found in the contents of the stomach of man and brutes in health and disease, where the

groups of four cells form smaller and larger aggregations. Occasionally small sarcinæ occur on boiled potatoes, egg albumen, and gelatine that have been exposed to the air. The cocci of these sarcinæ are smaller than those of the sarcina ventriculi; on cultivation the growth is of a yellow colour and represents the species known as sarcina lutea.

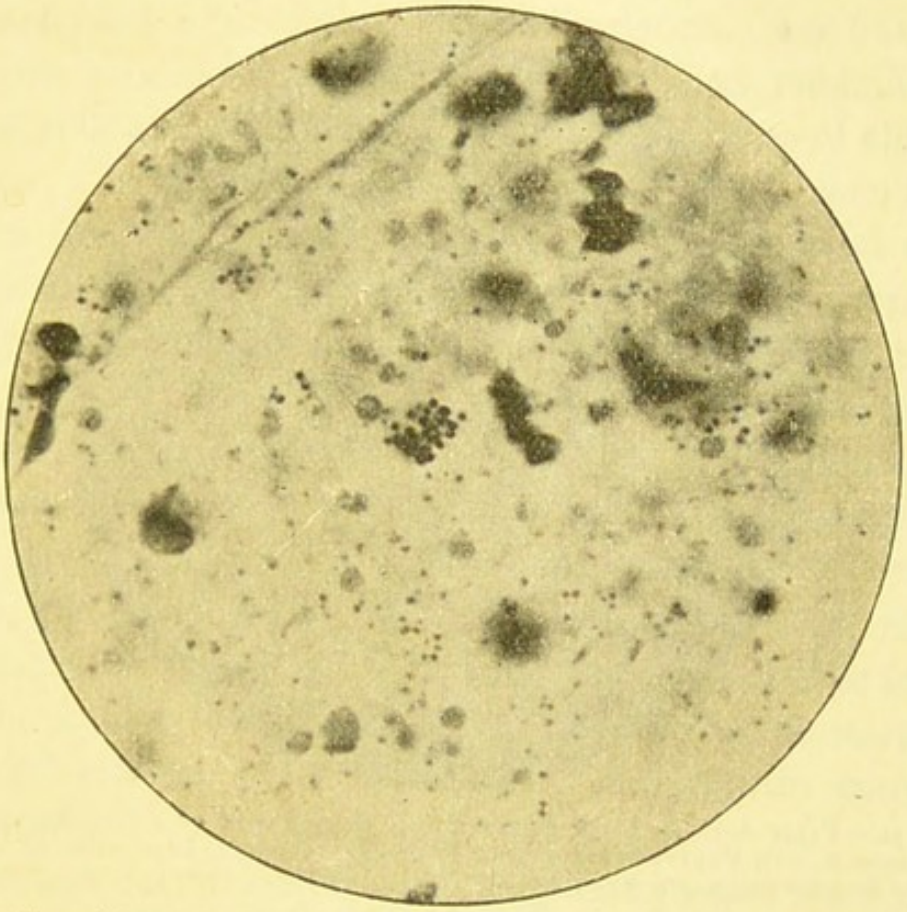

FIG. 31.—FILM SPECIMEN OF PUS FROM ACUTE ABSCESS: AMONGST MASSES OF NUCLEI, NUMEROUS COCCI, SINGLE, DIPLOCOCCI, AND GROUPS.
× 1000.

As stated just now, it is widely distributed in air, occasionally is also found in open waters.

Micrococci connected with disease, or capable of producing disease in man and animals:—

1. *Staphylococcus pyogenes* (Ogston).—In most purulent acute inflammations there occur numerous cocci which

when cultivated prove to belong to two well-defined species : staphylococcus pyogenes aureus and albus.

Staphylococcus pyogenes aureus.—This organism is common in acute suppurations and ulcerations, alike those in the skin or mucous membranes, serous membranes, or parenchymatous organs ; it is met with abundantly also in acute external inflammations, ulcerations (*e.g.* after vaccinia

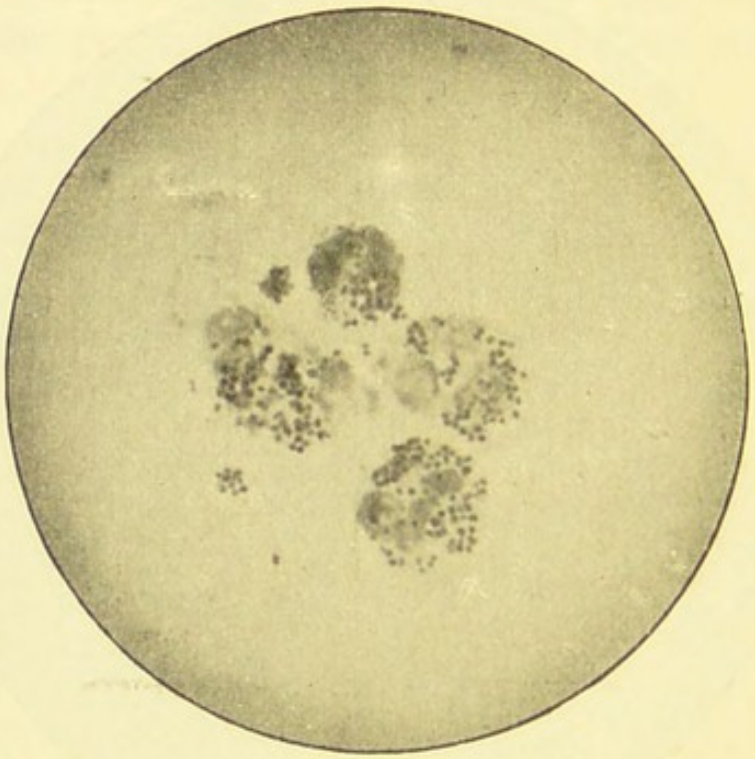

FIG. 32.—FILM SPECIMEN OF PERITONEAL EXUDATION OF A GUINEA-PIG, DEAD FROM ACUTE PERITONITIS AFTER INTRAPERITONEAL INJECTION OF CULTURE OF STAPHYLOCOCCUS AUREUS.

Four lymph-cells filled with the cocci. × 1000.

and variola, in diphtheritic inflammation of the fauces, in some cases of ulcerative endocarditis). In purulent inflammations (abscesses acute and chronic) this organism is present in large numbers in the pus as single cocci, as dumb-bells, and as large and small connected clusters. Many of the dumb-bells and connected masses show the individuals as crescents—that is as divided. A film of pus dried on a cover-glass, heated and stained in methyl-blue or gentian

violet shows the cocci as above, between, and also on the surface and in the interior of the pus cells. In catarrhal inflammation of the fauces they occur in numbers adhering to the surface of the detached scaly epithelial cells.

In gelatine plate cultivation kept at 20° C. the colonies are minute whitish dots, visible already after 24 hours; after 36 to 48 hours each dot is already of a yellowish tint, sunk in, as it were, into a pit of clear liquefied gelatine. The liquefaction now proceeds rapidly, each liquefied area containing a central yellowish granular mass which is made up of clusters of cocci. In gelatine stab cultures the line of inoculation is soon (after 24 hours) marked as a connected lineal mass of growth; liquefaction commences generally at the top and rapidly proceeds into the depth, the liquefied gelatine being fairly clear or very slightly turbid; at the bottom of the liquefied channel or funnel the main part of the growth is accumulated in the form of a yellowish powdery precipitate.

On agar it forms a characteristic yellow, pale orange yellow, or golden-yellow moist growth—hence its name. On subculture from generation to generation it will be found that the colour becomes paler than is the case at starting; the condensation water is uniformly turbid with granules and flocculi.

Although not invariably local suppuration and multiplication of the cocci are produced in rodents by injecting subcutaneously some of the growth, it nevertheless sometimes succeeds; it succeeds easier by injecting at the same time a 10 per cent. sugar solution.

The subcutaneous injection of a culture (broth culture) of staphylococcus aureus in large doses is occasionally followed by acute and general infection and death; the blood contains then a crop of the cocci; the serous membranes are inflamed, and their exudation is full of the cocci;

occasionally, if the disease lasts a few days, disseminated purulent abscesses are found in some of the viscera.

2. *Staphylococcus pyogenes albus* is also often present in purulent matter, particularly of acute abscess, either alone or associated with aureus. The liquescens albus differs from the aureus morphologically and culturally only in this that its growth on Agar possesses no colour, but forms a whitish mass. It liquefies rapidly gelatine, and the liquefied gelatine is fairly clear or slightly turbid, and at the bottom is a whitish, powdery, granular precipitate consisting of continuous masses of cocci, which in morphological respects cannot be distinguished from aureus. Its pathogenic action on subcutaneous injection into animals is the same as in the case of aureus; also occasionally a general acute infection with lethal end is producible in rodents.

Both aureus and albus grow rapidly in beef-broth, making it strongly and uniformly turbid with a powdery and flocculent granular precipitate.

The enormous rapidity with which staphylococcus aureus is able to grow at 37° C. has been detailed in a former chapter.

3. Occasionally in purulent and acute inflammatory foci is found a coccus which forms a distinctly white growth on Agar and on gelatine, and does not liquefy gelatine; this is the *staphylococcus albus non-liquescens*. A variety of this forms flat, white, rapidly spreading dry colonies and growth, and represents staphylococcus cereus albus. I have met with both these varieties in purulent matter of the sores after vaccination, also from variola in the suppurative stage.

4. *Streptococcus pyogenes albus*.—This is the microbe of acute phlegmon; it is also present in chronic abscess, in acute serous effusions. The principal morphological characters of this as also of other species of streptococci

are that the cocci by repeated division form linear series, thus producing shorter or longer chains, the latter more or less twisted and wavy; when growing in fluid media at 37° C.—broth, condensation fluid of solidified Agar, or blood-serum—the chains are rapidly formed and attain great length. On solid media—gelatine, Agar, blood-serum—the

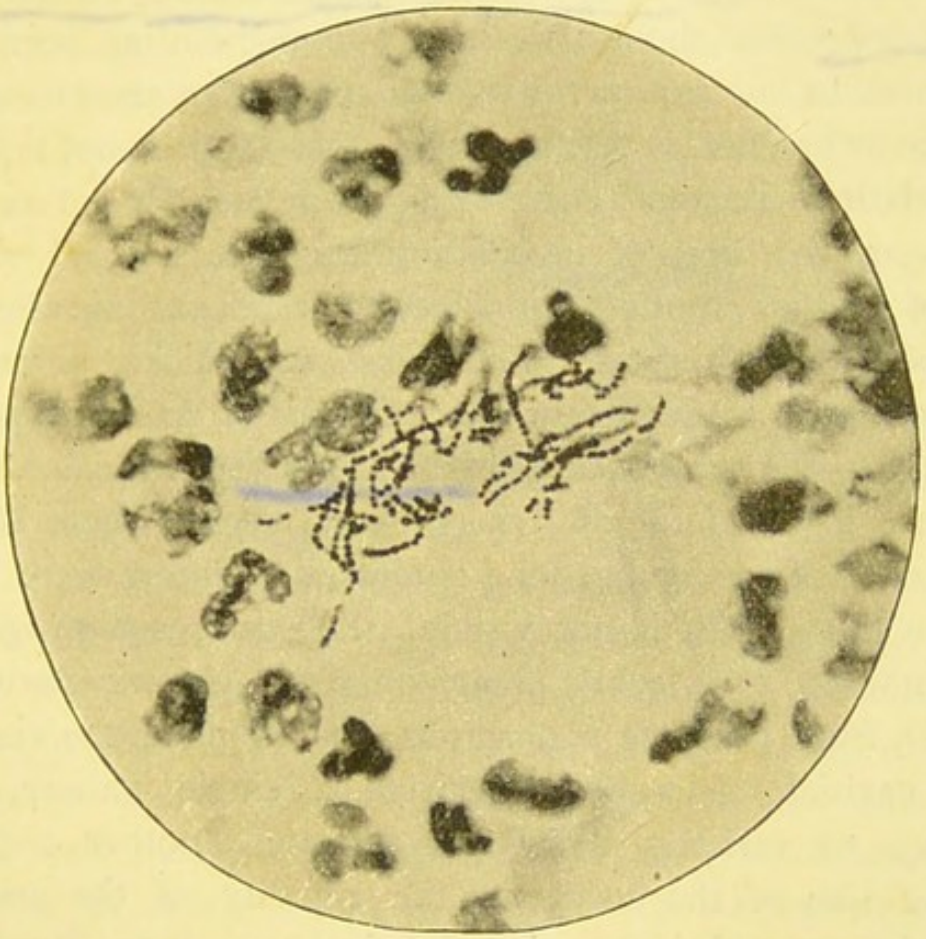

FIG. 33.—FILM SPECIMEN OF PUS OF CHRONIC ABSCESS. PUS CELLS, AMONGST THEM A GROUP OF STREPTOCOCCUS PYOGENES.

1000

chains are not so long, occasionally only composed of six or eight cocci. Examining the long chains of fluid media, one always notices an inequality in the size of the cocci, sometimes one or the other coccus—in the middle, or oftener at the end of the chain—being twice and thrice as big as the average coccus; in some chains, wholly or in part, the cocci

are distinctly arranged as a series of dumb-bells, in others there is no such distinct arrangement.

Streptococcus pyogenes forms in nutrient gelatine at 20° C. already after twenty-four to thirty-six hours minute, dot-like, grey, translucent, round colonies, which after two to three days' growth are large enough to show under a magnifying glass a darker, thicker centre and a thin, rounded, translucent periphery; after about a week or two the outline becomes irregular, to one side more than to the other, thus forming a more or less fan- or fern-shaped patch. It does not liquefy the gelatine. In streak culture on solid gelatine, blood-serum, or Agar, the line of inoculation becomes marked as a line of separate, rounded, translucent, or more or less whitish-grey colonies, which as a rule, unless very thickly sown, do not coalesce. In fluids—broth, condensation fluid of Agar or of serum—the growth causes slight turbidity of the fluid and is more in the form of stringy, flaky masses, these being composed of continuous long chains much interwoven. On potato the growth is not visible. Streptococcus pyogenes as obtained from acute phlegmon, from chronic purulent matter, from purulent and serous exudations of the viscera and cavities, does not constitute a single variety, but belongs to varieties differing from one another slightly in the size of the cocci, in the rapidity of the growth on gelatine, and in the length of the chains. Similarly varieties of streptococci are known to occur in the various normal secretions—fauces, bronchi, intestinal contents, soil, &c.—which in some or all the above respects more or less resemble the streptococcus pyogenes. The streptococcus pyogenes cultivated from pus shows on inoculation of a rabbit or mouse in many instances a tendency to form inflammation and abscess; in some instances, particularly on injecting large doses, general acute septicæmic infection

and death, with plugging of capillaries in the parenchymatous viscera with masses of streptococci, are observed; the blood yields on culture numerous colonies of streptococci.

Streptococci resembling in morphological and cultural respects the streptococcus pyogenes are found in connected masses in the ulcerated tissue and on the villous outgrowths of the cardiac valves in some forms of ulcerative endocarditis. In other cases of ulcerative endocarditis masses of staphylococcus aureus only occur. Also in puerperal septicæmia a streptococcus is cultivable from the blood and spleen which in cultural respects resembles the streptococcus pyogenes except that it is more virulent, producing on injection into the subcutaneous tissue of the rabbit's ear an extensive blush and occasionally acute septicæmic infection. It is difficult to say whether this streptococcus is a virulent variety of streptococcus pyogenes or a less virulent variety of the streptococcus erysipelatos.

It is an easily ascertained fact that the streptococcus pyogenes cultivated from phlegmon and various purulent exudations when tested on the animal (notably the rabbit's ear) does not behave in a uniform manner, inasmuch as in some instances it acts virulently, causing distinct and spreading blush and purulent exudation and even general infection, whereas in others it has no appreciable pathogenic action under the same conditions; and it is likewise a fact that a streptococcus, which is pathogenic at first, by repeated subculture loses this action.

5. *Streptococcus erysipelatos.*—Fehleisen first isolated this microbe from the progressing margin of erysipelas; it is a microbe which, as sections through the erysipelatous skin show, is abundantly present in the distended lymph-spaces and lymph-vessels of the marginal part. The morphological and cultural characters coincide with that of the

Karlinski gives as the result of a large number of observations on purulent matter of man the following list (*Centralbl. f. Bact. und Parasit.*, VII., No. 4, p. 115):—

Disease	Staphylococcus pyogenes aureus	Staphylococcus pyogenes citreus	Staphylococcus pyogenes albus	Streptococcus pyogenes	Micrococcus tetragonus	Bacillus pyogenes fœtidus	Bacillus of Friedländer	Bacillus anthracis
Mastitis, 36 cases . .	22	4	4	6	—	—	—	—
Subcutaneous abscess, 30 cases	10	2	8	6	2	2	—	—
Phlegmon, 24 cases . .	—	—	—	24	—	—	—	—
Furuncle, 20 cases . .	9	—	10	—	1	—	—	—
Bubo, 17 cases . . .	8	1	1	7	—	—	—	—
Subperiosteal abscess, 16 cases	6	—	10	—	—	—	—	—
Panaritium cutaneum, 16 cases	7	—	9	—	—	—	—	—
Abscess of gums, 10 cases .	1	—	4	1	3	1	—	—
Hordeolum, 10 cases . .	6	—	4	—	—	—	—	—
Otitis media, 4 cases . .	2	—	—	—	—	—	2	—
Carbuncle, 4 cases . .	2	—	1	1	—	—	—	4
Osteomyelitis, 3 cases . .	2	—	1	—	—	—	—	—
Summary . . .	75	7	52	45	6	3	2	4

streptococcus pyogenes and other streptococci;[1] this great similarity in morphological and cultural characters of most species of streptococci is no justification for assuming that the two are the same, and that they are mutually interchangeable. The streptococcus erysipelatos taken direct from the erysipelatous skin of a man or rabbit (serum squeezed out of the progressive margin), or from cultures on serum, or Agar, broth or gelatine, particularly the first, when inoculated into the skin of the root of the rabbit's ear, produces typical progressive erysipelas: after twenty-four hours there is distinct blush and swelling, starting from about

[1] The streptococcus erysipelatos forms more pronounced chains, even on solid media, than does the streptococcus pyogenes.

the seat of inoculation, and gradually extending towards the tip of the ear; in three to four days the whole ear is red, swollen, hot, and pendulous; later on, when the process retrogrades, the epidermis is raised in blisters and peels just as in erysipelas of man. The process is sometimes so severe that the ear sloughs, or general septicæmic infection occurs; occasionally not only the ear but also the skin of the neck

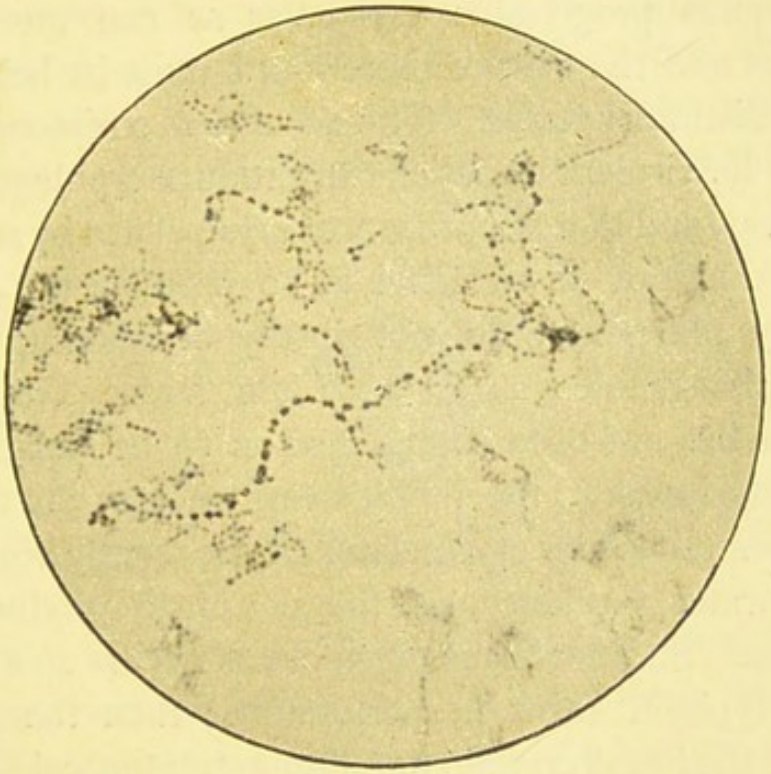

FIG. 34.—FILM SPECIMEN OF STREPTOCOCCUS SCARLATINÆ FROM A FLUID CULTURE. × 1000.

becomes involved. Sometimes on subcutaneous injection of the culture at the root of the ear an acute septicæmic infection is at once produced, the animal dying in twenty-four to thirty-six hours, and the blood containing the streptococci in large numbers. Cultivations with a droplet of the serum from the erysipelatous ear always yield numerous colonies of the streptococcus.

After repeated subcultures on gelatine or Agar the virulence

—that is, the power to produce typical erysipelas in the rabbit's ear—becomes less and less and ultimately is lost. But by starting a fresh culture on solidified blood-serum and using then a somewhat large dose erysipelas in the ear can be again produced, the lymph of this ear and its cultures again being capable of producing typical erysipelas. Until streptococcus pyogenes obtained from abscess or common phlegmon can be shown to produce in the rabbit's ear the same typical progressive erysipelas as can the lymph of erysipelas and the culture therefrom it must be held that the two are distinct species. The facts that streptococcus pyogenes in its virulent varieties can produce a phlegmon in the rabbit's ear, and that streptococcus erysipelatos by subcultures loses so much of its virulence as to produce not erysipelas but only phlegmon, do not justify considering the two as interchangeable; as far as I am aware, streptococcus pyogenes has not been so changed as to be capable of producing erysipelas in the rabbit's ear, whereas the attenuated form of streptococcus erysipelatos can be readily brought back to its former virulence, *i.e.* the power to produce typical erysipelas. Streptococcus erysipelatos occurs as a complication in typhoid fever in perforation; then the peritoneal fluid and the blood contain numerous streptococci the culture of which produces in rabbits typical erysipelas. The name "streptococcus erysipelatos" must therefore be reserved for that species of streptococcus which is found in genuine human erysipelas, and which can set up in the rabbit's ear typical spreading erysipelas, and must not be mixed up with streptococcus pyogenes, however much morphologically and culturally the two approach one another.

6. The same may be said of the streptococcus, which I described, of the contents of the vesicles and of the ulcers in foot-and-mouth disease of sheep. In culture it resembles

streptococci in general, inclusive of the streptococcus pyogenes, although on gelatine its colonies are markedly transparent, and it grows much slower than those of streptococcus pyogenes. Cultures injected into the skin of sheep produced a vesicle, and from it the same streptococcus was cultivated. Schottelius described a chain-coccus in foot-and-mouth disease which seems to me indistinguishable from the one which I described.

7. The streptococcus which I cultivated in a certain percentage of cases of scarlatina from the blood of patients during the acute febrile stage belongs to this group; when injected into rodents it produces in a large percentage acute

FIG. 35.—COLONIES OF STREPTOCOCCUS OF FOOT-AND-MOUTH DISEASE AS SEEN ON THE SURFACE OF GELATINE UNDER A MAGNIFYING GLASS.

septicæmic infection. That this streptococcus is of a secondary character and capable of producing the purulent and other additional phlegmonous changes indicating secondary infections in scarlatina, as is maintained by several observers, remains to be shown. As far as my observations go, I found the streptococcus in the blood of patients in the early febrile stages of pure scarlet fever in which of secondary infection nothing could be seen.

The same streptococcus was found in connection with an eruptive (ulcerative) disease on the teats and udder of milch cows at Hendon in 1886, to the consumption of whose milk an extensive outbreak of scarlet fever in the north of London was definitely traced (see Mr. Power's report for

1886 to the Local Government Board). This intimate relation between an eruptive (ulcerative) disease of the teats and udder of milch cows to the cause of human scarlet fever was subsequently to 1886 demonstrated in several other localities (Glasgow, New Cross). In the Hendon cows, above referred to, there was in addition disease of the lungs and kidneys, from which the streptococcus was obtained by culture. Cultivations of the streptococcus from the blood of human scarlet fever or from the eruption on the teats of cows produced in mice and calves a definite general infection; in healthy milch cows the injection of the streptococcus produced the eruption with subsequent ulceration on the teats and udder, as also the visceral disease observed in the Hendon cows. (*Reports of the Medical Officer of the Local Government Board for* 1886, 1887, 1888.)

8. Löffler[1] showed that in faucial diphtheria, and associated with the diphtheria bacilli, occur streptococci, some of which, at any rate, play an important part in the secondary infections—swollen and suppurative glands—as also in septicæmic infection. These streptococci when injected into animals cause occasionally disseminated inflammatory foci, principally in the joints, and general septicæmic infection.

9. Membranous exudations in, and inflammation of, the fauces occur which are not accompanied by diphtheria bacilli, and which therefore are not true diphtheria; they do not lead to post-diphtheritic paralysis and terminate in recovery; they resemble mild cases of diphtheria. Such cases represent the cases of pseudo- or cocco-diphtheria. The exudation is found to be crowded with cocci, often in larger or smaller masses, numerous leucocytes being also present. When cultivated one obtains colonies of staphylo-

[1] *Mittheilungen aus d. k. Gesundheitsamte, II.*

coccus aureus and albus and two species of strepto-cocci—one in which the chains are made of cocci of the size of those of streptococcus pyogenes, and another of much smaller cocci and forming shorter chains.

10. Schütz[1] discovered that acute pharyngeal abscess in the horse ("Druse") is caused by a streptococcus which culturally differs from the streptococcus pyogenes principally

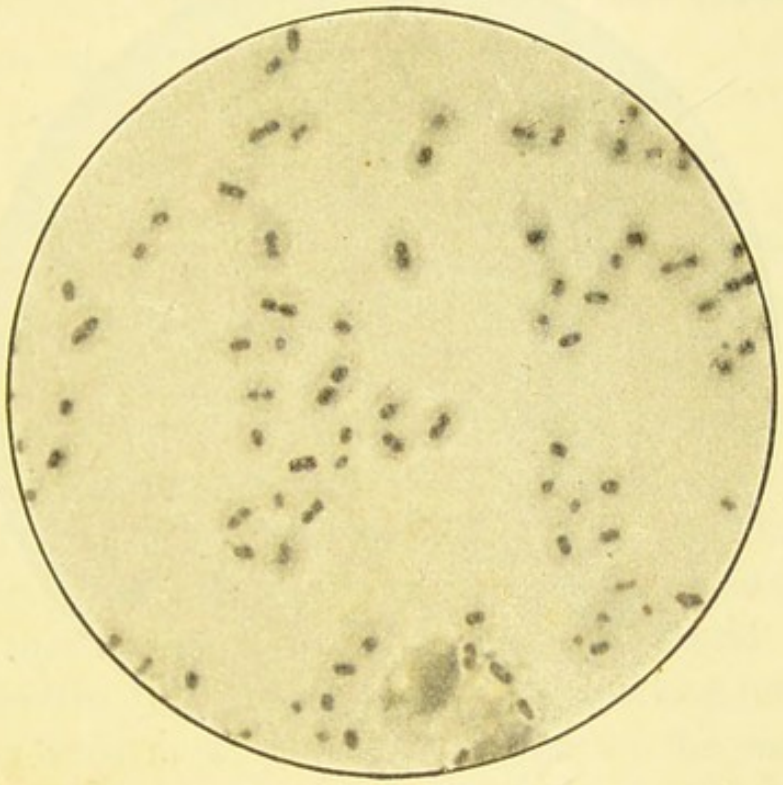

FIG. 36.—FILM SPECIMEN OF CAPSULATED DIPLOCOCCUS PNEUMONIÆ IN RUSTY SPUTUM OF ACUTE CROUPOUS PNEUMONIA.
× 1000. (A. Pringle.)

in this that the former does not grow below 22° C.; it acts virulently on rodents, and its culture produced in horses the typical pharyngeal abscess.

11. Cases of acute pneumonia occur which are associated with the copious presence of streptococci in the blood-vessels as also in the air-cells; they are considered by Finkler (*Die Lungenentzündungen*, &c.) to have caused the pneumonia.

[1] *Archiv f. wiss. und prakt. Thierheilk.* vol. 14, No. 3.

12. The *pneumococcus* or *diplococcus pneumoniæ* of Fraenkel and Weichselbaum. The principal morphological character of this microbe is that it occurs chiefly as dumb-bells or short chains of dumb-bells of cocci; the dumb-bells are invested in a gelatinous capsule easily stained when obtained directly from animal tissues. It occurs occa-

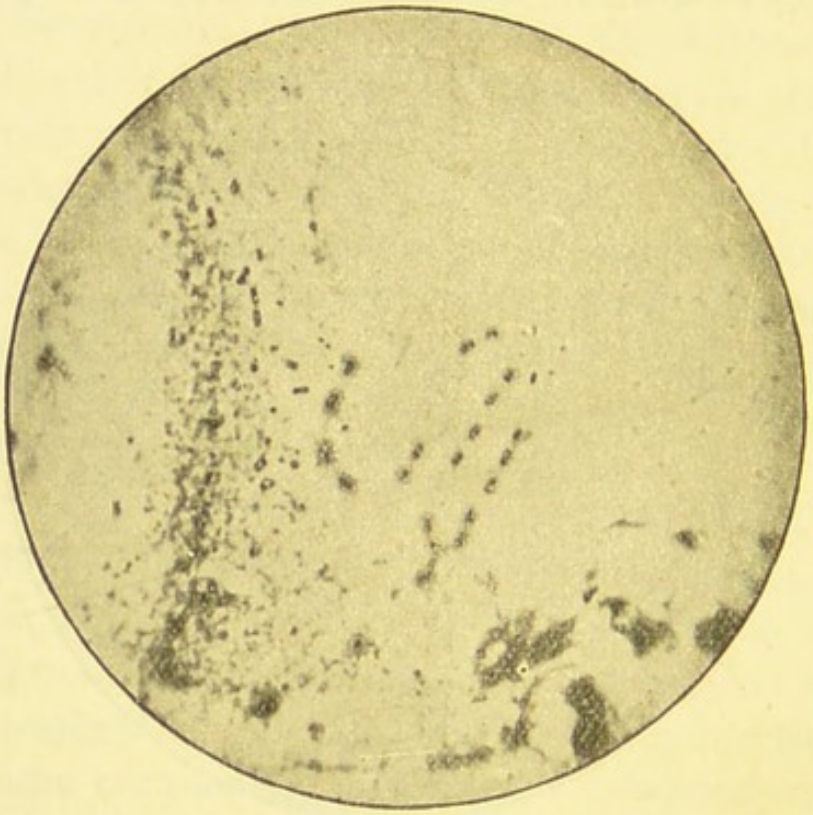

FIG. 37.—FILM SPECIMEN OF BRONCHIAL SPUTUM FROM A CASE OF ACUTE INFLUENZA, SHOWING CAPSULATED DIPLOCOCCUS PNEUMONIÆ.
× 1000.

sionally, but sparingly, also in normal bronchial expectoration; in the fluid of the mouth and nose (rarely); in the rusty sputum and the fluid of the lung in the acute stage (red hepatisation) of croupous pneumonia (large percentage of cases); in the peritoneal exudation in some cases of peritonitis; in the pericardial and pleural effusions in acute pericarditis and pleurisy; in the effusion in cerebro-spinal meningitis; in the purulent matter in inflammation of the

middle ear; in some cases of ulcerative endocarditis in which the valves contain masses of this diplococcus; in the bronchial sputum in influenza, and in catarrhal bronchitis. This diplococcus does not grow below 22° C. (*i.e.* not on ordinary nutrient gelatine solidified); it grows well above 28° C., best at 35° to 38° C. On Agar or on blood-serum it forms at 37° C., already after twenty-four hours, minute, translucent, round colonies, which after two to three days appear raised, moist-looking, whitish-grey, and round. In culture the capsule around the diplococci is absent altogether or only slightly indicated.

On account of its presence in large numbers—sometimes in pure culture—in the rusty sputum and in the blood-juice of the lung in the stage of red hepatisation in the great majority of cases of croupous pneumonia, prior to the height of the disease, it must be assumed that it has an intimate relation to the cause of this disease; that it is not the only cause of croupous pneumonia is shown by the fact that in some cases only streptococci* are present. In some epidemics (Middlesbrough) a motile bacillus was found in pure culture in the lung-juice in the red hepatised lung. But, assuming with most pathologists that in the majority of cases of genuine acute croupous pneumonia it is intimately related to the *causa vera*, it is not easily seen why the same microbe (the same in respect of morphological, cultural, and physiological characters) should in one instance cause croupous pneumonia, in another ulcerative endocarditis, in a third peritonitis, and in a fourth suppuration of the middle ear; or why it should be found fairly abundantly in some cases in the bronchial sputum (bronchitis, influenza) without producing pneumonia. All this is obscure and unintelligible if the diplococcus pneumoniæ be considered as the essential and sole cause of croupous pneumonia.

* The Streptococcus form of the Diplococcus?

Recent cultures of the diplococcus made from pneumonic sputum or other exudations (mentioned above) inoculated into mice or rabbits produce as a rule fatal septicæmic infection ; the viscera are greatly congested, and the blood and viscera contain abundantly the microbe. The same result is produced in the rabbit by injecting it with the rusty sputum of croupous pneumonia prior to the fifth or sixth day. In the blood and tissues of a mouse or rabbit that succumbed to

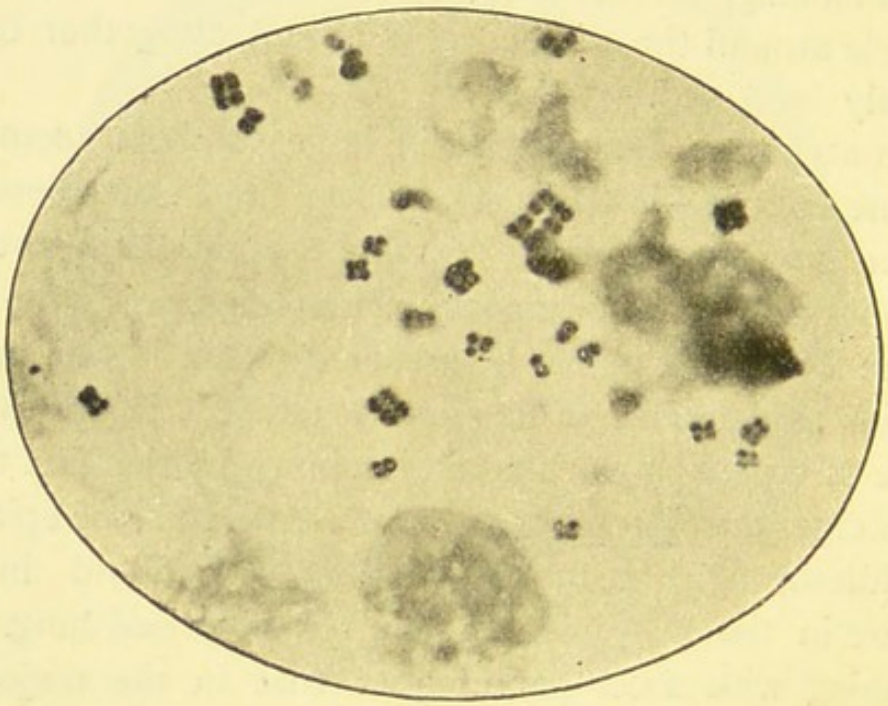

FIG. 38.—LUNG JUICE OF GUINEA-PIG DEAD AFTER INFECTION WITH MICROCOCCUS TETRAGENUS.
× 1000. (A. Pringle.)

infection, the diplococci are capsulated, and the capsules can be as easily stained as those in the sputum or bronchial exudation with eosin after the cocci themselves had been stained with methyl-blue. Staining with gentian-violet in alcoholic solution, and then carefully washing in water, shows the cocci stained deep purple, the capsules light violet.

Cultures that have been carried on for some generations gradually lose the power to produce infection in the rodents,

but on growing them again on serum or in broth to which a piece of boiled white of egg has been added the cultures regain virulence.

The capsulated, oval, rod-shaped, or cylindrical microbe described first by Friedländer as being the cause of croupous pneumonia occurs in the sputum only in a small percentage of cases, certainly not more than five per cent.; it occurs also occasionally in the bronchial secretions not connected with croupous pneumonia, and even in the fluid of the mouth in health. This *bacillus of Friedländer* is most probably identical with the capsulated microbe of the fluid of the mouth described by Sternberg. Inoculated in largish quantities into the rabbit, it causes acute septicæmic infection and death; in the blood and various viscera the microbe is then abundantly present.

13. *Micrococcus tetragenus.*—This microbe, related to sarcina-like cocci, was found by Gaffky in pulmonary tubercular expectoration and in the tissue of the tubercular lung. It occurs in groups of four cocci surrounded by a capsule. Cultivated in gelatine plates, it forms already after twenty-four hours minute white dots which during further incubation enlarge into prominent white moist discs. In streak cultures it forms a narrow, white, sticky growth along the line of inoculation. White mice are very susceptible to infection by subcutaneous injection of small quantities of culture. The animals begin to show illness after two days and generally die after three to six days. The blood and the spleen contain the microbe in large quantities. Also guinea-pigs are susceptible, but less so, since as a rule a local abscess is formed only, and occasionally a general fatal infection.

14. *Micrococcus of acute infectious osteomyelitis.*—Dr. Becker has made, in the laboratory of the Berlin Imperial Sanitary

Office, a series of important experiments on the micrococci discovered by Schüller and Rosenbach. He collected pus from five cases of acute osteomyelitis in which the abscesses had not been opened, and cultivated the micrococci on sterilised potatoes, coagulated serum, and gelatine-peptone. After 3–5 days the punctures made by the needles assumed the appearance of white streaks, around which the gelatine gradually liquefied and took an orange colour. The culture injected into the jugular vein caused acute septicæmia and death; but nothing abnormal was found in the bones in either case. A small quantity was then injected into the jugular veins of fifteen rabbits, after having, some days before, fractured or bruised the bone of one of the hind legs. At the end of the first week a swelling was formed at the seat of the bruise or fracture; the animals lost flesh and died after a few days. On dissection, large abscesses were found around and in the bones, and in several cases metastatic abscesses had formed in the lungs and kidneys. Numerous colonies of micrococci were discovered in the blood, which are identical with the staphylococcus pyogenes aureus.

15. Koch[1] described various kinds of micrococci intimately connected with certain pyæmic processes in mice and rabbits. (*a*) Micrococcus of *progressive necrosis* in mice. Injecting into the ear of mice putrid fluids, he observed a necrosis of the tissues of the ear (skin, cartilage) starting from the point of inoculation and gradually spreading on to the surrounding parts and killing the animal in about three days. As far as the necrosis reaches, the tissue is crowded with micrococci, chiefly in the form of chains and zoogloea. The individual cells are spherical, of about 5 μ in diameter.

[1] *Untersuchungen über die Aetiologie d. Wundinfections-Krankheiten*, Leipzig, 1878.

I have inoculated a number of white mice subcutaneously in the tail with a small micrococcus, due to accidental contamination. These micrococci, having been cultivated through several generations, were used in small doses for the inoculation of the mice. In two instances the inoculation was followed after two or three days by purulent inflammation at the seat of inoculation, but apparently not spreading beyond it. But, as time went on, inflammation and abscess in the lungs set in and the animals

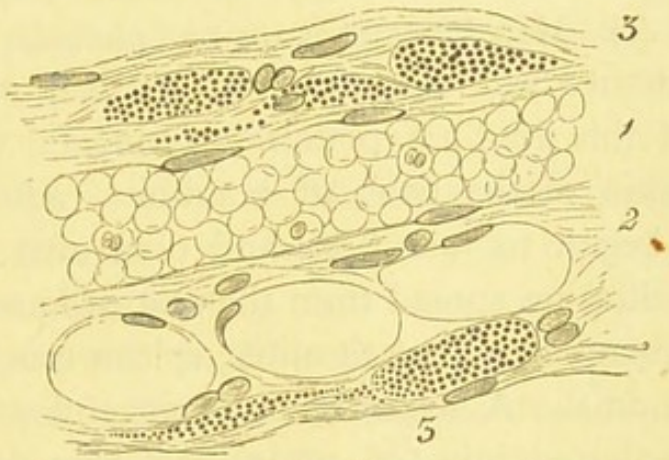

FIG. 39.—FROM A SECTION THROUGH THE TAIL OF A MOUSE INOCULATED INTO THE SUBCUTANEOUS TISSUE OF THE TAIL WITH ARTIFICIALLY CULTIVATED MICROCOCCUS.

The part here illustrated is a good distance from the ulceration.

1. A capillary blood-vessel filled with blood-corpuscles.
2. Fat cells.
3. Groups of micrococci filling the lymph-spaces of the connective tissue.

died after about a week. On making longitudinal sections through the tail, it was found that in most of the lymph-spaces and lymph-vessels of all parts of the cutis and subcutaneous tissue, far away from the seat of inflammation, there were densely crowded masses of the same minute micrococci as were used for inoculation. And these crowds of micrococci could be traced to the seat of inflammation, where they extended amongst the inflammatory products in great masses. The abscesses in the lungs were filled with

the same micrococci. Inoculated into the skin of fresh mice, it again produced death by pyæmia. This micrococcus may therefore be called the *micrococcus pyæmiæ* of mice. (*b*) Micrococcus causing *abscesses* in rabbits. Putrid blood injected into the subcutaneous tissue of the rabbit often produces suppurative abscess which, spreading, kills the animal in about twelve days. In the wall of the abscess are found continuous masses of zoogloea of micrococci. The pus is infectious. The micrococci are spherical, and of a very minute size, measuring only about 0·00015 mm. in diameter. (*c*) Micrococcus causing *pyæmia* in rabbits. Skin of a mouse was macerated in distilled water for two days, and of this fluid a hypodermic syringeful was injected under the skin of the back of a rabbit. After two days the animal began to lose flesh and died after 105 hours. Purulent infiltration spread from the seat of inoculation into the subcutaneous tissue; peritonitis; spleen much enlarged; slight pneumonia. A hypodermic syringeful of the blood of this animal was injected under the skin of a second rabbit, and this died after forty hours. Post-mortem examination showed the same lesions as in the first case. In the blood-vessels of the affected parts were present micrococci, single, as dumb-bells, and in zoogloea; they were spherical, about 0·00025 mm. in diameter. (*d*) Micrococcus causing *septicæmia* in rabbits. An infusion of meat was prepared; this was left to putrefy, and of this fluid a quantity was injected under the skin of the back in two cases. Extensive gangrene with much œdematous exudation followed, and death ensued in two days and a half. The blood, the capillaries of the kidney, and the enlarged spleen contained numerous oval micrococci. Two drops of the œdematous exudation-fluid were injected under the skin of the back of another rabbit. Death followed in twenty-two hours. There

was no gangrene here; but œdema was present, spreading from the seat of the inoculation. Sub-serous hæmorrhages appeared in the intestines; and minute hæmorrhages were also present in the œdematous tissue and in the muscles of the thigh and abdomen. The œdematous fluid, the cutaneous veins, the capillaries in the kidney, especially those of the glomeruli, in the lung, and in the spleen, contained numerous oval micrococci, singly, in dumb-bells, and in zooglœa. The micrococci measured about 0·8 to 1 μ in their long diameter. These micrococci (taken with the blood) produced in another rabbit and in a mouse the same fatal disease.

16. *Micrococcus bombycis* (Microzyma bombycis, Béchamp). —Oval micrococci, of about 1·5 μ in length, present in large numbers, singly, and as dumb-bells and chains (straight or curved), in the contents of the alimentary canal and in the gastric fluid of silkworms dead of the "maladie de mortsblancs, *flacherie*."—*Micrococcus ovatus*, Nosema bombycis. Present in large numbers in the blood and organs, ova included, of silkworms affected with the disease called "maladie des corpuscules," "pébrine," or Cornalia's disease. Cornalia first saw them, afterwards Lebert and Nägeli. Pasteur proved definitely that ingestion as well as inoculation of the silkworms with the micrococci produces the disease. The micrococci are comparatively large, 0·003 to 0·004 mm. long, 0·002 mm. broad; they are very bright and occur singly, or in dumb-bells, or in small groups.

17. *Micrococcus of gonorrhœa* (gonococcus). Neisser was the first who pointed out the constant presence, in the exudation in gonorrhœa, of peculiar micrococci, which occur as dumb-bells and as masses of dumb-bells, either

free in the serum, or frequently within the protoplasm of the pus cells, or adhering in smaller or larger numbers to the epithelial cells: these cocci he called gonococci. They are 1·25 μ in length as diplococci, 0·6–0·8 μ in transverse diameter, and they occur, as just stated, in the form of diplococci and as groups of four; the cocci are cres-

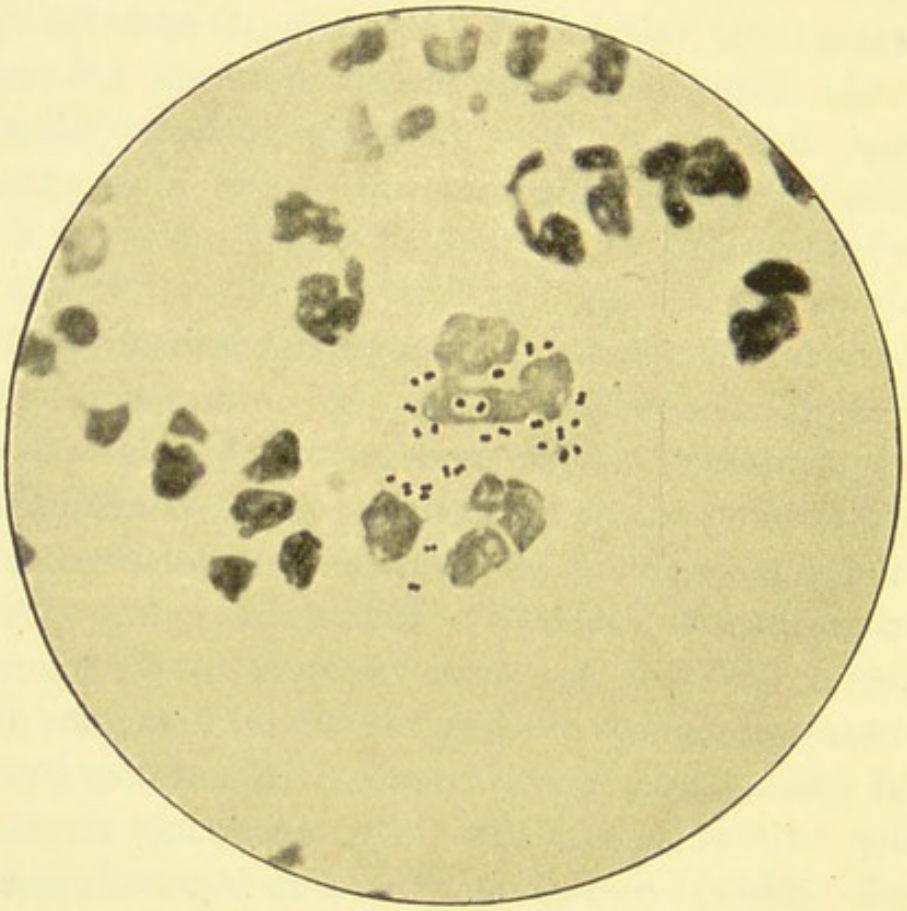

FIG. 40.—FILM SPECIMEN OF GONORRHŒAL PUS. IN THE CENTRE TWO PUS-CELLS CONTAINING IN THEIR INTERIOR NUMEROUS GONOCOCCI. × 1000. (E. C. Bousfield.)

centic and in this respect do not differ from many other species of cocci. Besides these diplococci, cocci often occur in the pus of gonorrhœa which are spherical and probably belong to the staphylococcus species (liquescens albus and liquescens aureus).

The gonococcus does not grow on nutrient gelatine, on

Agar mixture, or potato, and herein differs materially from the ordinary cocci occurring in pus. Bumm has proved that the gonococcus grows only on blood-serum, and Löffler and Krause have also succeeded in growing it on serum. In streak cultures on moderately solid blood-serum kept at 32° C., well moistened, the gonococcus, according to Bumm, grows in the form of a thin, narrow, greyish-yellow film 1-2 mm. in breadth, with smooth and moist-looking surface. The growth does not proceed for more than a few days and then dies. Animals are refractory against the gonococcus or the gonorrhœal secretion; dogs, rabbits, monkeys, horses, show no reaction, neither on the conjunctiva nor on the urethra. Bumm has, however, succeeded in producing in the human subject real gonorrhœa by inoculating, from a culture of the gonococcus, the urethral mucous membrane.

There can be no doubt about the fact that the gonococcus, which, as mentioned above, grows well on serum, is peculiar to gonorrhœa and cannot, therefore, be confounded with other pus micrococci. Probably Neisser's gonococcus was only a pus coccus, since it grew also on other media.

CHAPTER IX

BACILLUS (*Desmobacterium*, Cohn)

General Characters.—Bacilli are cylindrical or rod-shaped bacteria, which are rounded or square-cut at their extremities; they are longer in proportion to their thickness, and divide by fission, forming straight, curved, or zigzag chains of two, four, six, or more elements. Many species of bacilli in suitable nourishing material grow by repeated division into longer or shorter chains of bacillus—filaments or *leptothrix*—while other species have little or no tendency to form filaments. These appear straight or wavy and twisted, isolated or in bundles; and, although in the fresh condition they appear of a homogeneous aspect, when suitably prepared, as by drying and staining with aniline dyes, they show themselves composed of shorter or longer cubical, cylindrical, or rod-shaped protoplasmic elements, contained in linear series within a general hyaline sheath; between many of the elements is a fine transverse septum. The isolated bacilli are likewise composed of a membrane and protoplasmic contents. These latter appear homogeneous or finely granular and, when stained with aniline dyes, absorb the dye very easily and retain it better and longer than the sheath.

The protoplasm is either uniformly stained, or, as is not uncommon, shows at the ends of each rod much deeper staining than in the middle—that is to say, there is denser protoplasm at the ends of the rods than in the middle. In the short individuals this often gives a very characteristic appearance, inasmuch as each rod appears made up of three parts of equal size : two terminal stained granules and a middle clear unstained part. As just stated, this is not

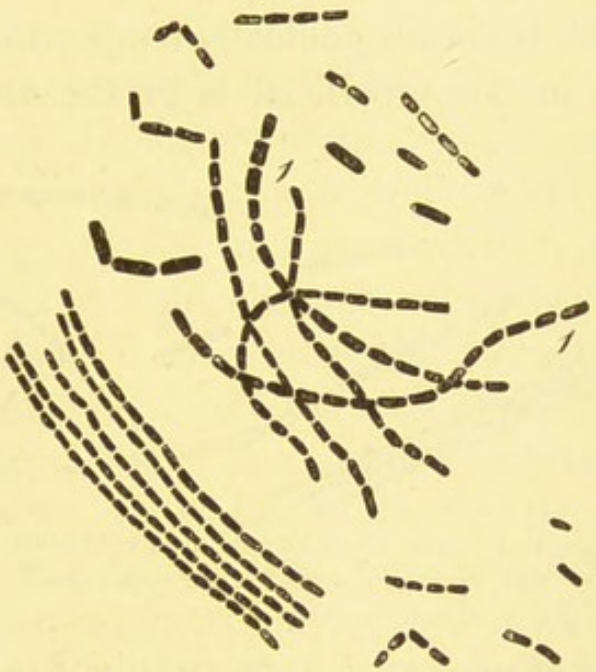

FIG. 41.—BACILLUS SUBTILIS GROWN IN PORK BROTH.
At 1, the elements are thickened. The preparation had been dried and stained with aniline purple.

peculiar to any one species, but can be noticed in all species ; it is particularly conspicuous in those in which the young elements are short, *e.g.* fowl cholera, fowl enteritis, septicæmia of rabbit, swine fever, &c., &c. But also amongst the longer, *i.e.* cylindrical, elements the middle part of the rod appears very often unstained and clear, while the protoplasm at the end is denser and stained ; the middle clear part is at the same time more or less well marked off with

rounded outline, spherical or oval in shape, and represents a vacuole; occasionally the stained protoplasm is central, while the unstained parts, the vacuoles, are terminal. Such vacuoles are very common in all species of bacilli; they (vacuoles) are, however, more frequently met with under conditions which imply want of sufficient nutritive material, as, for instance, when bacilli grow on solid media (gelatine, Agar mixture, potato) and when, owing to the continued growth into the depth of the medium, the first-formed or superficial layer becomes gradually removed from the nutritive material; in this superficial layer the vacuoles in the

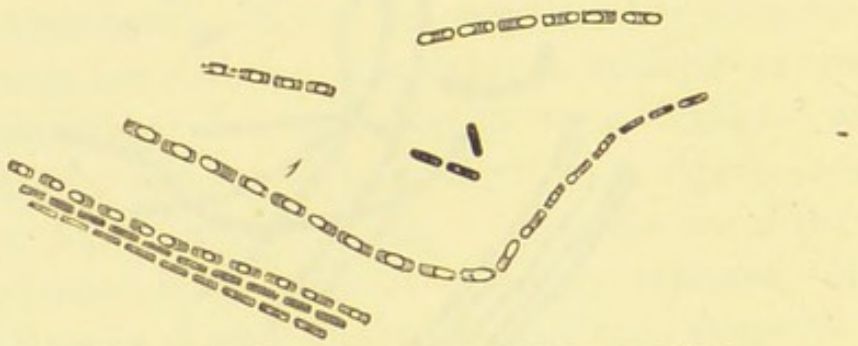

FIG. 42.—THE SAME BACILLUS AS IN PRECEDING FIGURE.
At 1, spores have made their appearance.

rods are very conspicuous; in preparations made of thread-forming bacilli under the above conditions of growth these appearances, *i.e.* of the presence of vacuoles regularly disposed in the individual rods, are very striking.

But, as stated before, the presence of vacuoles in the rods is also found under other than the above conditions, in some species more numerously than in others, and more often where rapid growth takes place than where this is not the case. This vacuolation is not indicative of any degenerative change, any more than it is in the mycelial threads of fungi where it is well known and typical, but seems, in some cases at any rate, to be due to the medium in which

the bacilli grow containing comparatively less nutritive material: not only in bacilli, but also in the individuals composing a spirillum, are these vacuoles to be observed. In cylindrical bacilli these vacuoles may be, and sometimes have been, mistaken for spores.

The ends of bacilli are generally rounded, occasionally straight, and less frequently more or less pointed or conical at one or both ends. In bacillus anthracis the ends are generally more or less straight; in the bacillus of diphtheria grown on gelatine many bacilli show one end pointed, the other rounded or straight and thick.

According to the stage and the rapidity of their growth, the bacilli vary much in length; this is the case not only with the single bacilli and short chains, but also in an eminent degree with the elements of a bacillus filament or leptothrix. In each case, indeed, it is possible to ascertain that all lengths occur, from the cubical or spherical element to the cylinder or rod. The former elongate into the latter and then divide. According to whether the division occurs in a short or long element, the daughter elements are cubical or spherical in the former, cylindrical or rod-shaped in the latter case. This applies to single bacilli, to short chains, and to the leptothrix forms.

There are a great many species of bacilli, differing morphologically from one another in the shape of the elements, in motility, in the power of forming filaments or leptothrix, and particularly in the thickness and length of the elements.

There are some species of bacilli—*e.g.* hay-bacillus, anthrax-bacillus, bacillus mesentericus, proteus vulgaris, bacillus of malignant œdema (Koch), &c.—in which in the single bacilli and in the chains and filaments the size of the elements varies from that of a cubical or spherical

mass of protoplasm not more than 0·5 or 0·8 μ in diameter to that of a cylinder or rod several times as long as it is thick. In some species (*e.g.* stained tubercle-bacilli) the elements of a chain are almost spherical. There are, on the other hand, other species (*e.g.* bacillus typhosus) where the elements are always rods or cylinders. In these cases of short bacilli it sometimes becomes difficult to say whether an

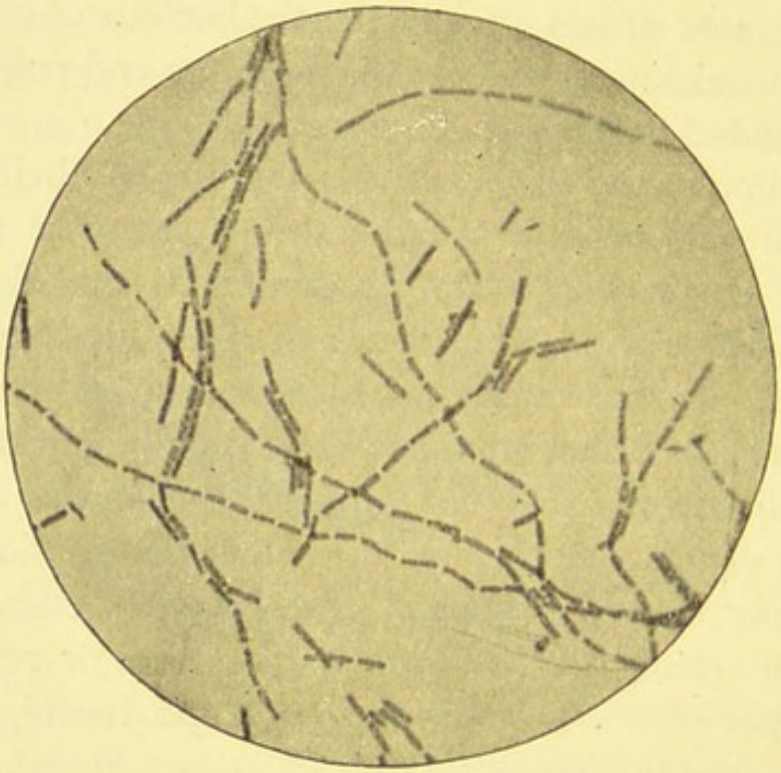

FIG. 43.—CHAINS OF BACILLI (BACILLUS FILAMENTOSUS) IN A STAINED FILM SPECIMEN.

individual is or is not a bacillus, but the growth of the bacilli into cylinders and leptothrix, and particularly their power of forming spores, is decisive, although neither of these events may happen, owing to peculiar conditions.

Flagella and motility of bacilli have been treated in a former chapter, and we need therefore not specially further concern ourselves about them.

Not all bacilli are capable of forming leptothrix-filaments.

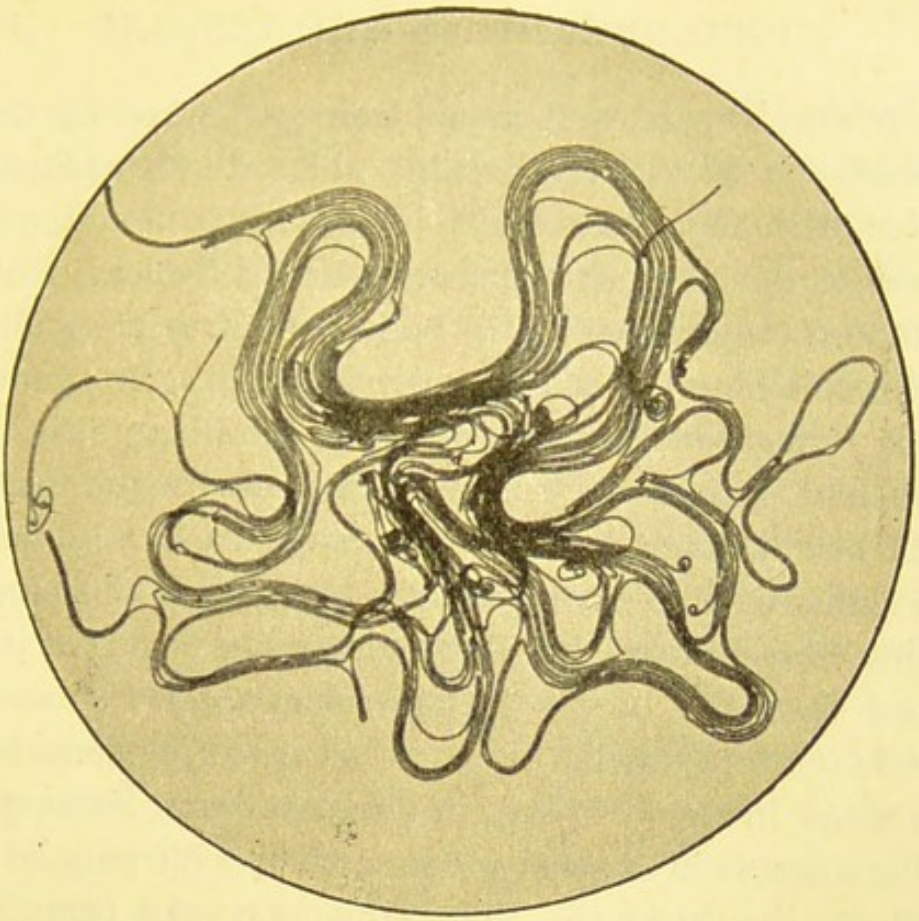

FIG. 44.—A COLONY OF FILAMENTOUS BACILLI (BACILLUS ANTHRACIS) AS SEEN UNDER MAGNIFYING GLASS.

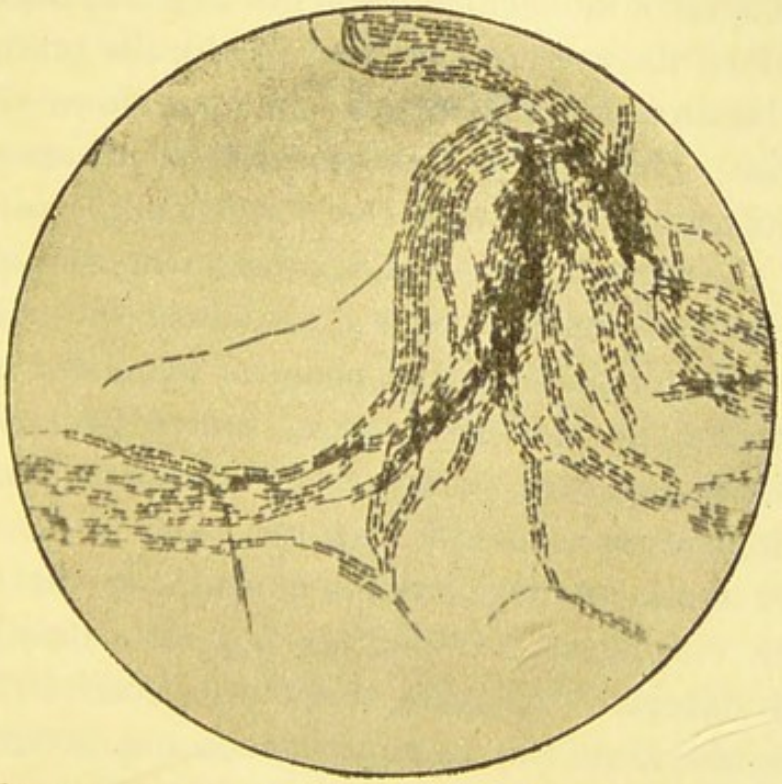

FIG. 45.—SAME SEEN UNDER A LOW MAGNIFYING POWER.

This power is possessed in an eminent degree by certain species, such as the hay-bacillus, the anthrax-bacillus, the bacillus of malignant œdema, the bacillus found on the surface of the mucous membrane lining the cavity of the mouth and tongue (leptothrix buccalis). Other bacilli (*e.g.* bacillus coli, leprosy-bacillus, tubercle-bacillus, &c.) generally do not, though exceptionally they do, form leptothrix.

Different species show great differences in the thickness of the bacilli, some being very fine, *e.g.* bacillus of mouse-septicæmia, bacillus of influenza; others thick and plump—bacillus amylobacter, bacillus megaterium; but it is also noticed that the bacilli of the same species growing in different culture media show in some cases considerable differences in this respect, in one medium forming thin bacilli, whereas in another medium the bacilli may be twice and thrice the thickness. The same may even occur in the same medium (see Fig. 41).

Many bacilli and bacillus-filaments (*e.g.* hay-bacillus, anthrax-bacillus) degenerate on growing old, the protoplasmic elements becoming granular and breaking down altogether into débris. This may occur to single elements within a chain or leptothrix; and then the corresponding part of the sheath of the chain, owing to the subsequent disappearance of the débris, becomes empty and devoid of protoplasm. Longer or shorter portions of a chain or leptothrix may thus degenerate and become deprived of protoplasm, the sheath only persisting. These portions become at the same time thicker, the sheath having swollen up.

Another mode of degeneration consists in the elements and sheath curling up, swelling up, and ultimately breaking down into débris. According to Cohn,[1] bacilli do not form zoogloœa in the same way as micrococcus and bacterium do.

[1] *Beitr. z. Biologie d. Pflanzen*, vol. ii.

With all due deference to the authority of Cohn, I must hold that some bacilli possessed of motility are capable of forming a true zoogloea. When one inoculates a fluid nourishing medium (*e.g.* broth) with hay-bacillus or other motile bacillus (*e.g.* bacillus mesentericus), after keeping it for twenty-four hours in the incubator one notices that the surface of the fluid is covered with a whitish film; this, as incubation goes on, thickens into a thick, resistant, not very friable pellicle. By shaking the fluid the pellicle becomes detached from the glass wall and sinks to the bottom of the fluid; after another day or two a new pellicle is formed, and so on until the material is exhausted.

Any part of this pellicle examined under the microscope shows itself to be a zoogloea in the true sense of the word, vast numbers of shorter or longer bacilli crossing and interlacing and lying embedded in a gelatinous hyaline matrix. As with proteus vulgaris, one occasionally notices at the margin of the mass one or other bacillus wriggling itself free and darting away. And in the case of non-motile bacilli, putrefactive and others, I have also seen distinct formations of zoogloea, having the shape of spherical or oval lumps of various sizes composed of a hyaline jelly-like matrix, in which are embedded the bacilli in active multiplication.

In those species in which the bacilli are capable of forming leptothrix (leptothrix buccalis, hay-bacillus, anthrax-bacillus) the filaments may form dense convolutions. When in these convoluted filaments spores are formed, and the sheaths of the filaments swell up and become agglutinated into a hyaline jelly-like substance, the spores appear to form a sort of zoogloea.

Bacilli are killed by drying, but it is necessary to bear in mind that they must be exposed to the drying process in thin layers (Koch). At the temperature of boiling water

they are invariably killed, but not their spores. Even heating them from half an hour to several hours at a temperature above 55° or 60° C. kills them. Freezing also kills them, but not their spores. Carbolic acid, corrosive sublimate, thymol, &c., kill them.

The formation of spores and the germination of these

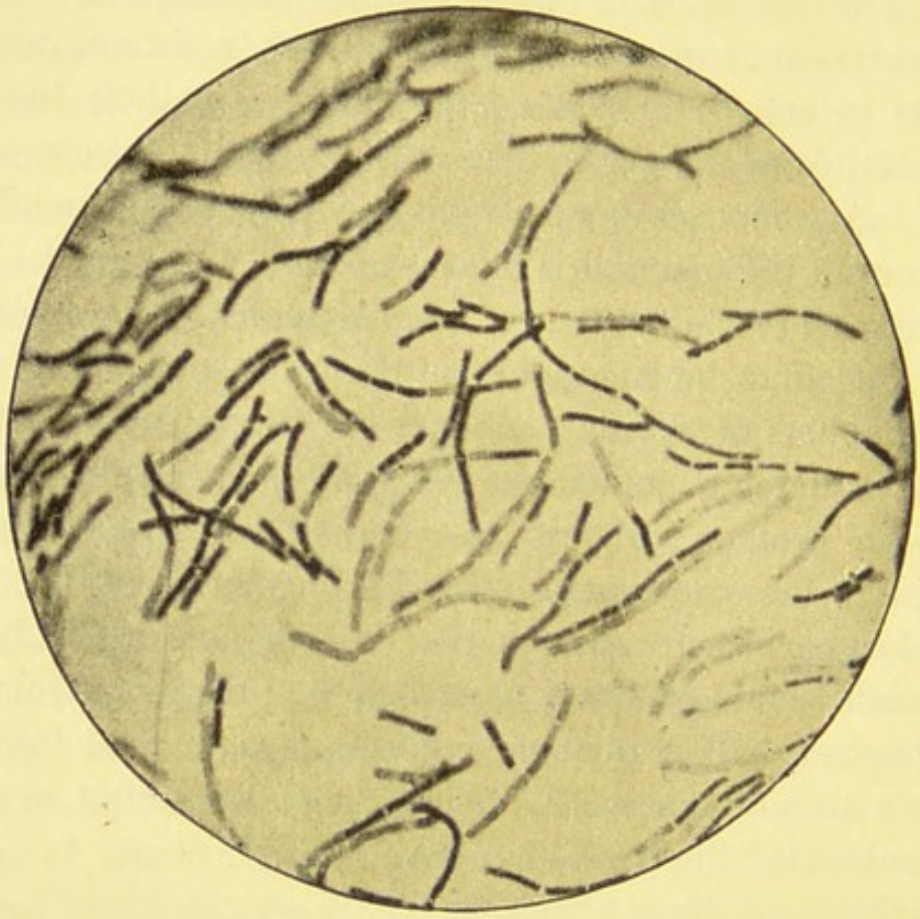

FIG. 46.—THREADS OF BACILLI (B. ANTHRACIS) SHOWING IN PARTS, OR AS A WHOLE, THE EMPTY SHEATH WITHOUT ANY STAINED BACILLARY PROTOPLASM.

× 600.

have been already described in a former chapter, and it now remains to describe the methods of staining them. When spores, either free or in bacilli, are stained in the usual way in film specimens, the spores do not take the stain, but remain conspicuous as clear oval bodies; in order to make them take the dye it is necessary, after drying in the usual

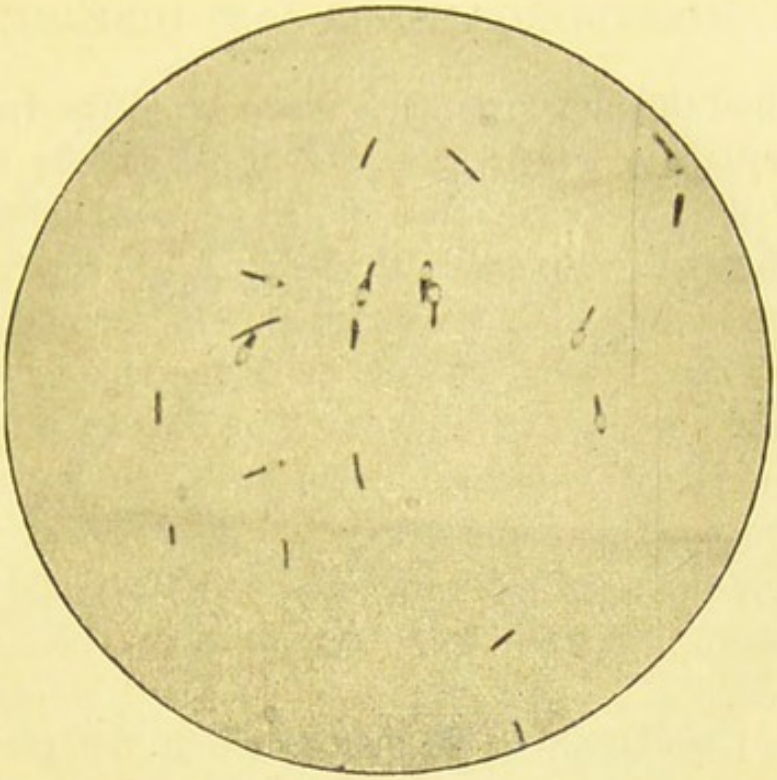

FIG. 47. — SPORE-BEARING BACILLI STAINED IN THE ORDINARY MANNER (BACILLUS OF SYMPTOMATIC CHARBON), THE SPORES BEING UNSTAINED.

× 1000.

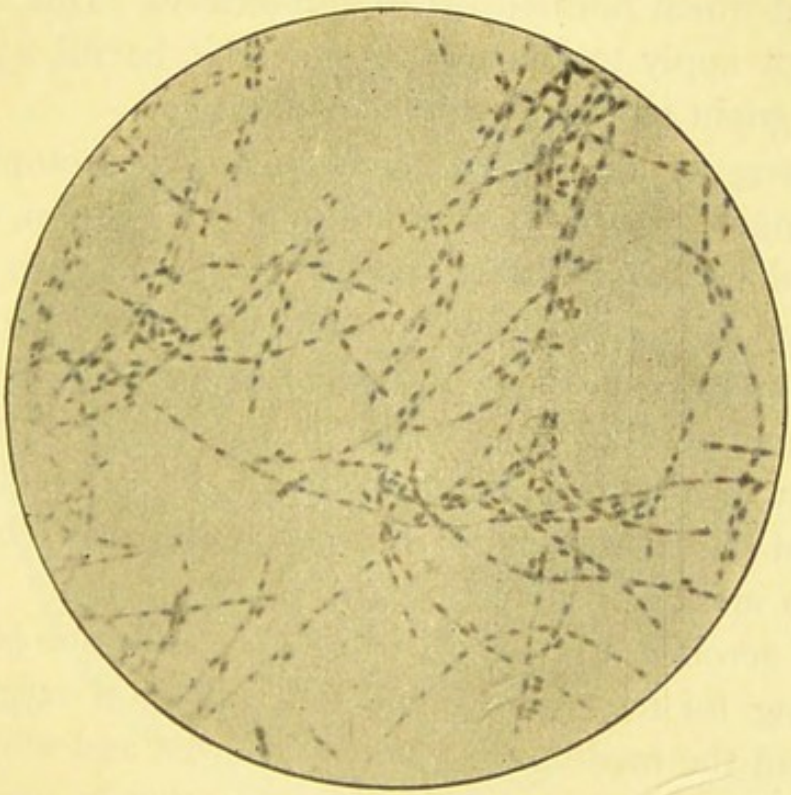

FIG. 48. — SPORE-BEARING FILAMENTS (BACILLUS ANTHRACIS); THE SPORES, STAINED AFTER BOILING IN CARBOL FUCHSIN, ARE DEEPLY STAINED, THE REST OF THE FILAMENTS ONLY FAINTLY SO.

× 600.

way, to boil the film cover-glass specimen in the dye (methyl-blue, gentian violet, or carbol fuchsin); hereby the spores become deeply stained and on subsequent good washing retain the dye with great persistence. By careful washing the point may be so hit off that the spores appear deeply stained, whereas the bacillary substance is only faintly so. The finest specimens are obtained by boiling the dried film specimen in carbol fuchsin; then wash well in water; then place the specimen in methyl-blue anilin water for half a minute to one minute; wash again well; dry and mount in xylol balsam: the bacillary substance appears blue, the spores bright red.

It has been shown by Engelmann that the presence and renewal of oxygen as well as a certain concentration of the nutritive material are essential for the motility of those bacteria that are possessed of cilia, *i.e.* that are possessed of locomotion and which normally grow aerobically. This, of course, does not apply to the motile anaerobic bacilli, *e.g.* bacillus of malignant œdema, tetanus, or butyricus.

As long as the bacteria are living, their protoplasm does not combine (stain) with nitrate of silver solution, only after death does this become possible. Hereby an index is furnished for ascertaining whether, and which, bacteria in a given sample are living, and which are dead. There is no difference in this respect, *i.e.* in respect of the different reaction of nitrate of silver on living and dead protoplasm, between the protoplasm of bacteria and that of other vegetable or animal tissues.

All aerobic bacteria, pathogenic and non-pathogenic, requiring for their growth and multiplication oxygen, obtain this from the medium in which they grow, and which oxygen is dissolved in those media, or after this is consumed or absent it is obtained by the bacteria in the process of the chemical decomposition of the carbohydrates and proteids

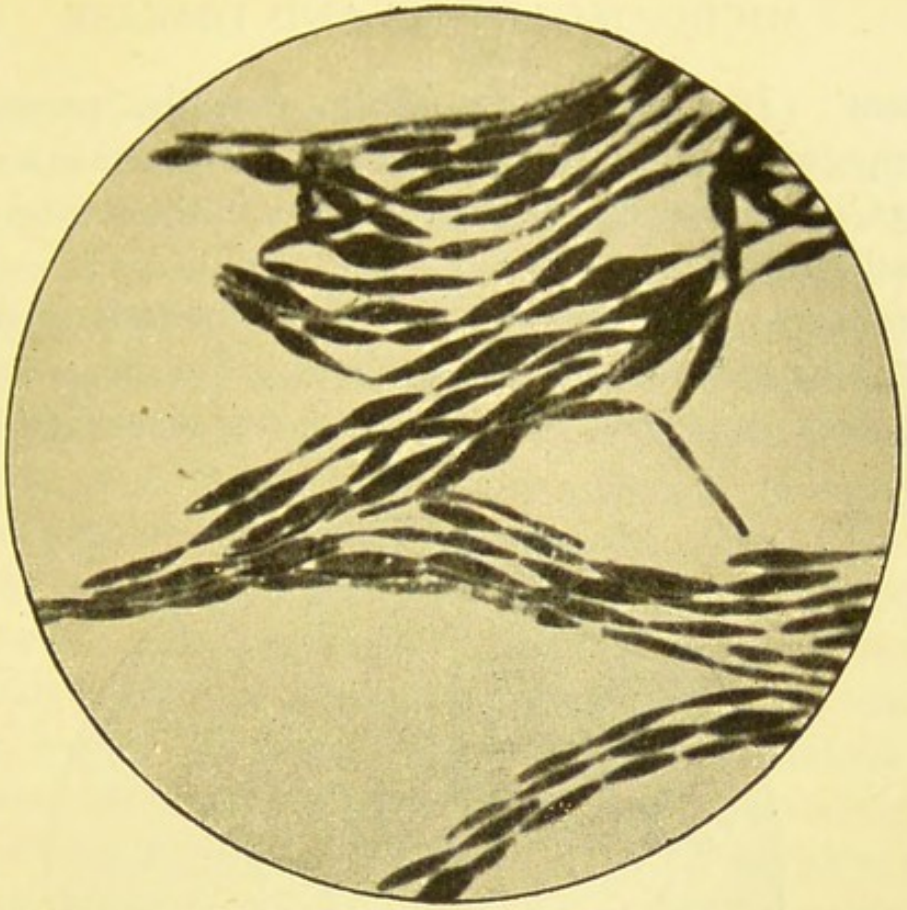

FIG. 49.—IMPRESSION SPECIMEN FROM A RECENT GELATINE-PLATE CULTURE OF BACILLUS ANTHRACIS. × 1000.

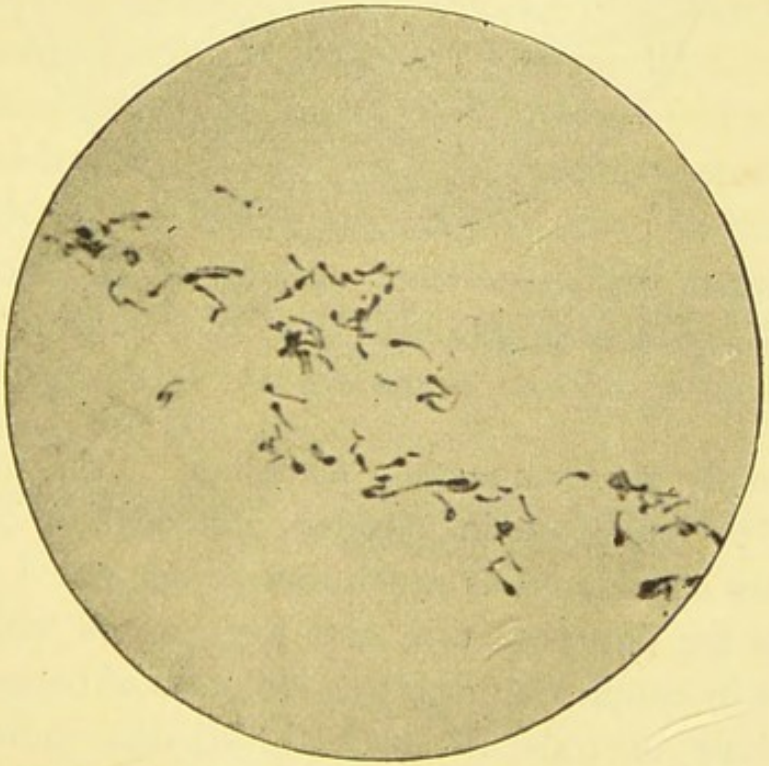

FIG. 50.—IMPRESSION SPECIMEN FROM A RECENT GELATINE-PLATE CULTURE OF BACILLUS DIPHTHERIÆ. × 1000.

present. Dr. Dupré[1] has shown that the presence or disappearance of oxygen (air) dissolved in water is a precise gauge, in the first case of the absence, in the second of the growth, of microphytes.

In many species one or both ends of the rods, or the free end of the rods forming the terminals in a chain, are swollen and thick, spherical, pear-shaped, or club-shaped (Fig. 50);

FIG. 51. — FILM SPECIMEN OF TUBERCLE-BACILLI FROM A GLYCERINE-AGAR CULTURE SOME WEEKS OLD; SHOWING BRANCHED MYCELIAL-LIKE FILAMENTS WITH CLUB-SHAPED SPROUTINGS.

× 1000.

occasionally there are some elements, in the middle of a shorter or longer chain, swollen, spherical, or oval. Such forms are considered as involution forms, but I have good grounds for doubting this, and the reasons will be stated later on in connection with the evolution of bacteria. When in a chain of rods, *i.e.* in a thread, the individual rods

[1] *Report of the Medical Officer of the Local Government Board*, 1884.

become so changed, an organism results which is totally unlike the typical thin smooth thread, but appears more like a varicose thread in which the individuals are torula-like, spherical, or oval cells, connected one with another by thin bridges, the cells being three or more times as thick as the typical rods (Fig. 49). In connection with this and the former appearance, another appearance deserves notice, viz. the segregation of the protoplasm in a chain or in individual rods as separate spherical or oval granules, whereby the rods and chains become transformed into varicose rods or fibres; in these the granules take and retain the dye easily, whereas the bridges between them are less stained; *e.g.*, in tubercle bacilli, leprosy bacilli, diphtheria bacilli, and others this appearance is sometimes very regular and characteristic.

Besides the above torula-like chains of the bacilli with or without terminal club-shaped or pear-shaped enlargements, another curious appearance deserves notice, that is the branching that is observed in threads of tubercle bacilli when grown for some time on glycerine Agar; we find here, besides torula-like threads, with club-shaped terminals, others which show distinct sprouting and gemmation of lateral cells,[1] these latter elongating into threads themselves with club-shaped terminal enlargement. This suggests that the tubercle bacilli are probably originally evolved from a mycelial fungus and under certain conditions have a tendency to revert to this state (Fig. 51).

[1] *Report of the Medical Officer of the Local Government Board*, 1890–91.

CHAPTER X

BACILLI : SPECIAL

BEFORE describing the various species of bacilli which in man or animals, or both, are associated with infectious disease we will describe the most common non-specific bacilli, as, owing to their wide distribution, they not uncommonly are found associated with the former.

These are the most widely distributed species of bacilli :— (1) Bacillus subtilis, or hay-bacillus; (2) bacillus mesentericus vulgatus; (3) proteus vulgaris; (4) proteus Zenkeri; (5) bacillus fluorescens liquescens; and (6) bacillus coli.

1. *Bacillus subtilis* (hay-bacillus).—The elementary rods are of various lengths from 0·002 to 0·006 mm., and are about 0·002 mm. in thickness. According to Cohn, at a temperature of 21° C. division into two requires about one hour and a quarter, at 35° C. only about twenty minutes.

The bacilli are capable of forming leptothrix filaments. The bacilli when single are possessed of one flagellum, or sometimes of two, one at each end. After division the individual bacilli remain connected, each possessing a flagellum at the free end. Each of them divides again into four, so that a chain of four is formed. But they may

separate again or may go on dividing, remaining united, and thus forming a longer or shorter filament. Not all bacilli possess motility, many of them being for a time in a resting state.

The bacilli form a dense resistant pellicle on the surface of the nourishing medium, and in this copious spore-formation takes place. If shaken when growing in a fluid,

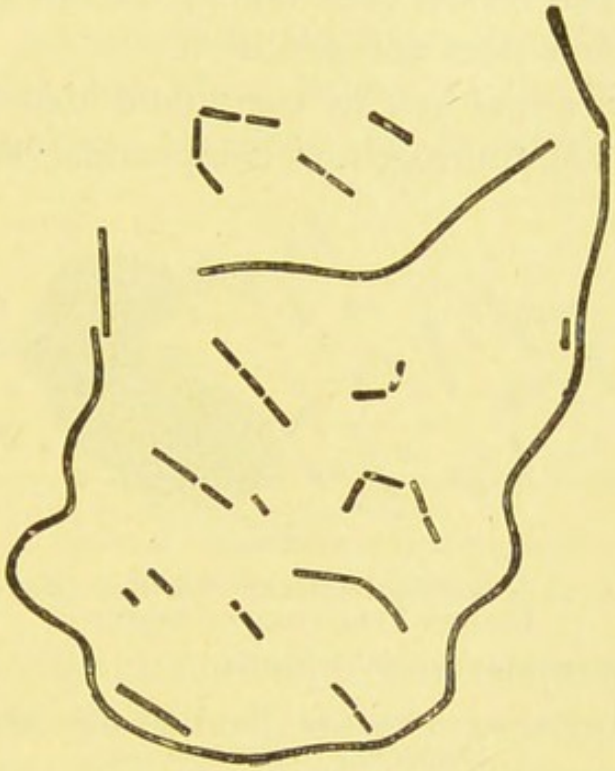

FIG. 52.—FROM A CULTURE OF BACILLUS SUBTILIS (HAY-BACILLUS). Various forms between single bacilli and leptothrix. Magnifying power about 700.

the pellicle falls to the bottom, and soon a new pellicle is formed.

Spore-formation is independent of any deficiency of nourishing material. The spores are oval, bright, of about 0·001 to 0·002 mm. in length, and about 0·0006 to 0·001 mm. in thickness. They do not stain in ordinary dyes, and hence form a great contrast to the bacilli.

This bacillus is very common and widely distributed; it

occurs in organic substances left exposed to the dust of air. The best material is hay-infusion. An infusion, cold or hot, of hay is made in a beaker or flask; the fluid is neutralised, then filtered, covered with a glass plate, and left to stand in a warm place. After a day or two it swarms with bacillus subtilis, which is also called hay-bacillus, since ordinary hay contains multitudes of its spores. For this reason even boiling of the fresh infusion for a few minutes does not sterilise it.

The bacillus grows well in every fluid that contains the necessary salts and nitrogenous compounds; thus all kinds

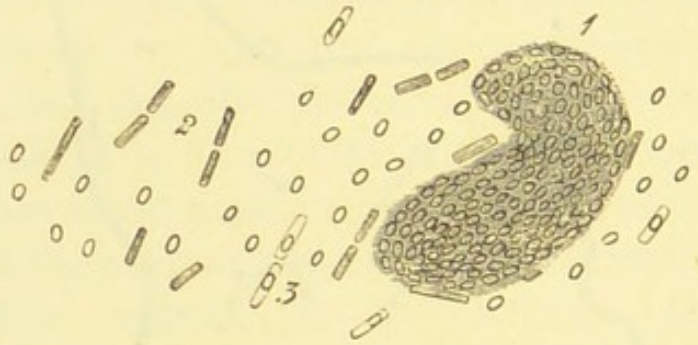

FIG. 53.—FROM A CULTURE OF BACILLUS SUBTILIS (HAY-BACILLUS), WITH COPIOUS FORMATION OF SPORES.

1. Mass of spores embedded in hyaline matrix.
2. Bacilli.
3. Single bacilli containing each a spore: the sheath of the bacilli is well seen.

Magnifying power about 700.

of broth, all kinds of animal fluids (hydrocele, blood-serum, &c.), gelatine, peptone solution, &c., are suitable nourishing media.

The spores of the hay-bacillus are widely distributed in the air, and contaminations by dust are due to its spores.

Hay-bacillus is an aerobic microbe.

In gelatine plates it forms liquefying colonies showing characteristic threads radiating from the centre. In stab and streak gelatine cultures it grows rapidly and liquefies the gelatine; solidified blood-serum is liquefied by the

growth. On potato it forms a thick, whitish, creamy growth rapidly covering the inoculated surface; here, as also on fluid media, it forms copious spores in a resistant, corrugated surface-pellicle.

In hay-infusion (neutralised) that had been kept in the incubator at 37° C. the spores which appear are not all belonging to the bacillus subtilis; those in the surface-pellicle are spores of this bacillus, but in the depth of the fluid spores occur which resemble the above in aspect, shape, and size, but which belong to the bacillus amylobacter or bacillus butyricus of Prazmovski, a strictly

FIG. 54.—GERMINATION OF SPORES INTO BACILLI.
a Spores of a small kind.
b. Spores of a larger kind of bacillus subtilis.
Magnifying power about 700.

anaerobic motile bacillus liquefying grape-sugar gelatine. Aerobic gelatine plates made of such an infusion, or of the surface-pellicle, after heating to 80° C. from five to ten minutes, bring forth the colonies of the hay-bacillus only. Anaerobic cultures in grape-sugar gelatine made of the fluid taken from the bottom yield growth of the bacillus amylobacter; the chief morphological character distinguishing it from the hay-bacillus and from other anaerobic bacilli (*e.g.* bacillus butyricus of Hueppe and of Botkin) is its change in shape during sporing; the cylindrical bacilli, as spores develop and grow in them, change into spindle- or tadpole-shaped forms three and more times thicker around the spore—clostridium.

2. *Bacillus mesentericus vulgatus, potato bacillus* (Löffler).—This bacillus is spore forming, aerobic, very motile, and is thicker than the former (bacillus subtilis); it occurs singly or in chains of two or more rods; it and its spores have a wide distribution; it is common in dust of air, and in many putrid organic substances (potato, milk); in milk and other organic fluids that have been exposed to air contamination it is often present.

It differs from bacillus subtilis, first, by the greater thickness of the bacilli, and, secondly, in the aspect of its colonies in gelatine plates: these being round, liquefying, and containing in the centre a membrane-like accumulation, but no radiation of fibres. Sown in broth and incubated at 37° C., it forms already in twenty-four hours a conspicuous, coherent, wrinkled pellicle, the broth remaining limpid. The pellicle is a network of filaments in which oval glistening spores soon make their appearance; the spores are of the size of those of hay-bacillus, but slightly thicker. On potato it forms rapidly a sticky, greyish-yellow mass, on nutrient Agar a wrinkled membranous growth; growing on the surface of gelatine, it liquefies this rapidly, forming, however, a coherent, wrinkled, membranous mass.

3. *Proteus vulgaris* (Hauser).—This is an aerobic motile non-sporing bacillus which, as Hauser has shown, is *the* microbe of putrefaction. It is found in all putrid organic substances; it is the principal microbe which is found in the putrid bodies of dead animals and man. It is present normally in the large intestine and from here after death soon extends (grows) through the walls of the intestine into the abdominal cavity, into the abdominal organs, then into the thoracic viscera, and through the blood-vessels into all other parts. It rapidly liquefies gelatine, and peptonises and destroys animal matter. In gelatine plates (at 20° C.) its colonies

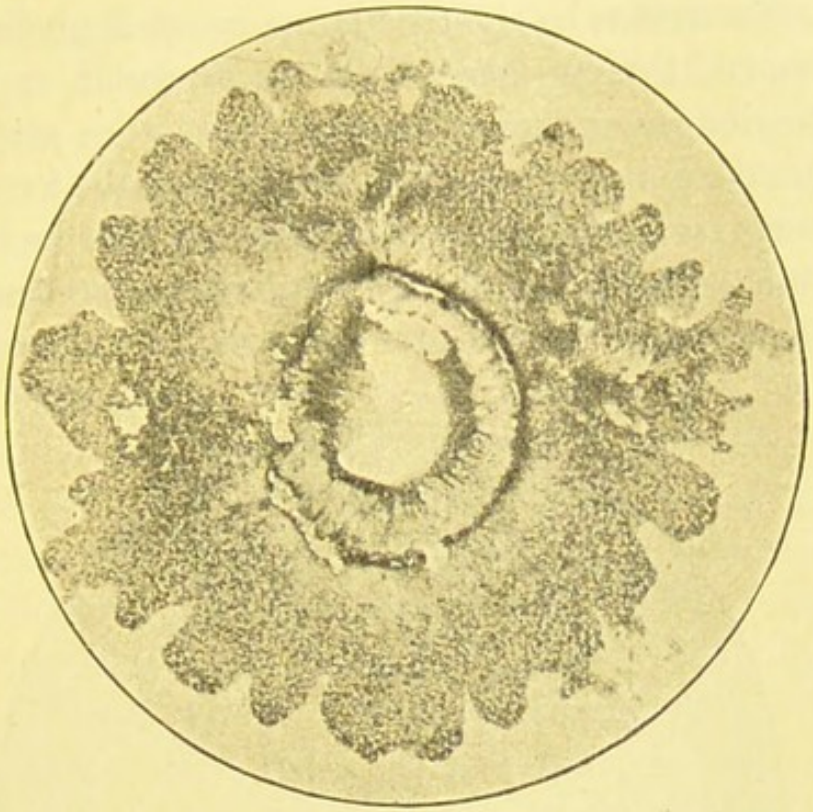

FIG. 55.—YOUNG COLONY IN GELATINE PLATE OF PROTEUS VULGARIS. As seen under a Magnifying Glass.

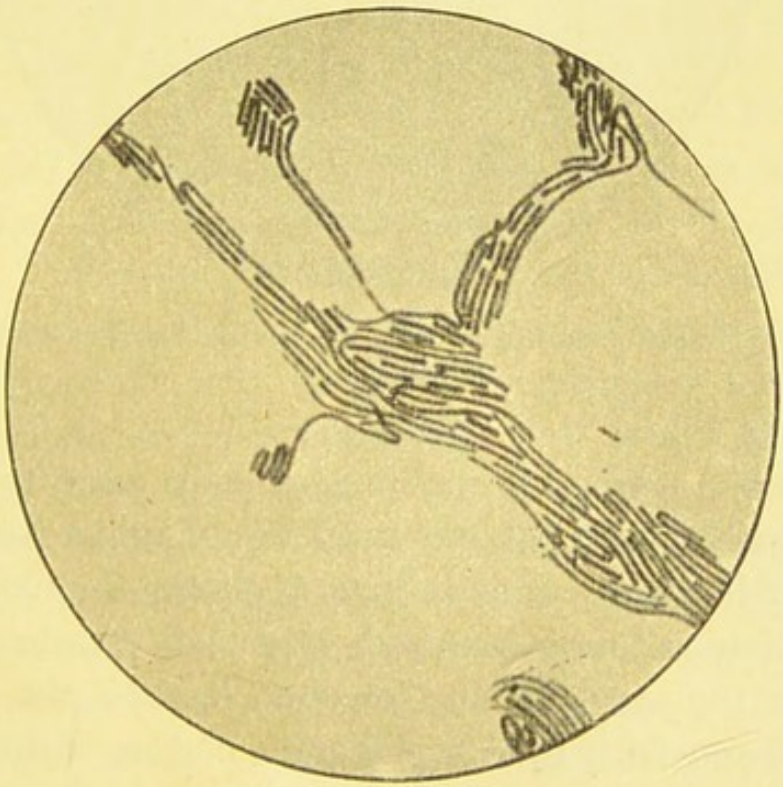

FIG. 56.—IMPRESSION SPECIMEN OF "SWARMERS" OF A YOUNG COLONY OF PROTEUS VULGARIS. × 1000.

appear after sixteen to eighteen hours as small greyish dots; when looked at under glass they are irregular in outline, possessing longer or shorter angular filamentous projections. These are composed of motile bacilli and are the forerunners —swarmers—for further outgrowths, so that after twenty-four hours or later many neighbouring colonies are connected by these filaments and coalesce, the older colonies showing rapid

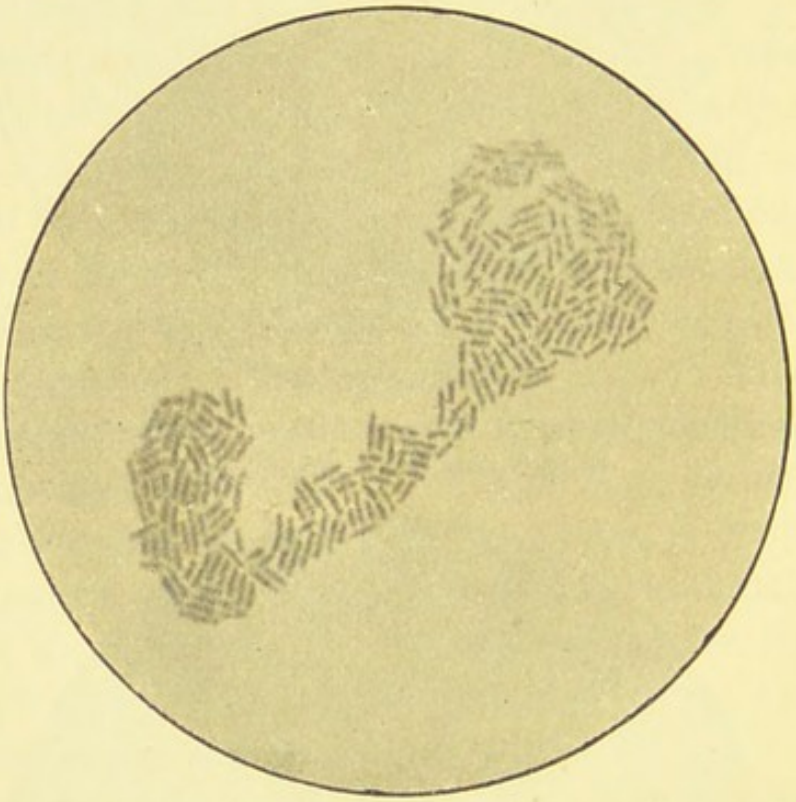

FIG. 57.—FILM SPECIMEN FROM A COLONY OF PROTEUS VULGARIS. × 1000.

liquefaction. In stab culture, already after twenty-four hours liquefaction is pronounced in the upper parts of the stab, which considerably increases during the next day, by which time a funnel-shaped, liquefied, translucent mass occupies the original line of inoculation; the liquefied gelatine being fairly translucent, at the bottom of the liquefied mass is seen a floccular, granular, white precipitate. On the

surface of Agar (streak) at 37° C. the growth is moist, sticky, and grey. Broth is made uniformly turbid in twenty-four hours; later on, an imperfect sort of pellicle is noticed. Film specimens (impression) made of young colonies on gelatine, before liquefaction has set in, show beautiful filaments of bacilli, some of considerable length and unsegmented, others made up of short rods; the filaments are straight or twisted and at their ends show rapid division into cylindrical bacilli. Fig. 56 shows such an impression film of the swarmers of a young colony (sixteen hours old); Fig. 55 an impression of a colony twenty-four hours old, the centre already liquefied. When the liquefaction has well progressed (say after two to three days) and a drop is examined fresh under the microscope most of the bacilli are actively motile, either short ovals—single or in dumb-bells—or cylindrical and even filamentous. There are also individuals so short that they cannot be distinguished from cocci—single cocci and diplococci; and, further, some of the cylindrical bacilli are more or less curved like vibrios, while some of the filaments are wavy and even spiral-like. It is because the microbe appears in such older cultures under all known shapes (*i.e.* protean) that Hauser gave it the name of "proteus." Proteus vulgaris is not, however, a single species.

The liquefied gelatine and the broth cultures possess distinctly a putrid smell. The bacilli possess a single short spiral flagellum, and it is astonishing how briskly they move in the fresh state and therein stand in striking contrast with some other bacilli, *e.g.* bacillus coli, which, though some individuals are provided with several flagella, show only very feeble movement in the fresh state. In some varieties the bacilli possess quite a number of flagella.

4. *Proteus Zenkeri.*—This is an aerobic, non-sporing, motile bacillus of about 0·4 μ thickness and 1 to 1·5 μ length;

it occurs frequently in putrid organic matter; in meat that has been exposed to air and is undergoing putrefaction it is often associated with proteus vulgaris. Its colonies in nutrient gelatine are very characteristic (Fig. 58): already after twenty-four, better after forty-eight, hours' incubation, whitish dots are seen which are made up of numerous

FIG. 58.—IMPRESSION OF A COLONY IN GELATINE PLATE OF PROTEUS ZENKERI. Magnified with a glass.

bundles of more or less beaded filaments radiating from a shorter or longer line situated in the depth; in addition to this, irregular grey groups of plate-like masses seem to pass out and to spread from the central mass on the surface. The mass of threads resemble a mycelium of fungus; the presence of the grey plate-like masses makes it at once distinct. Under

the microscope, in stained specimens the growth is made up of threads which consist of rows—generally more than one—of short bacilli; in many places the bacilli form clusters in the threads (Fig. 59). It does not liquefy gelatine. On the surface of gelatine it forms a filamentous expansion, the filaments growing from the central streak of inoculation like the filaments in the fan of a feather; the same kind of

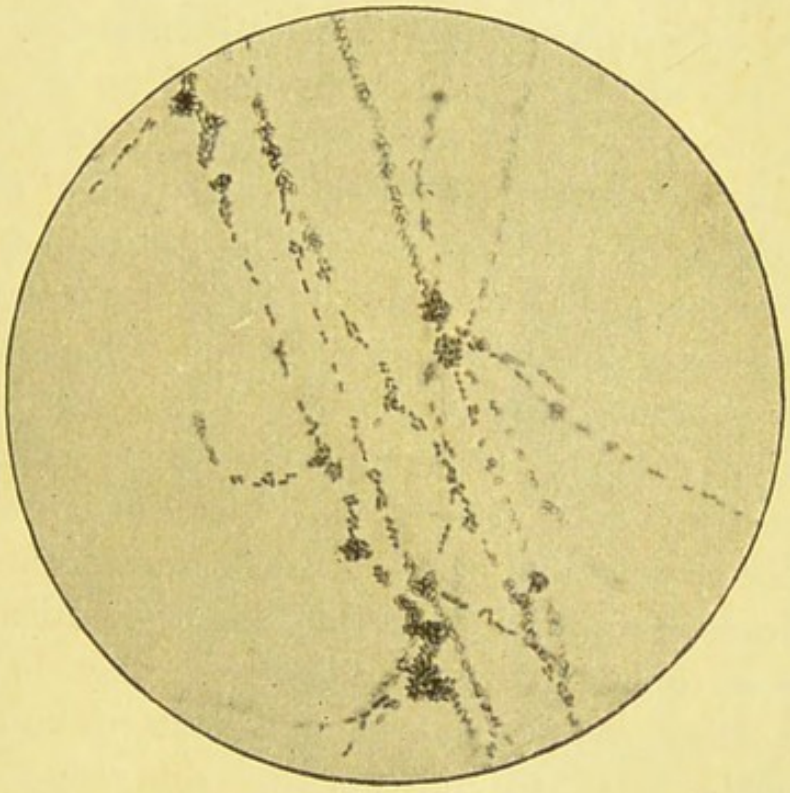

FIG. 59.—IMPRESSION SPECIMEN OF THE FILAMENTS OF PROTEUS ZENKERI. × 300.

growth, only not so distinct, is formed by the microbe on Agar; it grows better at 20° than at 37° C.

5. *Bacillus fluorescens liquescens.*—This is a typical water bacillus; it occurs in most waters—river, lake, pond, well—and in all or most organic substances to which such water had been added. It is a motile, aerobic, non-spore-forming bacillus, liquefying gelatine rapidly and producing a fluorescent greenish or diffuse greenish-blue colouration. Its

character in gelatine plates is sufficient to identify it: after twenty-four hours at 20° C. it first forms grey, circular colonies, already depressed and liquefying; after forty-eight hours the colonies have much increased and are now liquefied, depressed, turbid, circular patches with a distinct greenish tinge of colour. When the colonies are numerous and closely placed the plate may by this time be altogether liquefied, the fluid gelatine turbid and of greenish, fluorescent tint. In gelatine stab culture the liquefaction proceeds from the upper part of the stab, the lower part being made up of a row of greyish-white dots. The appearance of a plate culture and of a stab culture after twenty-four to thirty-six or forty-eight hours' incubation, as also of the individual bacilli seen under the microscope, looks exactly like those figures of the Bacillus radicicola mentioned in a former chapter (Chapter VI), the liquefied gelatine being fluorescent, greenish. Soon the liquefaction extends throughout the whole culture. On Agar also the greenish, fluorescent colouration is pronounced, the surface growth itself being brownish, translucent. Under the microscope the bacilli are thin and cylindrical, motile, singly or in dumb-bells, or in filaments; they do not form spores. They grow best at lower temperatures up to 22° C., but grow also at 37° C., only not so well in comparison.

6. *Bacillus coli communis* (Escherich).—The typical bacillus of fæcal matter, of the intestinal contents of man and animals; and occurs also in all solids and fluids to which intestinal discharges have had access. It is sometimes present in nasal, oral, and pectoral discharges. It occurs (due to secondary invasion from the intestine) in abdominal inflammatory processes: abscess of the liver, spleen, peritoneum; in pulmonary and bronchial suppurations; in ulceration and abscesses of the skin and mucous membranes open to contamination with filth. Its primary home ap-

pears to be the normal large intestine; in acute and chronic diseases of the small intestine it may be very copiously present in the ileum.

Bacillus coli is a motile, aerobic (facultative anaerobic), non-sporing, non-liquefying rod; it is killed by thorough drying and by a temperature of 66° C. in five minutes.

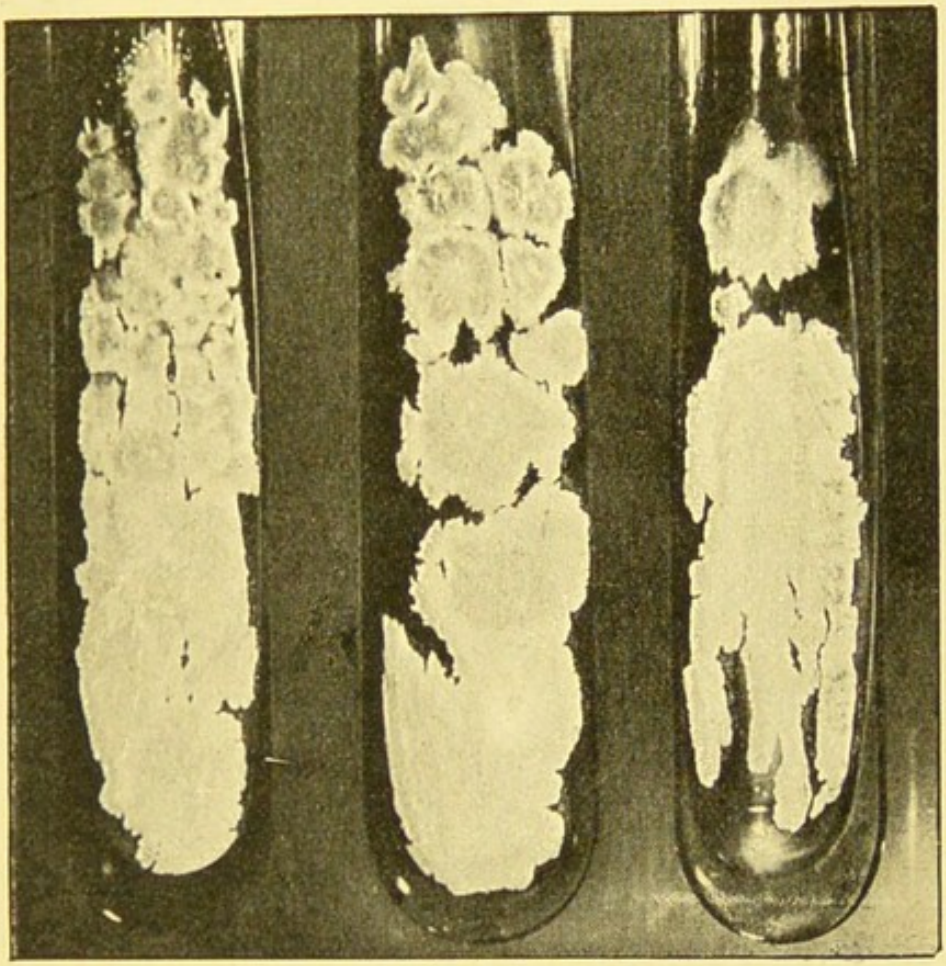

FIG. 60.—SURFACE GROWTH ON GELATINE OF BACILLUS COLI, SHOWING ISOLATED, CONFLUENT COLONIES.

The length of the individuals varies between 0·8 μ and 1·5 μ–3 μ, though in later stages in culture longer or shorter filaments are met with; its thickness is about 0·4–0·5 μ. When examined fresh from the intestinal contents in health and disease only a minority are as a rule found to be possessed of motility, though in some cases (English cholera)

motility may be observed on many individuals. The same holds good for artificial cultures—plates, surface gelatine and surface Agar, broth and milk cultures—for here also in young cultures, as a rule, only a minority show motility, in old cultures the motile individuals are rare.

Bacillus coli forms typical colonies on the surface of gelatine at 20° C.; after twenty-four hours they are recognisable

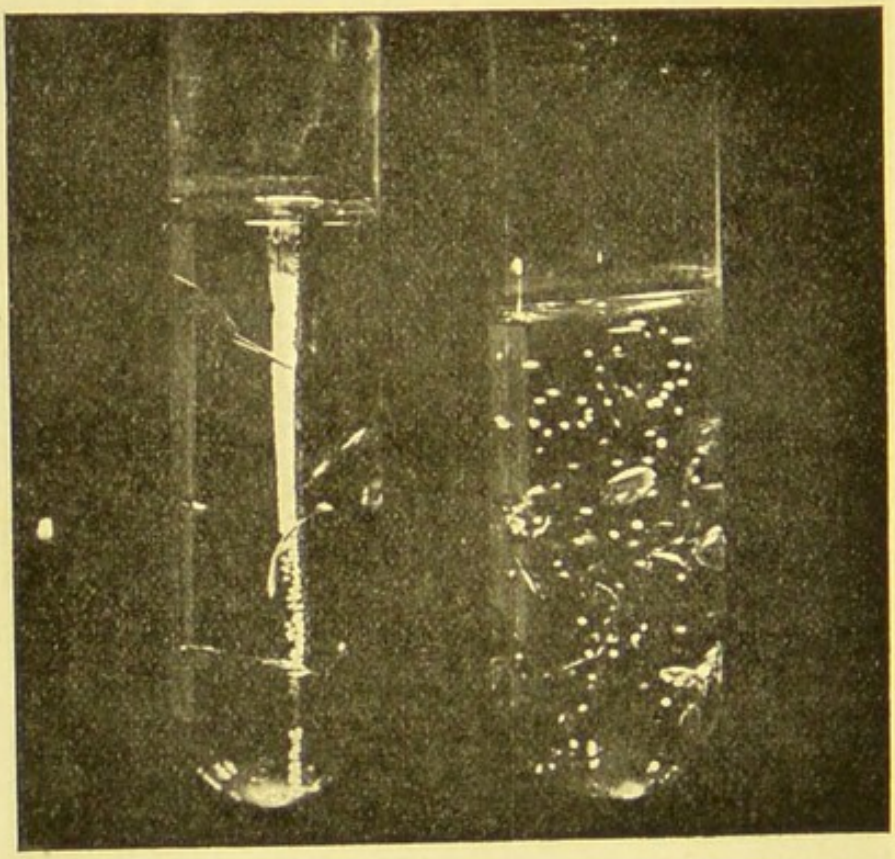

FIG. 61.—A STAB CULTURE AND A SHAKE CULTURE IN GELATINE OF BACILLUS COLI, WITH GAS BUBBLES.

as flat, translucent, greyish, roundish, but angular patches, slightly thickened in the middle part or near one margin; after forty-eight hours the patches are considerably enlarged, angular, thin and filmy, and translucent in the marginal, thick and less translucent in the middle part. The whole patch is dry, whitish in reflected light, and under a magnifying glass appears fairly homogeneous, though after several

days it commences to show some kind of concentric differentiation.

The colonies in the depth of the gelatine appear as spherical small dots, white in reflected, brownish in transmitted light. Fig. 72 is a good illustration of a gelatine plate culture of bacillus coli; compare also Fig. 60.

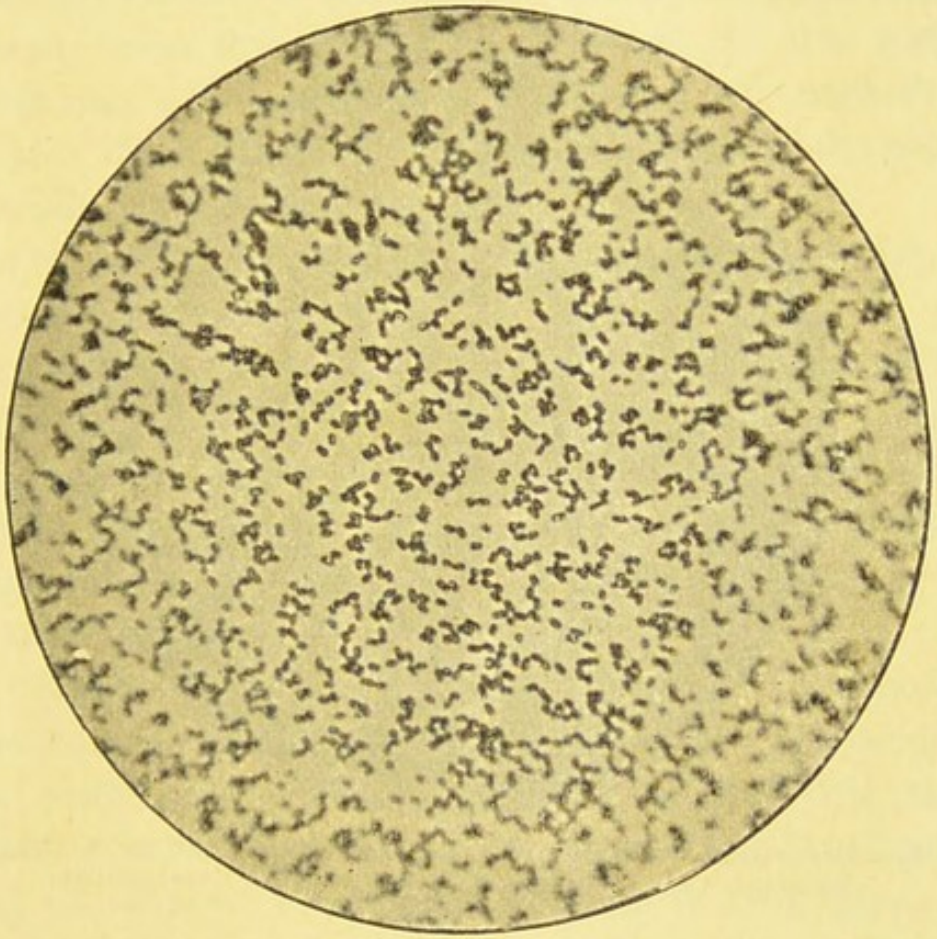

Fig. 62.—Film Specimen of a Variety of Bacillus Coli, the individual Bacilli chiefly Oval Rods, some few Cylindrical.

× 1000.

Equally characteristic is the streak culture on the slanting surface of gelatine; after twenty-four hours a greyish band, thicker in the line of inoculation—grey, filmy, knobbed, or crenated in the marginal part—after forty-eight hours it has spread considerably in breadth, but has retained the above aspect, except that the middle part is more thickened, and

the whole growth appears more white in reflected light. After 3–4 days the band has spread over the greater part of the surface of the gelatine, but is still dry, filmy, crenate, and irregular in the marginal part; the whole band examined under a glass appears more or less homogeneous.

When ordinary nutrient gelatine is inoculated from a culture, then melted and shaken and allowed to set again,

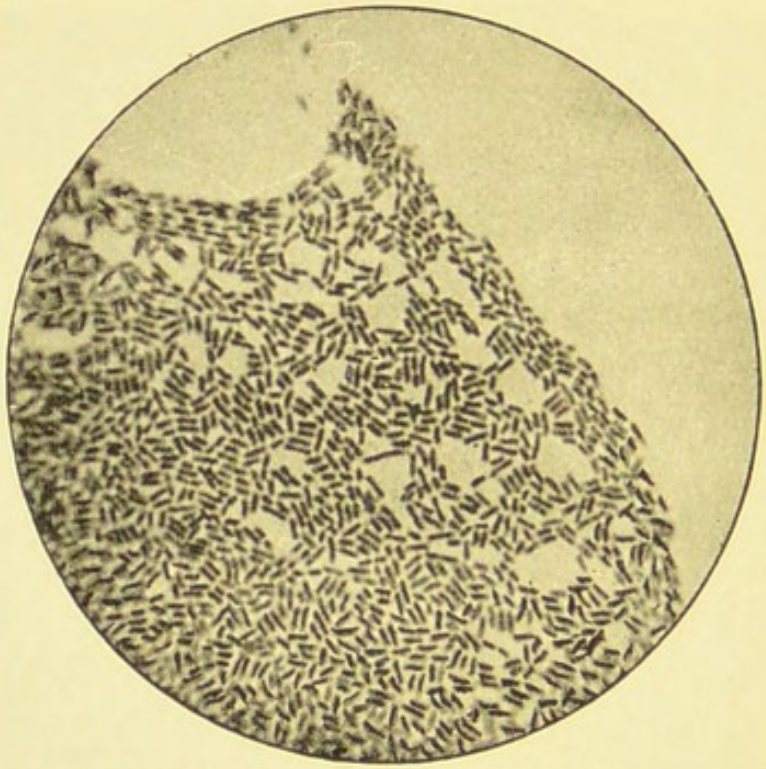

FIG. 63.—IMPRESSION SPECIMEN OF THE MARGINAL PART OF A COLONY OF BACILLUS COLI; MOST OF THE BACILLI ARE CYLINDRICAL.

× 1000.

and incubated, it will be found after 24–36 hours that this shake culture is permeated by minute spherical colonies in all its depth, and in connection with each colony is a spherical or lenticular gas bubble (methan gas); this gives to the culture a very characteristic aspect; the same is observed if, instead of ordinary nutrient gelatine, grape-sugar gelatine is used for the shake culture. In gelatine stab culture the stab becomes on incubation marked as a row or rows of minute dots,

white in reflected, brownish in transmitted light ; on the top of the stab is a translucent, plate-like expansion of the growth. After two, three, or four days' incubation this expansion covers the whole upper surface of the gelatine, while in connection with the stab there are a few large flat gas bubbles hanging on, as it were, to the growth in the stab.

The gas bubbles in the upper layers of the shake culture gradually break through and escape on to the free surface, so that after a time only the deeper layers still contain gas bubbles. Neutral litmus-whey is turned red by the growth of bacillus coli (Petruschki).

On the surface of Agar the growth is a grey, dry film, not possessing any special character.

On potato it forms a light yellowish-brown expansion. Alkaline broth becomes strongly and uniformly turbid at 37° C. already after twenty-four hours ; later, while the turbidity increases, a whitish, floccular, granular precipitate appears in the depth, and on the surface an attempt at the formation of a white, imperfect pellicle.

If after 3—5 days' incubation a few drops of potassium nitrite solution, and then a small quantity of nitric acid, are added to the broth culture, a characteristic pink colouration appears, due to nitroso-indol, the typical bacillus being a strong decomposer of albumen, forming thereby indol. In milk incubated at 37° C. the typical bacillus coli grows copiously, and clots and solidifies the milk already in 30—48 hours, or latest three days ; after clotting a separation of the clot from the whey takes place. These are the principal morphological and cultural characters of the typical bacillus coli, and it remains to be added that, stained for flagella after van Ermengem's method, the bacilli contain at one or both ends several flagella—two, three, up to eight altogether ; the flagella are wavy, whip-

like, or even spiral, but not very long. Bacillus coli grows well in gelatine and broth to which phenol has been added to the amount of 0·05 per cent. While these are in general the characters of the typical bacillus, such as can be isolated from stools normal and pathological, there occur in the intestinal contents and discharges, as also in various other substances—pathological secretions, dust, water, sewage, &c.—bacilli which, examined as regards all the above points, coincide in some, but differ in others. Owing to their general morphological similarity—rods of the shape and size of bacillus coli, and flagella, two to eight—and owing to the non-liquefaction of gelatine and the power to grow well in phenolated gelatine and broth, and the identical appearances and rapidity of growth in gelatine plates and in gelatine streak and stab, on Agar, potato, and broth cultures, they must for the present be considered as bacillus coli, but on account of their differing from the typical bacilli in respect of gas-production in gelatine shake culture, clotting of milk, and indol-reaction, they must be considered as varieties of bacillus coli. (1) As to size, the figures given above are open to considerable alterations, since there are varieties of bacillus coli of which the elementary rods as taken from a young colony on gelatine or Agar appear distinctly and uniformly cylindrical, whereas in some other varieties the great majority are under the same conditions very short ovals. (2) As to motility, there exist also great differences. While in some, *e.g.* the typical bacillus coli of the intestine taken from a young colony, only here and there a bacillus shows motility—darting to and fro, and spinning round—there are varieties of which almost all the bacilli, at any rate the majority, show active motility. And similarly as to the number of flagella: for, while in some two or three flagella at one or both ends are

discoverable, in others their number mounts up to eight or even in single cases to ten flagella. The production of gas bubbles in shake cultures notifies great differences. While the typical bacillus coli forms gas bubbles copiously and rapidly in 24—48 hours, there are varieties which produce gas bubbles under these conditions later, or very late—8—10 days or not at all. The same holds good as to milk curdling: varieties exist which either curdle milk at 37° C. after several days, or after many days—as late as 20—25 days. And, finally, the indol reaction of broth cultures is in some varieties to be obtained after many days' growth, and in others, otherwise behaving like typical bacillus coli, is not at all obtainable.

Mr. Mervyn Gordon, who has devoted in my laboratory special attention to these varieties, has isolated from the intestinal contents in health and disease, from waters, and from sewage, a number of varieties which in respect of length, motility, and number of flagella, of the power of gas-formation, of the power of curdling milk, and of the power of indol-formation in broth cultures, furnish quite a respectable number. Thus he found varieties which in all respects compare with the typical bacillus coli except that it has eight flagella, or that it is pronounced cylindrical, or that it does not form gas, or that it does not curdle milk till very late, or that it does not form indol in broth culture; then he found varieties which, except in two of these characters combined, have all other characters; and so on to a variety which by the mode of growth in plate and streak and on potato, and by the flagella, is bacillus coli, but has no other character of bacillus coli, in that it does not form gas, does not curdle milk, and does not form indol.

The typical bacillus coli is a strong producer of acid. This can be shown very strikingly by using for culture

medium ascitic fluid made strongly alkaline ; then glycerine is added, and the whole sterilised before inoculation with the microbe. Incubating the cultivation at 37° C., it will be found to have become completely solidified in forty-eight hours, this solidification being due to neutralisation and further coagulation of the alkali albumen. The same phenomenon is observed with the typhoid bacillus sown in the alkaline ascitic fluid and glycerine. So that both these microbes are strong producers of acid ; and yet there exists this striking difference between the typical bacillus coli and the typical bacillus of typhoid with which the above rapid coagulation of alkali albumen is produced that the former curdles milk in 36—48 hours while the latter does nothing of the kind ; no coagulation of milk can be produced with this particular typhoid bacillus that was used for the above experiment. The conclusion which I think can be drawn from these facts is that the curdling of the milk so conspicuous in the case of bacillus coli cannot be due solely to the acid formed, but must be due to ferment action, and further that those varieties of bacillus coli which have the power of curdling milk in an imperfect degree (very late curdling), or not at all, owe this deficiency to a want, not of acid-production, but of ferment-production. A species of non-sporing, non-liquefying aerobic bacillus occurs in a small percentage of intestinal discharges and in a somewhat larger percentage (30 per cent.) of sewage, which in so far is of interest and importance as its distribution seems to be limited to these two materials ; at any rate I have not met with them otherwise, and I have met with them in water which had received in a conspicuous degree sewage, and for this reason I am inclined to think that, if this species be found in water, such water has most probably been polluted with sewage ; and, further, I think the presence of this species in

water is of even greater importance than that of the bacillus coli. For it must be obvious that, since bacillus coli is often present in many materials besides sewage, its presence alone in water, particularly in limited numbers, does not justify the conclusion that such water had been directly polluted with sewage. If, however, bacillus coli and proteus vulgaris should be present in considerable numbers, such a conclusion as to probable sewage pollution would be most probably a correct one. The bacillus which I am about to describe being of rarer distribution outside sewage, and being present in sewage, it is clear that for diagnostic purposes it is of importance. Now, this bacillus has certain characters in cultivation in common with bacillus coli, and from the aspect of its colonies in gelatine and in streak cultures on gelatine might be mistaken for it: it grows as rapidly as, if not more so than, bacillus coli, and forms the same kind of flat, dry, translucent, angular, patch-like colonies; in gelatine streak it forms the same kind of translucent band with filmy, irregular, or crenate and knobbed margin. *Like bacillus coli, it grows well in phenolated gelatine and in phenolated broth*, it differs, however, from bacillus coli in the following respects:—

It grows quicker in plates and in streak culture in gelatine; its colonies are flatter and show, when examined with a magnifying glass, already after twenty-four hours, better after forty-eight hours, in reflected light very characteristic white granules scattered through the middle part of the patch; the same white granules are noticed along the middle of the band in streak culture; later, say after three days, the number of the granules increase considerably and extend from the middle to near the margin both in the colonies of the plate as also in the band of the streak, so that thereby the growth becomes whitish in reflected, opaque

and brownish in transmitted light. This bacillus is non-motile; it is markedly cylindrical, forming short and long chains and filaments, the above white granules in the young colonies being due to collections of such chains and filaments; it does not curdle milk, does not form gas-bubbles in gelatine shake cultures, and does not form indol in broth:

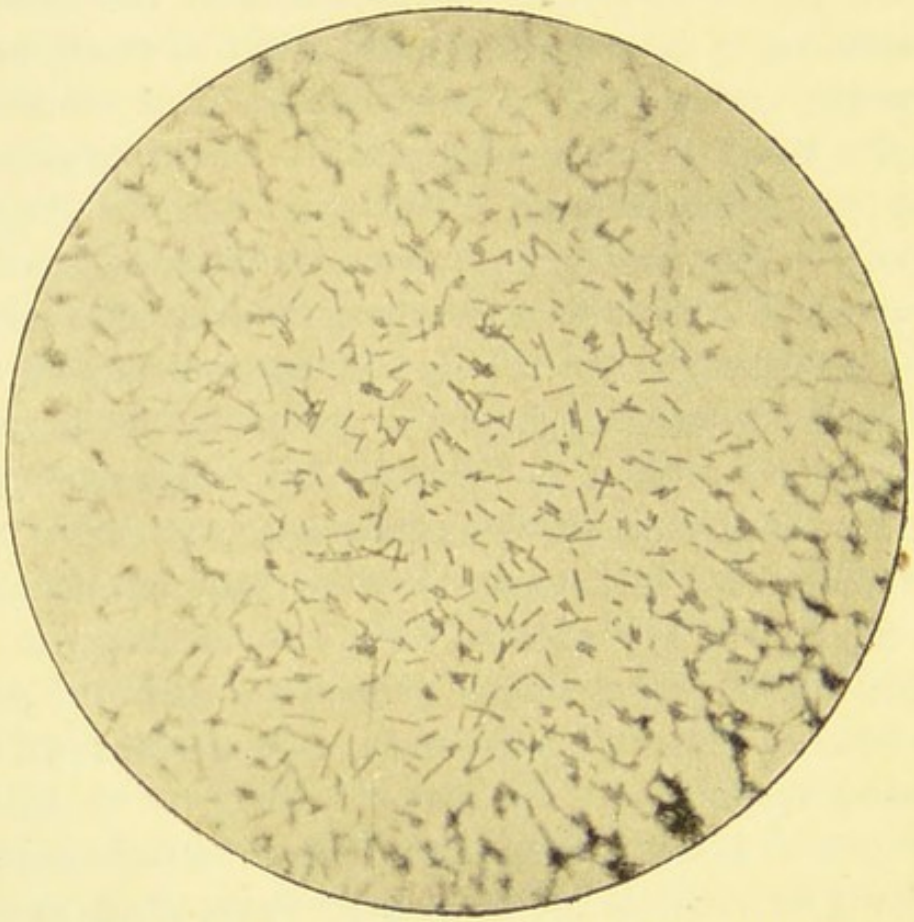

FIG. 63*a*.—FILM SPECIMEN OF BACILLUS PYOCYANEUS.
× 1000.

it is, therefore, easily distinguishable from bacillus coli. It approaches the proteus Zenkeri inasmuch as in streak culture in gelatine after some days it forms threads and filaments radiating from the centre of the streak which recall the growth of proteus Zenkeri; we therefore call it (in the laboratory) the sewage variety of proteus Zenkeri, though, as mentioned above, it resembles more the bacillus coli than

the proteus Zenkeri; from this latter it differs in almost all other respects, our bacillus being more cylindrical, not motile, and its colonies on gelatine being filmy, translucent, granular patches.

Another bacillus which I found occasionally, but rarely, in sewage, and which I have not found elsewhere, is a bacillus which on account of its eminent tendency to form long

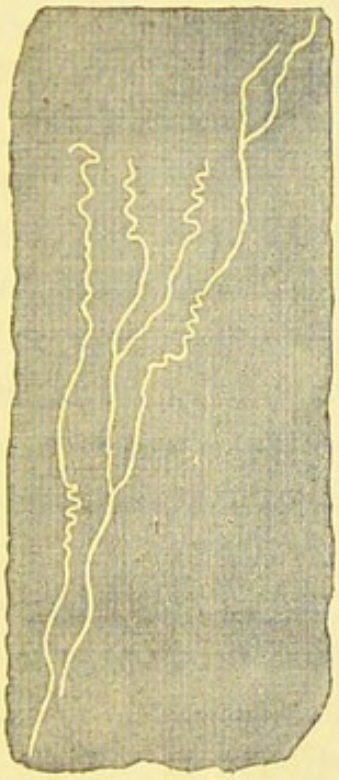

Fig. 64.—Streptothrix Foersteri (after Cohn).

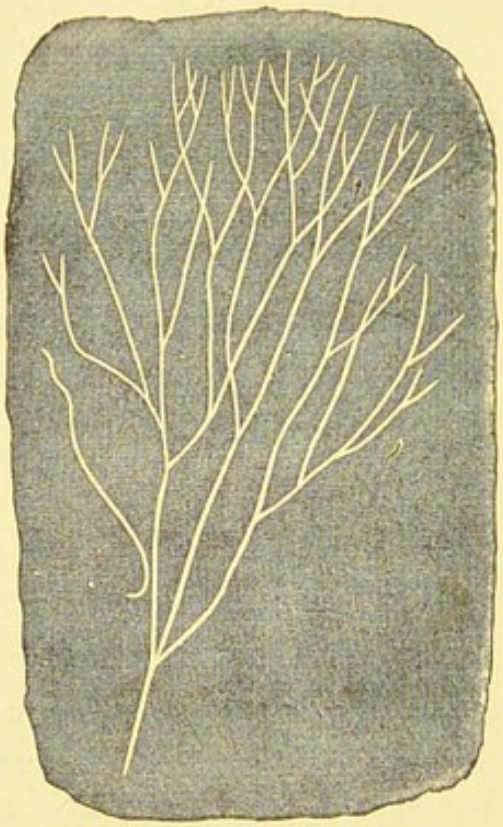

Fig. 65.—Cladothrix Dichotoma (after Cohn).

threads I have called bacillus filamentosus; this is a strongly aerobic, non-motile microbe, consisting of cylindrical bacilli with square ends like bacillus anthracis; in stab and streak it forms a marked feathery, filamentous growth; it liquefies gelatine very rapidly, the liquefied gelatine being quite limpid, and it rapidly forms bright, glistening, oval spores in size, shape, and position the same as bacillus anthracis.

7. Of less common occurrence is the *Bacillus prodigiosus*, forming a characteristic bright-red or bright-pink growth. This microbe occurs occasionally in air, in water, and in soil. On Agar plates and Agar surface it forms round colonies which have a bright pink colour; on gelatine the colonies appear round, at first faintly red and rapidly liquefying, making the liquefied gelatine turbid and of a pale-red tint. On potato it grows rapidly, forming a bright pink expansion. The microbe grows best at 20° C.; it does not grow at 37° C. The growth is composed of non-motile, oval, or even spherical or cylindrical rods, singly or in dumb-bells, or in short chains. The pink colour is noticed only in aggregations of the microbe. I have seen a wholesale infection of food-stuffs (beef, mutton, fish) occurring in a City establishment next to which an old churchyard had been disturbed, owing to old graves having been dug up previously; the larder in which the infection occurred was overlooking the said churchyard. By means of alcohol or chloroform the pigment can be easily extracted.

8. *Bacillus pyocyaneus* is the microbe found in blue-green pus—in fact, it is the organism which produces the blue-green colour. Gessard and Charrin (Gessard: Thèse de Paris, 1882. Charrin: Communication à la Société Anatomique, December 1884) first described the microbe. Gessard particularly isolated the blue pigment produced by it, pyocyanin. When isolated by gelatine plates the microbe grows as translucent colonies irregular in outline and showing a fine radial striation, the gelatine gradually assuming a greenish colour. The gelatine is liquefied and of a uniformly greenish colour; on Agar it forms a white film, while the Agar becomes tinted greenish; on potato it forms a brownish film, while the substance of the potato underneath assumes a greenish colour. It has pathogenic action on

guinea-pigs.[1] Under the microscope it is an extremely minute and thin cylindrical rod (*see* Fig. 63*a*).

This microbe has a much wider distribution than green pus, for I have isolated it several times from the contents of the intestine both in acute diarrhœa and in cholera. Dr. F. W. Andrewes has made some interesting experiments with the blue pigment, showing that a solution of it turns bright red on the addition of acid, and it assumes again the deep blue colour on adding sufficient alkali.

I append here, as morphologically interesting forms, three micro-organisms of which the position amongst bacteria is not definitely determined yet.

(*a*) Streptothrix.—Cohn[2] found in a concretion of the human lacrymal canals long, pale, smooth, apparently branched threads, either straight or twisted; they were finer than the threads of leptothrix buccalis; he called them *Streptothrix Foersteri*.

(*b*) Cladothrix dichotoma (Zopf).—This occurs in pond-water containing decomposing organic matter. It consists of long whitish threads fixed on chlorophyll-containing algæ. The threads when fresh appear smooth, pale, occasionally granular, and on staining they are seen to be composed of shorter or longer bacilli, just like the leptothrix form of bacillus subtilis; but they are thicker than the bacillus subtilis. Occasionally the ends of the threads are seen, not as linear series of bacillar rods, but, like bacillus anthracis, as chains of torula-like spherical elements. From the threads single motile bacilli are seen to come off. The threads are

[1] When a few divisions up to half a Pravaz syringe of the broth culture is injected subcutaneously, the animals become ill and die in from two to four days, showing peritonitis, pericarditis, and pleuritis, with copious membranous and purulent exudation, which contains abundantly the bacilli.

[2] *Beitr. z. Biol. d. Pflanzen*, vol. i. p. 186.

only apparently branched, since the branches are threads merely stuck on to other threads sideways at an acute angle. A bacillus may be seen to stick to a thread and then to

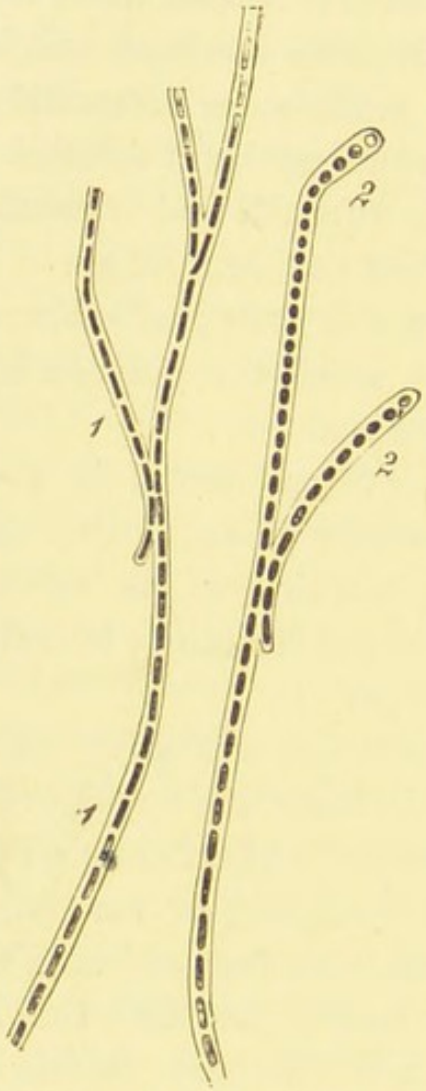

FIG. 66.—THREADS OF CLADOTHRIX DICHOTOMA HIGHLY MAGNIFIED AND STAINED WITH SPILLER'S PURPLE.

1. Threads of bacilli.
2. Torula-forms.

The sheath is everywhere well seen.

grow out by continuous divisions into a long chain of bacilli, thus forming, as it were, a side-branch. Some of the threads are wavy and curved; most of them are, however, straight. Zopf[1] states to have observed that the

[1] *Zur Morphologie der Spaltpflanzen*, Leipzig, 1882; see also Cienkowski.

threads of the cladothrix gave rise to micrococcus, bacterium, bacillus, and spirillum; and further that each of these is again capable of growing into the threads of the cladothrix.

(*c*) *Beggiatoa.*—In stagnant water, particularly in sulphur-containing water, peculiar oscillating colourless threads are met with of the thickness of 0·0001 to 0·016 mm.; they contain highly refractive granules, which Cohn (*Beiträge zur Biol. d. Pfl.* i. 3) has shown to be composed of sulphur. After dissolving these granules it is seen that each thread is septate, being composed of a sheath and transverse septa at regular intervals, by which the threads appear made up of a series of short cylindrical elements. There are a number of species varying from one another in the thickness of the threads.

CHAPTER XI.

BACILLI SPECIFICALLY PATHOGENIC TO MAN OR ANIMALS.

Group A.—Amongst these a group of bacilli is first to be considered which comprises several species, all of which have certain characters in common. (1) All of them are short oval rods, some more cylindrical than others, occurring singly, in dumb-bells, or even in short chains. (2) They do not liquefy gelatine and do not form spores. (3) They produce uniform turbidity of broth already after 24–36 hours at 37° C., although the amount of turbidity varies in the different species, and also as regards presence or absence of a pellicle. (4) They are killed by a temperature of 60° C. in five minutes. (5) They all produce in one or the other rodent, on subcutaneous injection of small quantities of culture—recent broth culture best—or of blood and tissues containing the microbe, acute septicæmic infection, the blood of the general circulation of the infected rodent containing more or less copiously the injected microbe; the viscera are hyperæmic, the spleen, the liver, the lungs, the kidneys, and particularly the peritoneum, containing small hæmorrhages with peritoneal, pericardial, and pleural exudation. They differ from one another (1) in the species of animals in which originally they are associated with acute

specific disease; (2) in the rodent in which they produce the acute hæmorrhagic septicæmia; (3) in the rapidity of growth, aspect, and size of their colonies on gelatine in the plate and streak culture; and (4) in the presence or absence of motility.

To this group belong:—(1) Bacillus of Davaine Septicæmia. (2) Bacillus of Fowl Cholera. (3) Bacillus of Frettchenseuche. (4) Bacillus of Duck Cholera. (5) Bacillus of Fowl Enteritis. (6) Bacillus of Grouse Disease. (7) Bacillus of Swine Fever, or Hog Cholera (and Swine Plague). (8) Bacillus of Wildseuche. And (9) Bacillus of Oriental Plague of Man.

The following short account is copied from Klein's article, Infectious Diseases, in Stevenson and Murphy, II., pp. 97, 98, 103, 104, 105, 106, 107, and 108:—

1. *Bacillus of Davaine septicæmia.*—This is a septicæmia which Davaine first produced by injecting into rabbits putrid ox's blood. It is known now that a small motile bacillus is the microbe, which by its great multiplication and universal distribution in the circulating blood causes the disease and death. The microbe is present in the blood in great numbers, nearly as great as that of the blood-corpuscles; in stained specimens the rods, which are short and oval, show a stained granule at each end with a clear space in the middle; the length of the rods is about 1·5μ, in thickness about half. The rods are motile, and from the heart's blood and all other tissues pure cultures can easily be made. In plate cultures after about two days minute white dots are visible; under a glass they appear as flat circular discs, white in reflected, yellow brown in transmitted light. After several days the colonies are larger, and appear thicker and broader in the centre than in the periphery, which itself appears more or less concentric owing to regular differences in thickness. At maximum growth the

colony does not exceed one to two millimetres. In stab culture the stab is occupied by a whitish line; under a glass this is seen to be made up of minute droplets and dots, whitish in reflected, yellow-brown in transmitted light. In streak cultures the streak is represented by a narrow whitish band of irregular outline and thicker in the middle than at the margin. Gelatine is not liquefied by the growth.

Rabbits, mice, fowls, pigeons, and sparrows are very susceptible (Koch) to the inoculation of very minute doses of culture or of blood of an animal previously dead of the disease; guinea-pigs and rats are unsusceptible (Koch). When inoculated with a trace of the blood of a rabbit dead of the disease, or with a trace of culture, rabbits show already after ten to twenty-one hours a distinct rise of temperature; in severe cases the animals show spasms, rapid fall of temperature, already before the end of the first sixteen hours, and are dead before the day is over; but in some cases, particularly after inoculation with minute traces of culture, death does not take place before thirty-six to forty-eight hours. The bacilli are found very numerously in the blood-vessels of all organs. Spleen and liver, lymph glands and lungs, are highly congested, so also the intestines; extravasations are only rarely found, and then only in the omentum and lungs; peritonitis is only noticed in a small percentage of cases, and then only when the omentum shows the extravasations; the serous coverings of the intestines are greatly injected. As a rule, these symptoms are greatly more pronounced if death does not occur before the second day.

A bacillus closely related to this is the one which causes acute septicæmia in guinea-pigs and mice, and which I obtained from the pleural exudation of mice and guinea-pigs that had died spontaneously from septicæmia—that is to say, in which no primary cause could be assigned, and in which

the *post-mortem* appearances showed the symptoms of septicæmia: viz., great congestion of the lungs, liver, and kidney, inflamed peritoneum, pleural and pericardial exudation, the spleen dark and slightly enlarged in the mice, the intestines relaxed, congested in the mucous and serous coats, the cavity of the small intestine filled with sanguineous mucus. Inoculation of guinea-pigs or mice with the gelatine cultures proved fatal in the mice within one, two, or three days; in the guinea-pigs larger doses had to be used to produce death in a day or two. When small doses are used there is noticed already in twenty-four hours, about the seat of inoculation, a firm thickening which gradually extends into wider areas; and death ensues after several days to a week. In all cases the bacilli can be easily demonstrated in the heart's blood and in the congested organs by cover-glass specimens and by culture. In sections through the liver and kidney the bacilli are found in masses occluding like emboli the capillary blood-vessels; in the liver the central vein of a lobule and numerous capillaries leading into it are found filled with and distended by continuous masses of the bacilli, the surrounding liver tissue being in a necrotic state; in the kidney numerous capillaries between the convoluted tubes of the cortex and in the glomeruli are found occluded by the bacilli. The bacilli taken from the blood are rounded at their ends, and motile; in cultures, notably in broth or other fluids, some of the bacilli are short like cocci, others are oval, others again cylindrical; there are also numerous longer and shorter chains, which show active motility; in these chains the joints or elements are of all shapes—cylindrical, oval, or coccus-like. That all these forms belong to the same species can be easily proved by plate cultivation; for in these all colonies are of exactly the same kind.

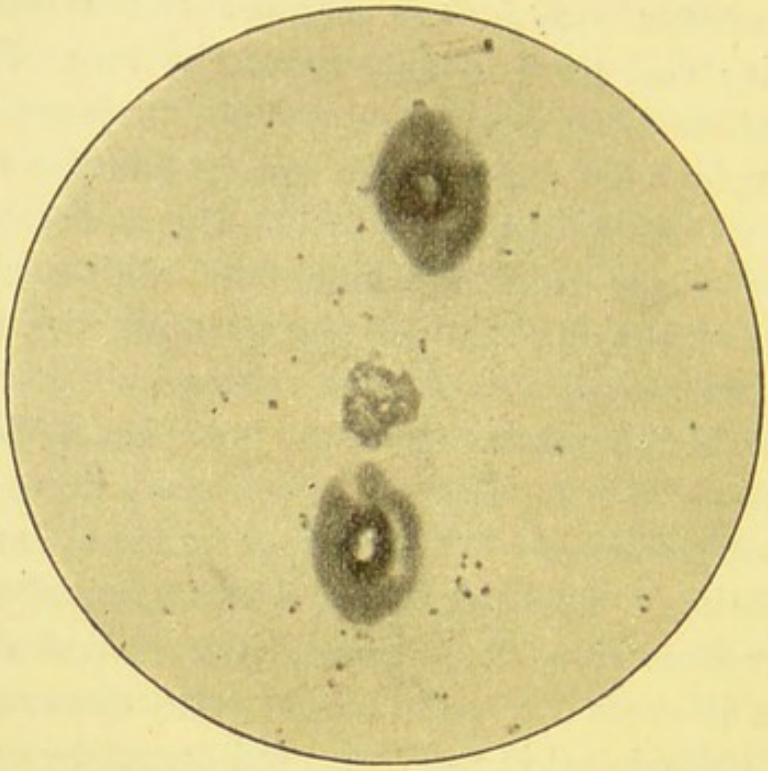

FIG. 67 — FILM SPECIMEN OF HEART'S BLOOD OF FOWL DEAD OF FOWL CHOLERA. NUMEROUS OVAL BACILLI WITH POLAR STAINING AMONGST THE RED BLOOD DISCS.

× 1000.

FIG. 68.—GELATINE PLATE CULTIVATION OF BACILLUS OF FOWL CHOLERA AFTER THREE DAYS' INCUBATION AT 20° C.

Natural size.

2. *Fowl cholera.*—This disease causes great devastation amongst poultry. The malady, well known by the researches of Perroncito, Toussaint, Pasteur, Kitt, and others, affects fowls, pigeons, and rabbits. In the fowl, after an incubative period varying between sixteen or eighteen hours to twenty-four hours, the disease declares itself by diarrhœa of fluid, greenish evacuations, great drowsiness, and sleepiness of the animal. In about twenty to forty-eight hours the animals are found dead; the blood in the heart and general circulation, and in the vessels of all organs, the intestinal contents, and evacuations teem with short, oval, non-motile bacilli measuring 1–1·2 μ in length; in stained preparations they show at each end a stained granule, while the middle part is clear and unstained. On *post-mortem* examination the viscera are found greatly congested and containing hæmorrhages; the mucous membrane of the upper part of the intestine is found congested; often small hæmorrhages occur in its mucous membrane; the contents are fluid fæces, the spleen is enlarged. Fowls, rabbits, and pigeons inoculated with a droplet of the blood of a fowl dead of the disease, or inoculated with the artificial culture of the bacilli, die of the disease in between thirty-six to forty-eight hours, the blood teeming with the bacilli. Feeding with the intestinal contents of fowls, the disease is reproduced in them. From this the conclusion is justified that also under natural condition infection is carried out by the healthy fowls picking up the contagium with the food from soil tainted with the evacuations of diseased animals.

Cultures of the bacilli show the following characters: In plate cultivations the colonies appear before forty-eight hours as minute yellowish-white dots, irregularly outlined or round; seen under a glass they are discs faintly granular; the centre is yellow and transparent, then follows a brown

zone, and then a transparent marginal part. In stab culture the line of inoculation becomes marked as a white line made up of more or less confluent yellowish-white droplets; on the surface of the stab is a thin, irregularly outlined plate; in streak cultures the growth appears after two or three days as a yellowish-white band with irregular or knobbed outlines, thin in the centre and margin, thicker and brownish in the intermediate parts; on potato the microbe grows only at higher temperatures, 28°–38° C. It grows slowly and forms a waxy grey-white film.

By inoculation of minute quantities, a drop of culture into the subcutaneous tissue, or by feeding of fowls, rabbits, mice, or pigeons with culture, the disease is easily reproduced. In guinea-pigs and sheep it produces a local abscess at the seat of inoculation.

By keeping broth cultures for some months Pasteur has succeeded in producing by inoculation of fowls a local œdematous inflammation; the animals became only slightly affected, but recovered and showed themselves refractory against a second inoculation. Pasteur thought that the influence of the oxygen of the air produced the attenuation; it is now proved, however, that this is not so (Kitt), but that Pasteur had impurities (accidental microbes) in his broth cultures, which at first attenuated the bacilli of fowl cholera and, as time went on, altogether suppressed these; hence the broth cultures of Pasteur after the lapse of some months proved barren of all pathogenic action.

Pasteur has shown that by injection of large quantities of broth cultures from which the bacilli of fowl cholera have been previously removed by filtration a transitory illness can be produced, and that such animals show themselves afterwards refractory against inoculation with virulent material. Marchiafava and Celli showed that the microbe passes from

the mother to the fœtus, probably owing to ruptures (hæmorrhages) in the vessels of the maternal placenta.

3. Eberth and Schimmelbusch (*Fortschritte d. Medicin*, Bd. VI., No. 8, p. 295) described an acute infectious disease in mustela furo—*Frettchenseuche*—chiefly showing itself as pneumonia with enlarged spleen; in the heart's blood, in the inflamed lung, the liver, and enlarged spleen there are present numerous motile bacilli, similar in many repects to the bacillus of swine fever, fowl cholera, and Wildseuche. The cultures act very virulently on sparrows, less virulently on pigeons; fowls are refractory; in rabbits the inoculation produces only a local inflammation of a temporary character, and the same results are obtained, only milder, in guinea-pigs.

4. *Duck cholera.*—As such, Cornil describes a fatal infectious disease affecting ducks, and in its symptoms and causation similar to fowl cholera; but there is this difference between them, that the disease of the duck is not transmissible to the fowl. The bacilli are, however, similar in many respects to those of fowl cholera.

5. *Fowl enteritis.*—This is an acute fatal infectious disease affecting fowls, but not pigeons and rabbits, and by this alone its differentiation from fowl cholera is established; besides the microbe and its distribution, the course and symptoms of the disease are quite distinct from fowl cholera. I have met with the fowl enteritis on a poultry farm in England, where it caused great mortality. The disease has been prevalent also in Ireland during the last few years. The fowls when affected show diarrhœa of fluid greenish evacuations, are quiet, but never show sleepiness or drowsiness. In a day or two after the diarrhœa has set in they are found dead. The mucous membrane of the intestine is found congested, but without hæmorrhage; the internal

surface of the mucous membrane is coated with grey or yellowish mucus, which under the microscope contains numerous leucocytes and detached epithelial cells; the liver is congested and brittle, the spleen much enlarged, the lungs are normal. In the heart's blood are present relatively few bacilli, which are a little longer and thicker than in fowl cholera; the spleen contains the bacilli numerously, and

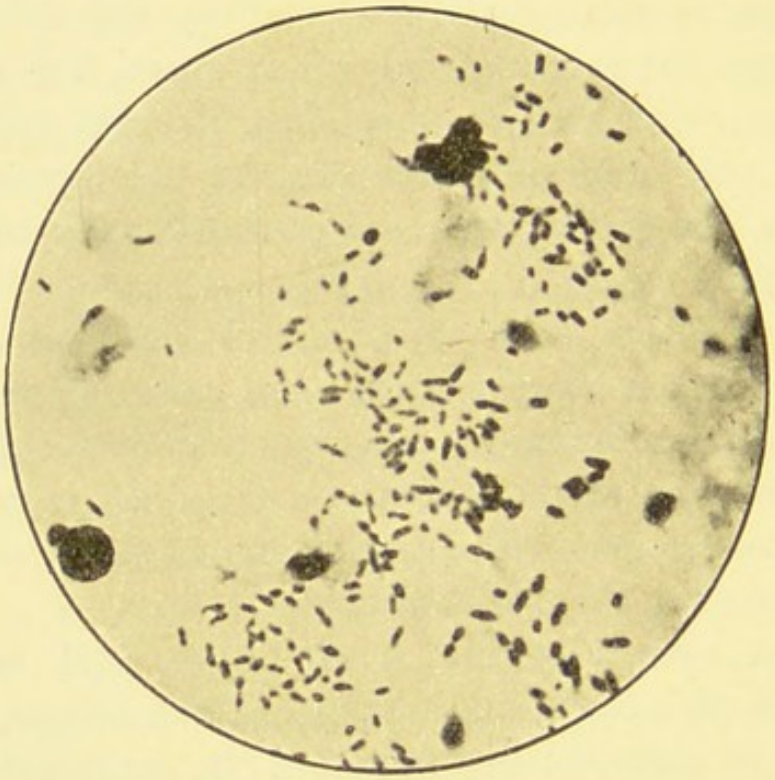

FIG. 69.—FILM SPECIMEN OF INTESTINAL MUCUS OF A FOWL DEAD OF FOWL ENTERITIS. PURE CULTURE OF BACILLUS OF FOWL ENTERITIS.

× 1000.

also the vessels of the liver; the mucus of the intestine contains the bacilli in almost pure culture. In cultural respects the microbe resembles the bacillus of fowl cholera, except that its colonies are disc-like when growing on the surface; further, that the microbe of fowl enteritis is more cylindrical, and that in gelatine streak culture it grows much faster and forms a broader, less translucent band than that

of fowl cholera. Pigeons are unsusceptible, rabbits only very slightly susceptible. By feeding of fowls with the contents of the intestine the disease can be reproduced; by subcutaneous inoculation the disease can be produced, both with the blood or spleen tissue of a fowl dead of the disease as also by artificial cultures of the microbe. In all cases

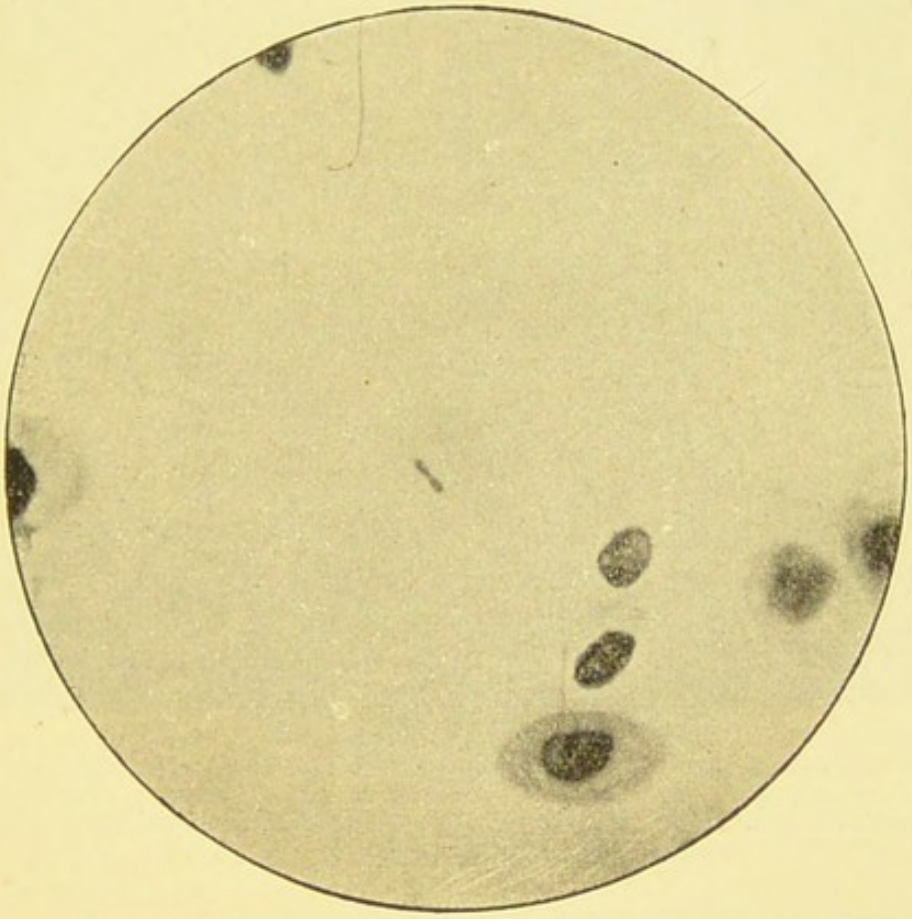

FIG. 70.—FILM SPECIMEN OF BLOOD OF FOWL DEAD OF FOWL ENTERITIS, SHOWING BLOOD CORPUSCLES AND ONE BACILLUS OF FOWL ENTERITIS.

× 1000.

the animals do not show any illness till the third or fourth day (this is also an important distinction from fowl cholera), or more generally till the fifth day: they suffer then from diarrhœa and are quiet; on the sixth or seventh day most of them are found dead, rarely do they survive till the eighth

day, nor do they die before the fifth day. The course of the disease, the symptoms and the appearances after death, the morphology and cultural characters of the microbe, distinguish this disease from fowl cholera.

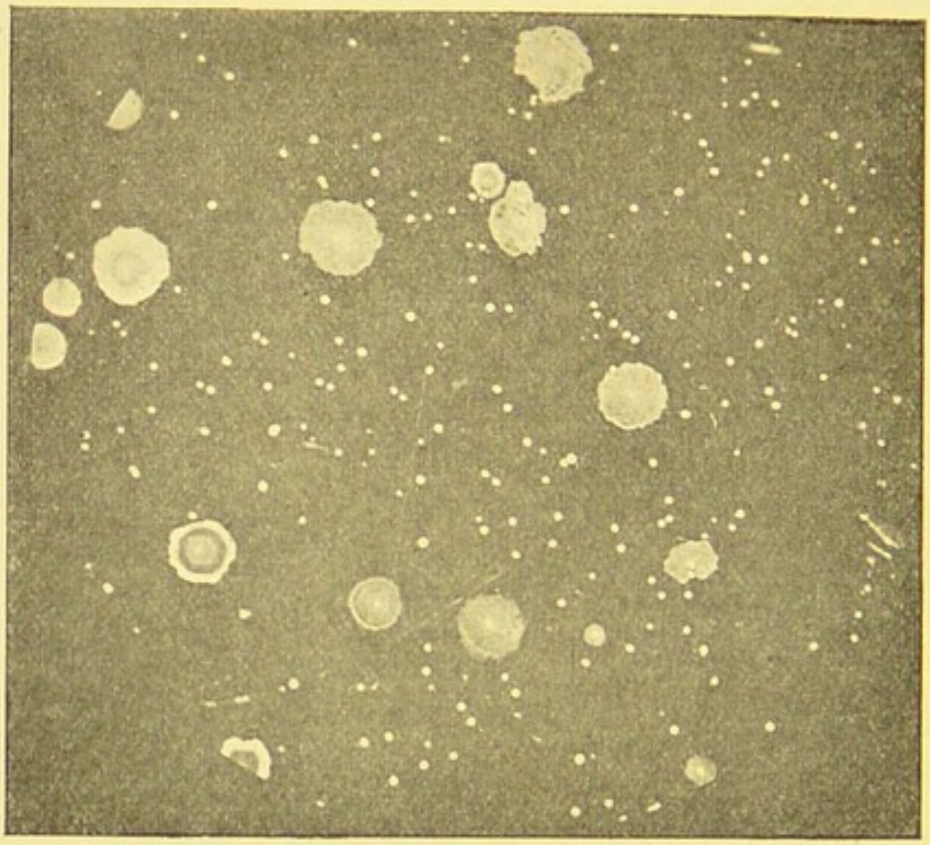

FIG. 71.—PLATE CULTIVATION OF THE BACILLUS OF FOWL ENTERITIS, SHOWING NUMEROUS DOT-LIKE COLONIES IN THE DEPTH OF THE GELATINE, AND DISC-SHAPED COLONIES ON THE SURFACE, THUS SHOWING A STRIKING CONTRAST TO FIG. 68 OF A PLATE CULTURE OF BACILLUS OF FOWL CHOLERA. Natural size.

6. *Grouse disease.*—The fatal disease which affects red grouse, and known as the *grouse disease*, is an acute infectious disease, of which the chief, and we may say the essential, pathological character is that of pneumonia, the lungs being greatly congested, and sometimes one or the other portion almost in a state of red hepatisation with engorgement of the blood-vessels and extravasation of blood into the air-spaces; the serosa and mucosa of the intestine show patchy redness; the liver is greatly congested and dark; the

spleen is not enlarged. In the diseased lung and in the liver there occur in the vessels and in the extravasated blood numerous bacilli singly, or more commonly in larger or smaller groups, sometimes forming emboli in the capil-

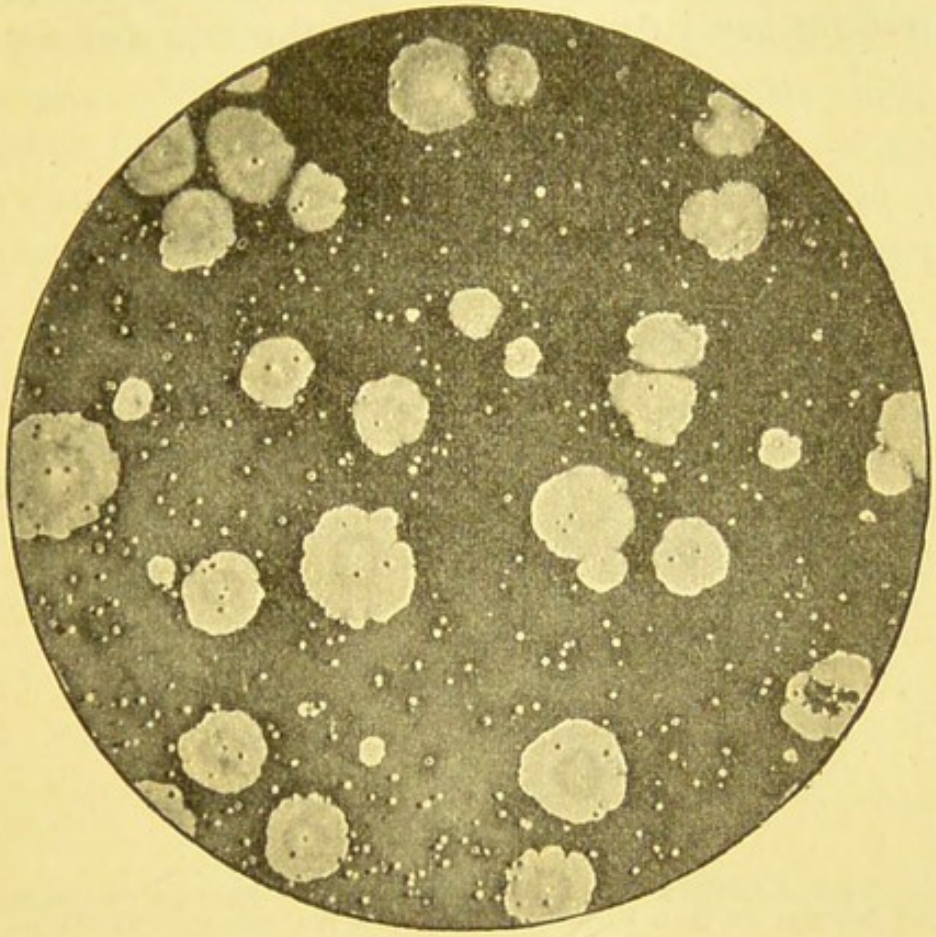

FIG. 72.—PLATE CULTIVATION OF BACILLUS OF GROUSE DISEASE, SHOWING DOT-LIKE COLONIES IN THE DEPTH, PATCH-LIKE COLONIES ON THE SURFACE.

This illustration may serve also to show the character of a gelatine-plate culture of the typical Bacillus coli.

Natural size.

lary blood-vessels. These bacilli belong to one and the same species; they are motile, either oval or even coccus-like; some few are rod-shaped. By cultivation on gelatine they can be easily obtained in numerous colonies from the sanguineous juice of the lung and liver; only in few cases are they to be seen in the heart's blood, both in cover-glass

specimens and in culture. The morphological and cultural characters of the microbe are shown in Figs. 72 and 73.

The microbe when examined from a cultivation is often rod-shaped—more often than in the tissue of the grouse. The motile forms are common in recent cultivations; in cultivations some days old most of the microbes are non-

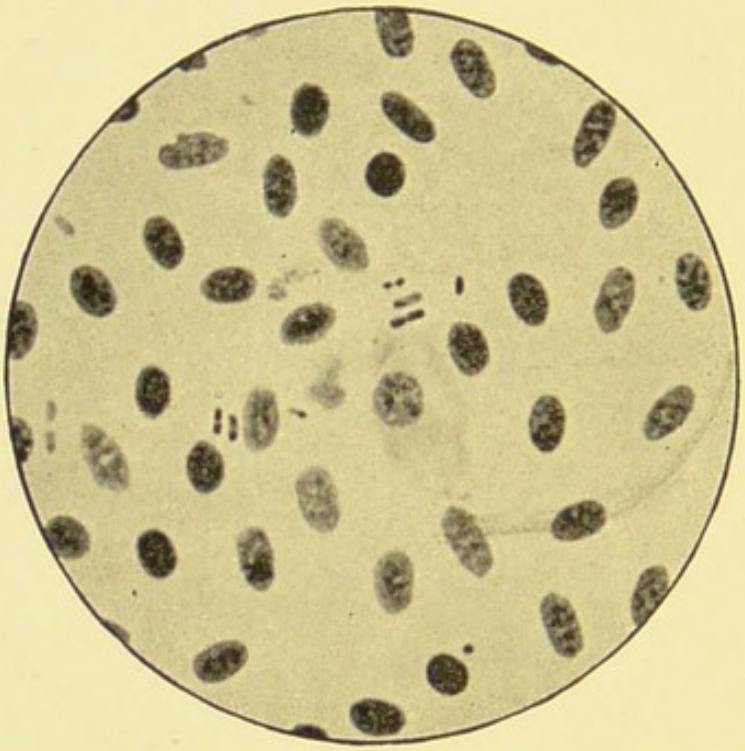

FIG. 73.—FILM SPECIMEN OF BLOOD OF GROUSE IN GROUSE DISEASE, SHOWING THE NUCLEI OF RED BLOOD DISCS AND A NUMBER OF THE BACILLI.

× 1000.

motile. Cultures inoculated into mice and guinea-pigs produce general infection, and rapidly death, mice being more susceptible than guinea-pigs: in both animals the disease produced is a double-sided pneumonia. Sparrows are also susceptible, but less so than the common bunting and yellow-ammer, which animals are highly susceptible; also in these the disease produced is a double-sided pneumonia. The microbe is present in numbers in the heart's blood, but particularly in the diseased lung.

Fowls, pigeons, and rabbits are unsusceptible to the disease.

As far as the appearances in gelatine plate and gelatine streak go, there is a considerable similarity between the microbe of grouse disease and bacillus coli; this is also strengthened by the fact that the former, like the latter, forms gas bubbles in shake culture and curdles milk, the difference being chiefly this—that the microbe of grouse disease on subcutaneous injection is highly virulent to mice, and particularly to the yellow-ammer; less so to guinea-pigs.

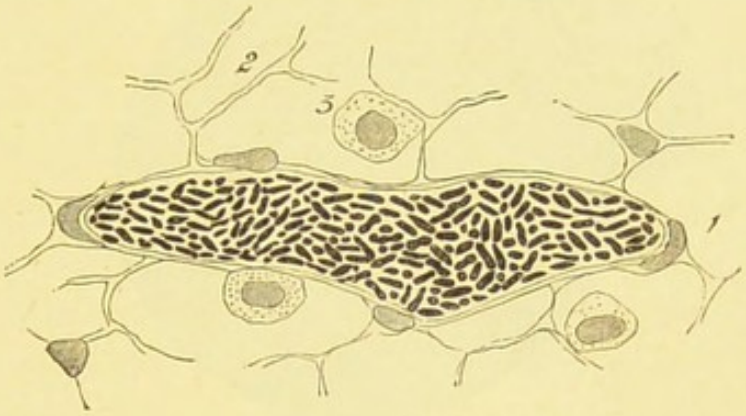

FIG. 74.—FROM A SECTION THROUGH THE ENLARGED INGUINAL LYMPH-GLAND OF A PIG DEAD OF SWINE FEVER.

1. A capillary blood-vessel filled with bacilli.
2. Reticulum of adenoid tissue.
3. A lymph-cell.

Magnifying power 700.

7. *Bacillus of swine fever.*—This disease prevails largely in this country; in America it is known as hog cholera, on the continent of Europe as swine plague. It is a highly infectious disease, spreading from animal to animal by air, food, water, the lungs and bronchi and the intestines being the chief places of disease, and containing the virus. The infection is, under natural conditions, attributable to the virus being derived from and spread by the expectoration of the lungs and evacuations of the bowels. Alike by feeding,

respiration, and by inoculation with the diseased particles of lung and intestine the disease is easily reproduced in healthy swine. After an incubation period varying from between two days and six to seven days the animals are quiet and refuse food, the body temperature shows slight rise, red patches of transitory nature are noticed on the belly and thighs; cough and occasionally diarrhœa of fluid evacuations declare themselves soon; the inguinal lymph-glands appear enlarged. In severe cases the diarrhœa

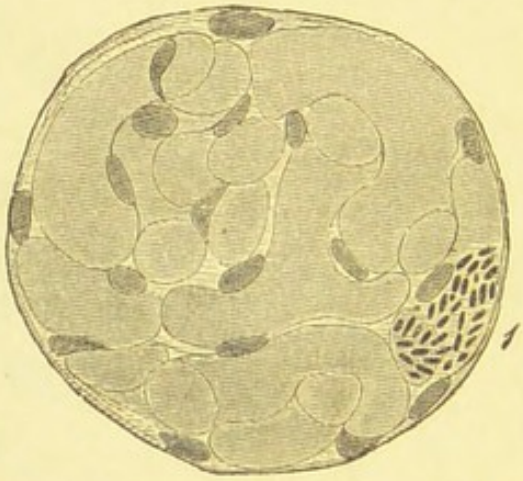

FIG. 75.—FROM A SECTION THROUGH THE KIDNEY OF RABBIT DEAD OF SWINE FEVER, SHOWING A MALPIGHIAN CORPUSCLE, THE CAPILLARIES OF THE GLOMERULUS BEING TRANSFORMED INTO HYALINE IMPERMEABLE CYLINDERS.

1. Bacilli.

Magnifying power 500.

increases, the fever continues, the cough becomes more pronounced; this state lasts for a few days, seldom more than a week, and under general prostration the animal succumbs. In a large percentage (50) of cases the animals recover. In mild cases, representing a considerable percentage, the disease is diagnosed only with difficulty; the rise of temperature is only slight and transitory, lasting only a day or two; the animals feed fairly well; show only, very occasionally at long intervals, a slight cough; the inguinal glands are slightly enlarged. These symptoms are so slight

and so little marked that it requires careful examination to diagnose the disease; nevertheless, on auscultation of the chest distinct lung disease may be recognised. On *post-mortem* examination of such slight cases the symptoms of the disease of the lung are easily confirmed.

In the well-pronounced cases dying naturally the *post-mortem* examination shows the following. The lungs of both sides show severe, extensive, lobar pneumonia, involving sometimes the greater part of the lung; the lobules show

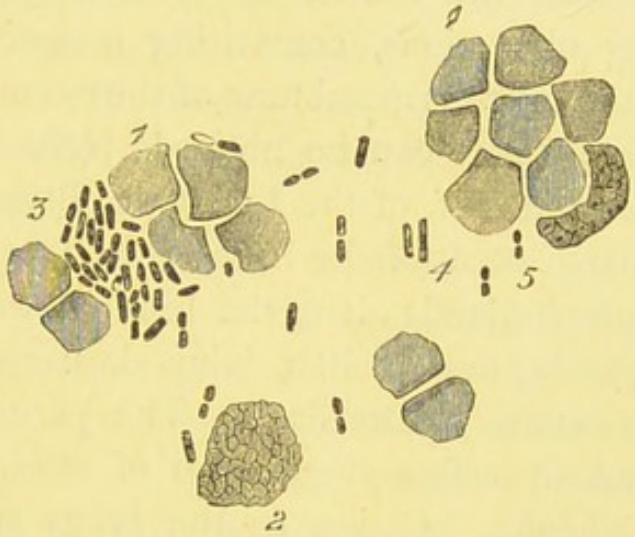

FIG. 76.—BLOOD OF FRESH SPLEEN OF A MOUSE THAT DIED OF SWINE FEVER.

1 Blood discs.
2 A large nucleus.
3. Groups of minute bacilli.
4. Long bacilli.
5. Dumb-bells of bacilli.

Magnifying power 700. (Stained with gentian violet.)

in recent cases all stages between congestion (punctiform hæmorrhages) and hepatisation; the lobes that are longer affected show more consolidation, and, the older this is, the more grey, and solid, and necrotic, dry and friable, is this part of the lung; the septa between the lobes are œdematous and well-marked; the bronchi and trachea contain grey and sanguineous muco-purulent matter; the endocard of the left and occasionally the right ventricle, near the

atrio-ventricular valves, and also these latter, show patchy and punctiform hæmorrhages; the liver is congested and occasionally shows dark-red patches due to hæmorrhage; the spleen is enlarged and dark; the colon and cæcum, particularly the former, contain punctiform hæmorrhages; in most cases they contain prominent round or oval, isolated, and in severe cases more or less confluent, ulcers (necrosis), showing an infiltrated base, and are stained greenish-black by altered bile-pigment; between a few small round ulcers near the ileo-cæcal valve to very numerous extensive long ulcerations, comprising occasionally extensive areas of the mucous membrane of the cæcum and colon, all intermediate stages can be noticed. (*See* Klein, in the Report of the Med. Off. of the Loc. Gov. Board for 1878.)

In the stomach occasionally hæmorrhagic patches can be seen. The lymph glands along the bronchi, the mesenteric and pelvic glands, are swollen, juicy, dark red, in part or wholly, and contain hæmorrhage. The peritoneum is inflamed, and on its surface are clumps of solid lymph composed of leucocytes. Owing to the lungs and intestines being found constantly affected, the disease has been designated by me pneumo-enteritis; but in Germany (Schütz) and in America (Salmon) it is asserted that the above disease is really two: one a disease of the lung, the other of the intestine; but from experiments made on a large scale with diseased lung and with diseased intestine, and from the *post-mortem* appearances in well-defined localised outbreaks that I have made, I am of opinion that this division cannot be maintained, but that the swine fever in this country is one single disease, viz. pneumo-enteritis. Microscopic examination of the lung and intestine shows that the disease really commences with congestion, stasis, and hæmorrhage, leading to infiltration and necrosis of the affected parts.

The cause of the disease is a bacillus, which in the affected tissues of the pig appears, as a rule, as a short rod, often constricted in the middle; in fluid cultivations (broth) and in animals (rabbits, mice) as a cylindrical rod, singly or in dumb-bells, occasionally growing to considerable length, and forming longer or shorter chains; but there can be always found short forms almost like oval cocci, rods, and cylindrical bacilli. Cover-glass specimens and cultures of the lung, spleen, lymph glands, and the sub-mucous tissue of the affected intestine demonstrate the presence of the bacilli. These bacilli are motile, though the motility is observed in a minority; in cultivations of broth, gelatine and Agar Agar many of the bacilli are motile during the first few days, but lose their motility later.

In plate cultivations the colonies are first noticed as greyish dots just visible to the eye already after twenty-four hours; in two or three days they are already conspicuous as whitish, round, angular specks of about the size of a large pin's head; in transmitted light they appear brownish, granular. In stab culture the stab of inoculation becomes marked as a white line made up (when seen under a glass) of minute globules closely placed side by side; on the surface of the stab is a small, irregularly outlined, whitish plate. In streak culture the line of inoculation is occupied in a few days by a grey band, knobbed or crenated in its outline. On Agar the growth (at 37° C.) is a greyish-brown smeary film, rapidly spreading over the surface of the Agar. In alkaline broth at 37° C. uniform turbidity is produced; after a few days a voluminous greyish-white precipitate is noticed at the bottom of the tube. No distinct pellicle is formed on the surface.

Inoculation of swine with cultures produces the disease, but this does not lead to death, and such animals after re-

covery show themselves refractory against inoculation with material of the diseased lung or intestine.

Inoculations into guinea-pigs with material from the diseased swine produce at the seat of inoculation hæmorrhagic infiltration and thickening, sometimes leading to death in two or three days; often, however, the thickening passes off in a week or so; cultures injected subcutaneously in guinea-pigs produce thickening at the seat of inoculation, but rarely death.

Inoculation into mice of minute particles of material of the diseased lung, or intestine, or of gelatine, or broth culture of the bacillus of swine fever causes disease and death in four to eight days; the spleen is found enlarged and dark; the liver is mottled with grey dots, streaks, and patches of necrotic tissue; the peritoneum is inflamed, and so are the kidneys and both lungs. Cover-glass specimens and cultures from the heart's blood and liver, kidney, and particularly the spleen, demonstrate the presence of large numbers of the bacilli (*see* Fig. 76). Among the bacilli in the spleen numerous long cylindrical rods can be seen. In the kidneys many of the capillaries of the glomeruli are plugged by the bacilli, so also in the liver.

In the rabbit inoculation produces disease and death in a few days: the spleen is slightly enlarged, the lungs are inflamed, the kidney is much congested in the cortex. Here also the bacilli can be easily demonstrated in the heart's blood, the liver, and the kidney; in this latter many Malpighian corpuscles show the capillaries of the glomeruli plugged with masses of the bacilli.

8. *Bacillus of Wildseuche.*—A disease amongst cattle (Rinderseuche) and horses, and amongst deer (Wildseuche), manifesting itself in diffuse pneumonia and hæmorrhagic enteritis, but without necrotic change (consolidation and

dryness) of the lung, and without ulceration of the intestine, has been first recognised by Bollinger. Kitt has shown that this affection is caused by a bacillus in many respects (morphological and cultural) similar to that of fowl cholera, rabbit septicæmia, and swine fever; and Kitt and Hueppe maintain, indeed, the identity of all these microbes; but the evidence to prove this is not sufficiently satisfactory. True, rabbits inoculated with the microbes obtained from Davaine's septicæmia, fowl cholera, swine fever, or Wildseuche succumb under the symptoms of Davaine septicæmia; it is likewise true that pigeons inoculated with cultures derived from either of these diseases succumb to fowl cholera; still a great deal remains yet to prove the identity as regards the action on swine of the bacteria of rabbit septicæmia, fowl cholera, and Wildseuche. To mention only one series of difficulties. Fowls, as mentioned above, are highly susceptible to the microbe of fowl cholera, but they are unsusceptible to the microbes of swine fever or Wildseuche. Billings (Texas fever, Lincoln, Nebraska, 1888) describes a species of small motile bacilli closely related to the bacilli of swine fever, alike as to morphology, cultural, and pathogenic characters, as the cause of the cattle plague in Texas and southern countries of the States.

Löffler (*Centralblatt f. Bakt. und Parasit.*, vol. xi., p. 134) described a fatal epidemic amongst mice kept in the laboratory. From the enlarged spleen of the dead mice a motile short bacillus was isolated, which evidently belongs to this group of swine fever—Wildseuche bacilli. Its culture proved very virulent on tame as well as wild mice, producing on subcutaneous inoculation, as also on ingestion, acute fatal septicæmia, the blood and the enlarged spleen particularly teeming with the microbe. On account of the microbe bearing a certain cultural resemblance to the

bacillus of human typhoid on gelatine, Agar, in milk and potato, Löffler called it bacillus typhi murium. Successful experiments with cultures were made in Thessaly to produce wholesale infection and destruction of field mice then infesting the agricultural districts of that country.

Of the same nature appears to be the bacillus isolated by H. Laser (*Centralblatt f. Bakt. und Parasit.*, vol. xi., p. 184), and which he found in a fatal epidemic amongst field mice kept in the laboratory. The morphological and cultural characters of the microbe, its virulence on mice, and the post-mortem appearances in these animals coincide with Löffler's bacillus typhi murium.

9. *Bacillus of Oriental or bubonic plague.*—This is at present the only known species of this group which affects the human subject. As shown by Kitasato and Yersin, the bacillus of the inflamed lymph-glands (bubo) and also of the blood, but principally the former, contain in pure culture an abundance of short rod-like bacilli, which in shape and size, in cultural characters, in plate and in streak on gelatine, and in their effect on rodents belong clearly to the above group of non-sporing, non-liquefying bacilli. The bacillus is non-motile, and its effect on the rodent (guinea-pig) is to produce acute hæmorrhagic, septicæmic infection and death.

Group B.—A second group comprises species which in many points resemble the bacillus coli,[1] but differ from it in this particular that they are capable of producing acute infection and death of the animal body. Like bacillus coli, the microbes of this group grow rapidly in gelatine plate and gelatine streak and stab, and the appearances herein

[1] It must be distinctly understood that I do not and cannot say whether the various species I am about to describe are or are not varieties of bacillus coli, for the characters by which this last is identified are, after all, only comparatively few, besides being artificial; it is more for convenience that we speak of "varieties" of b. coli.

produced are not essentially different from those of bacillus coli; they also form gas-bubbles in gelatine shake culture, curdle milk, and produce indol in broth. The appearances of the growth on Agar and on potato are the same as those of the bacillus coli. As to flagella, they possess two or three flagella, and taken from recent culture many individuals show active

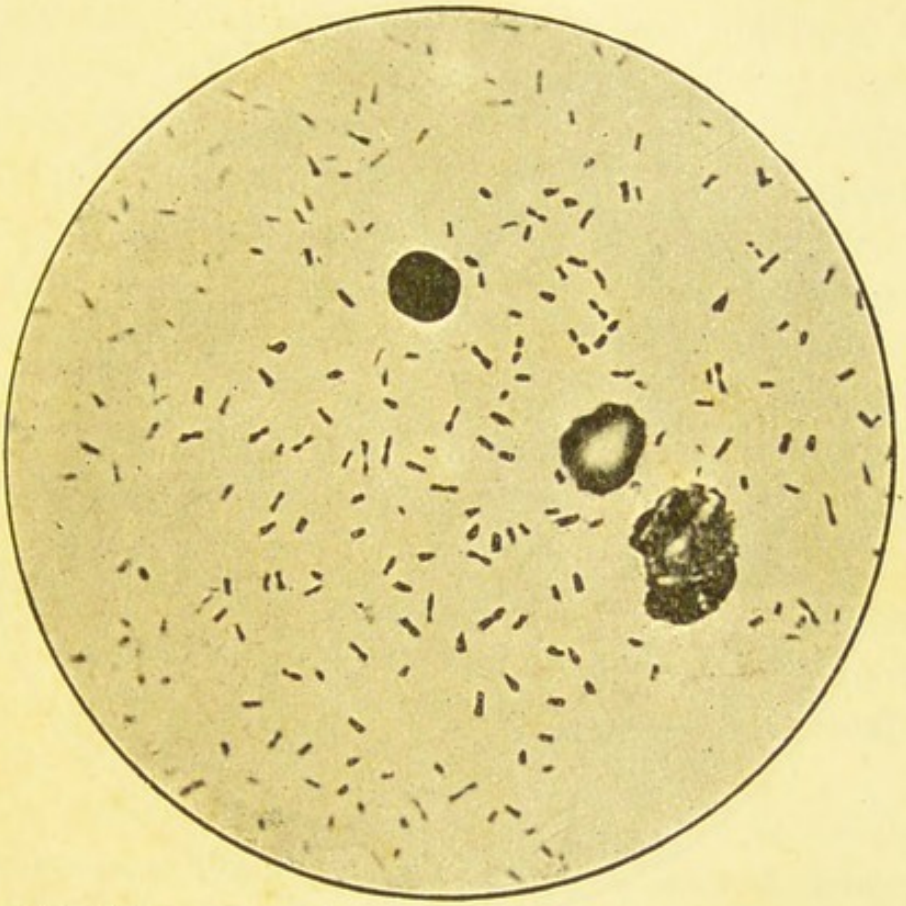

FIG. 77.—FILM SPECIMEN OF THE JUICE OF A BUBO IN ORIENTAL PLAGUE; BESIDES A FEW NUCLEI THE FILM CONTAINS THE BACILLUS OF PLAGUE IN PURE CULTURE.

× 1000.

locomotion. Morphologically they occur as short ovals, singly and in dumb-bells, or as cylindrical individuals with tendency to form chains. As stated just now, the chief difference lies in the fact that, whereas bacillus coli injected subcutaneously into guinea-pigs and mice in small doses causes transitory local swelling only, and in large doses

general infection, the microbes in question cause on subcutaneous injection, already in small doses (a few drops of a recent broth culture), acute septicæmic infection; the blood contains copiously the microbe; the lungs and liver, and particularly the spleen, is hyperæmic and enlarged, and full of the microbe.

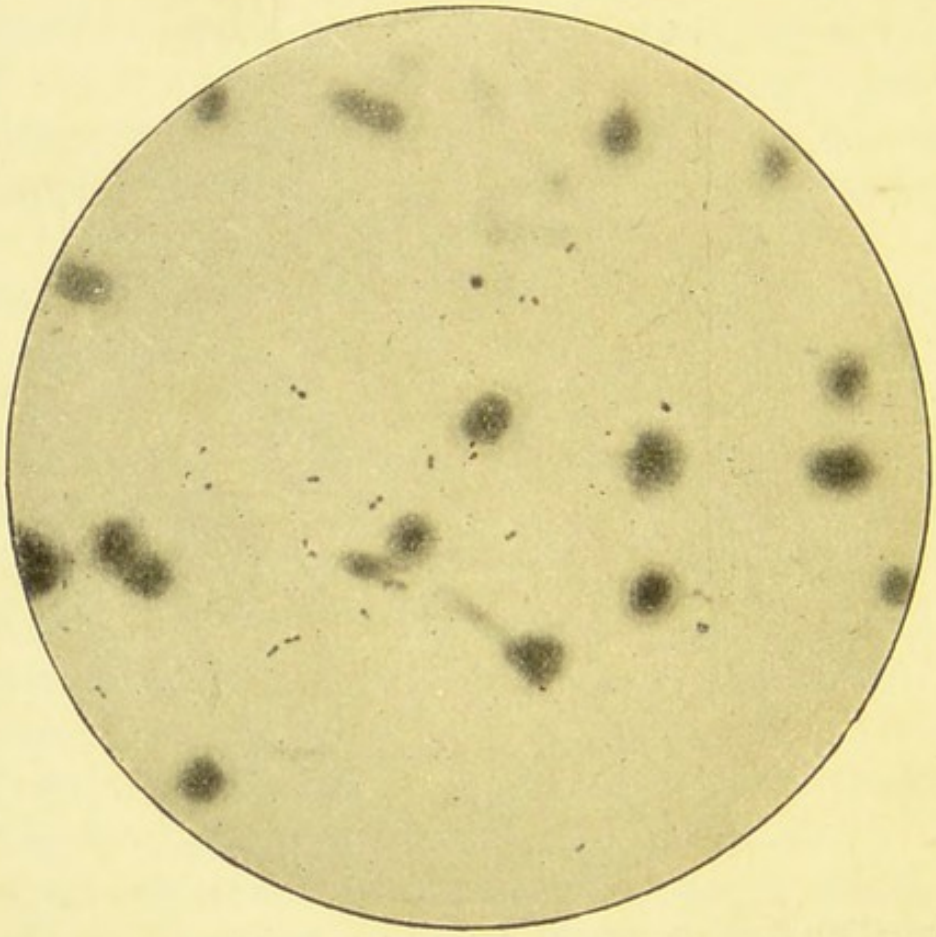

FIG. 78.—FILM SPECIMEN OF LUNG JUICE IN MIDDLESBROUGH PNEUMONIA AMONGST RED BLOOD DISCS, NUMEROUS BACILLI.

× 1000.

To this group belong:—1. The bacillus that I found in pure culture very copiously in the juice of the congested lungs in an *epidemic of fatal pneumonia* that occurred in *Middlesbrough* (Dr. Ballard's Report to the M.O. of the Local Government Board, 1889).

The general morphological and cultural characters are those of bacillus coli. As stated just now, from the lung juice pure cultures were obtained, the organism being present in the lungs in great abundance (see Fig. 78). The bacilli are 0·3–0·4μ thick, 0·8–1·6μ long.

The cultures as also the lung juice act virulently on mice and guinea-pigs, on the former more than on the latter. Subcutaneous inoculation produces disease and death in the course of thirty to one hundred hours. On *post-mortem* examination both lungs are found intensely inflamed, some portions in a state of red hepatisation; generally there are present pleurisy and pericarditis and peritonitis, with more or less sanguineous exudation. The spleen is enlarged in mice, but not in guinea-pigs. The bacilli can be easily demonstrated in very large numbers both by cover-glass specimens and cultures in the heart's blood, the lung juice, and the spleen of mice, and in the lung juice of guinea-pigs.

The lung juice, or cultures derived from the tissues of the infected mice or guinea-pigs, inoculated into further mice or guinea-pigs, produce the same disease and death with the symptoms just described.

While working with cultures of these bacilli on mice and guinea-pigs there occurred amongst normal mice and guinea-pigs kept in the same stalls as the experimental animals an epidemic of pneumonia, leading to the death of a great many of them; on *post-mortem* examination all showed exactly the same appearances as those experimental mice and guinea-pigs, and the juice of the inflamed lungs contained the bacilli in crowds.

Three monkeys, kept on the same premises, and which most probably became accidentally infected by food, died of pneumonia. In the inflamed lungs the bacilli could be

easily demonstrated by cover-glass specimens and by culture.

2. Guinea-pigs injected intraperitoneally with large doses of various species of non-pathogenic bacteria taken from the surface of Agar cultures, *e.g.* bac. prodigiosus, bacillus coli, vibrio of Finkler, &c., &c., succumb, as has been pointed out in a former chapter, to acute fatal peritonitis. The more or less copious, more or less sanguineous peritoneal fluid is crowded with the microbe injected, but in one or the other such case, although rarely, contains in addition a number of bacilli which in morphological and cultural respects coincide with the bacillus coli. Gärtner has met with this bacillus in the peritoneal exudation after intraperitoneal injection with pus coccus; I have met with it after prodigiosus injection. Cultures of this peritoneal bacillus prove it to be bacillus coli, and I explained its presence in the peritoneal cavity by the nearness of the intestine—its original habitat—to the inflamed peritoneum. This peritoneal bacillus differs, however, from the intestinal bacillus coli in its high virulence since small doses of the former injected subcutaneously into guinea-pigs produce acute general septicæmic infection.

3. In a good many cases of fatal *English cholera* I have found the mucus flakes and the fluid of the small intestine containing a bacillus copiously and almost in pure culture, which in its general morphological and cultural characters (gas-formation, curdling of milk, and indol production) coincides with the bacillus coli; it is, however, more motile and more cylindrical than the typical bacillus coli. In the mucus flakes it is in some cases present in continuous streaks and masses, and not seldom arranged linearly in the manner characteristic of the cholera vibrio in the mucus flakes of the rice-water contents of the ileum in Asiatic

cholera (see chapter on Cholera). The colon variety obtained from these cases of English cholera possesses, however, considerable virulence on the guinea-pig, producing in this animal after subcutaneous injection of small or moderate doses acute septicæmic infection and death. Of this character appears to be the *Bacillus neapolitanus* isolated by Emmerich from the intestinal fluid in cases of Asiatic cholera.

4. The *aerobic bacillus of malignant œdema*, which I obtained from recently manured garden earth, produces on subcutaneous inoculation into guinea-pigs and mice extensive gangrene of the skin and muscle, with sanguineous, malodorous exudation, and death in twenty-four to thirty-six hours—a condition similar to that produced by the anaerobic malignant œdema bacillus of Koch (also obtainable from manured garden earth); in cultural respects, in its motility and flagella, it is not distinguishable from bacillus coli; the chief difference from the latter consists in the great virulence of the former. The subcutaneous exudation as also the skin itself is crowded with the bacilli.

5. A bacillus which in morphological and cultural respects is closely related to the bacillus coli was found in abundance in *beef-pie* (*Portsmouth*), which had caused in those who partook of it severe gastro-enteritic symptoms. By feeding mice with the pie or with the broth cultures of the bacillus acute gastro-enteritis was produced (Report of the M.O. of the Loc. Gov. Board, 1890–91), and thereby its difference from the bacillus coli was established, for such a result is not to be obtained with the cultures of the bacillus coli derived from the intestinal contents.

6. A bacillus of which the cultural characters have not been ascertained (it occurred at a time before solid culture media were used), and of which therefore I am unable to

say to what group of bacilli it does belong, though from its size it could not belong to the group of bacillus coli, is the

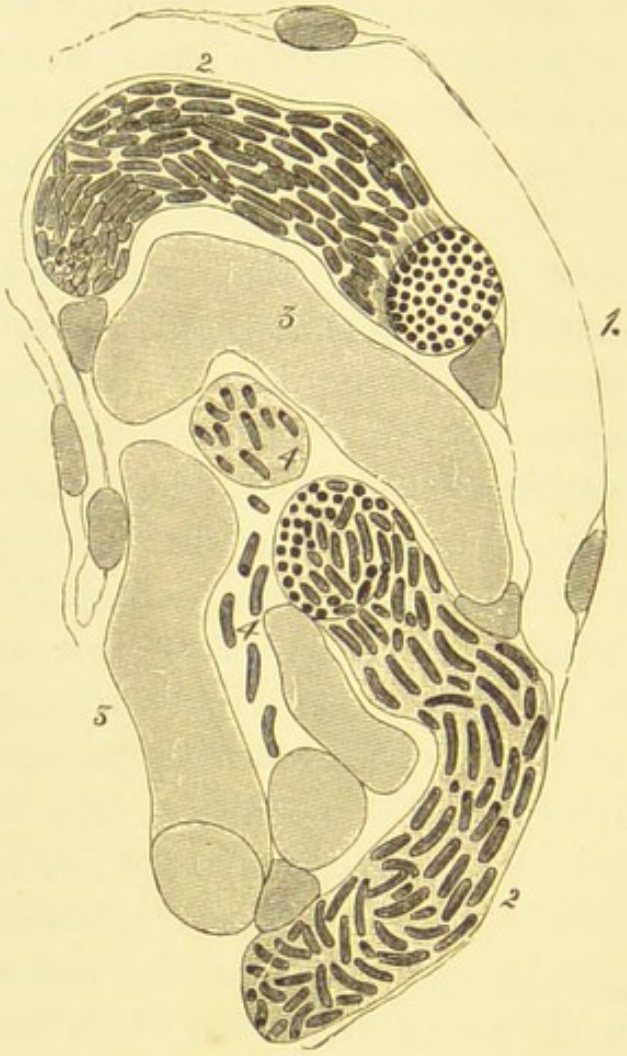

FIG 79.—FROM A SECTION THROUGH THE KIDNEY OF A CASE THAT DIED AFTER MEAT-POISONING AT WELBECK.

The figure represents part of a glomerulus of a Malpighian corpuscle, in which some of the capillary blood-vessels are filled with the bacilli. Magnifying power 700.

1. Capsule of Malpighian corpuscle.
2. Capillaries filled with bacilli.
3. Capillaries empty.
4. Bacilli contained between capillaries.

microbe found in connection with outbreaks of choleraic diarrhœa in Welbeck and in Nottingham.

Bacillus of choleraic diarrhœa from meat-poisoning.—In

July, 1880, there occurred in Welbeck, Notts, an extensive outbreak of diarrhœa among over seventy-two persons who had partaken of beef and ham sandwiches sold at Welbeck on the occasion of a sale of timber and machinery on the estate of the Duke of Portland. The infection showed itself after an incubation-period varying from twelve hours or less to forty-eight hours or more. The first symptoms were a sudden feeling of languor, nausea, griping in the abdomen, in some cases giddiness and fainting, and pain in the trunk. Then followed pain in the abdomen, diarrhœa, and vomiting, the diarrhœa being most constant. Four cases ended fatally. On *post-mortem* examination enteritis and pneumonia were most prominent. Part of the kidney

FIG. 80.—ISOLATED BACILLI IN A SMALL ARTERY OF THE SAME KIDNEY AS IN PRECEDING FIGURE.
Some of the bacilli contain spores.

was examined in microscopic sections, and it was found that many of the tubuli uriniferi contained hyaline casts; that the capillaries of the glomeruli of the Malpighian corpuscles, and the afferent arterioles, contained numbers of bacilli, some of the capillaries being distended by and plugged with masses of bacilli densely aggregated. In February, 1881, a similar but less extensive outbreak occurred at Nottingham, among fifteen persons that had partaken of certain baked pork. The symptoms were similar to those in the Welbeck outbreak. One case ended fatally. *Post-mortem:* bloody exudation in pericardium, intense pneumonia, mesenteric glands enlarged, enteritis, Peyer's glands enlarged. Bacilli similar to those

of the above case were found in the blood, in the pericardial exudation, in the juice and in the bloody fluid filling the alveolar cavities of the inflamed lung, in the vessels of the kidney, in the submucosa of the inflamed Peyer's glands of the small intestine, and in the blood-vessels of the spleen and around them.

The bacilli vary in length between 0·003 and 0·009 mm.; their thickness is about 0·0013 mm. They are rounded at their extremities, single or in chains of two, and some contain a bright oval spore, situated in the centre or at one end, and about 0·001 mm. thick. This was the case with the bacilli in the glomeruli of the kidney of the Welbeck case. The bacilli containing spores were thicker than those without them.

Experiments by feeding and inoculation made on dogs and cats, rabbits, guinea-pigs, and mice with the ham that had done the mischief in the Welbeck case produced positive results. In all cases we found pneumonia and hæmorrhage in the liver, peritonitis in some, spleen enlarged in most. The bacilli found in this ham were cultivated in the incubator in white of egg, and after two days' cultivation four white rats and several guinea-pigs and white mice were inoculated, and they became ill after twenty-four hours; they were quiet, did not feed well, and were more or less soporous. When killed the spleen was found enlarged, and in the lungs were found hæmorrhage and hyperæmia, and in some cases extensive pneumonia.

Blood, pericardial exudation, and lung juice from the fatal Nottingham case inoculated into ten animals (guinea-pigs and white mice) produced fatal results in six, the other four were killed: but in all there was severe pneumonia, in eight out of the ten there was peritonitis, in four also pleuritis, and in two in addition enlargement of the liver

and spleen. Bacilli were found in the blood and exudations of these animals. On cultivating blood and lung juice from the above case a crop of bacilli was produced which on inoculation proved very poisonous in the same way as in the previous cases.

7. *Bacillus enteritidis of Gärtner.*[1]—This microbe was found in the flesh of a cow that had been killed after ailing with diarrhœa; and the bacillus was also found in the spleen of a man who died twelve hours after eating of the above beef. The morphological and cultural characters—as far as investigated—coincide with those of bacillus coli; on rodents the cultures proved virulent on mice after feeding, and on rabbits and guinea-pigs after subcutaneous injection. It seems to me quite probable that this is the same microbe that I mentioned *sub* (5) as the beef-pie (Portsmouth) bacillus.

8. Of the same nature, *i.e.* allied morphologically and culturally to a variety of bacillus coli, is the bacillus described by H. Laser (*Centralblatt f. Bacteriologie und Parasit.*, xiii. Band, No. 7, p. 217) as a "gas-forming ærobic bacillus," and which was met with in and cultivated from the lung and liver of a young calf that had died, with several others, from some unknown malady. The characters of the microbe in microscopic specimens and in culture on the various media show that it belongs to the colon group. The cultures possess on subcutaneous injection into rodents a moderate degree of virulence and produce in a small percentage of them septicæmic infection.

9. A bacillus possessed of the power, to a considerable degree, of forming gas has been obtained from the dead body, and described by Welch and Nutall under the name of

[1] *Correspondenzblätter d. allgem. Aerztl. Vereins von Thüringen*, 1888, *No.* 9.

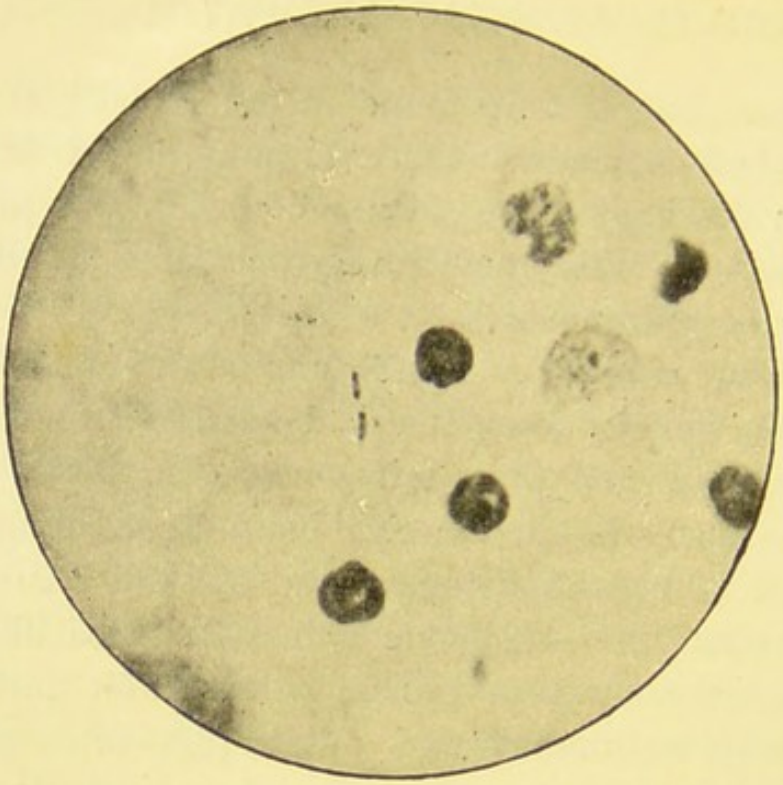

FIG. 81.—FILM SPECIMEN OF SPLEEN IN TYPHOID FEVER; NUCLEI AND CELLS OF SPLEEN PULP, TWO TYPHOID BACILLI.

× 1000.

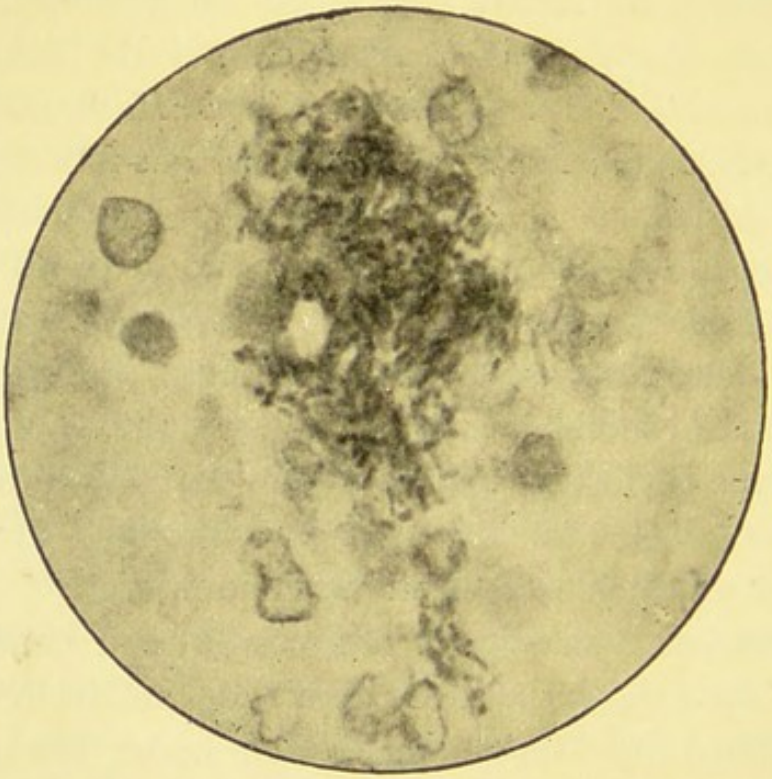

FIG. 82.—FROM A SECTION THROUGH THE SPLEEN IN TYPHOID FEVER, SHOWING A COLLECTION IN THE PULP OF TYPHOID BACILLI.

× 1000.

bacillus ærogenes capsulatus, and by A. Fraenkel under the name of bacillus gasoformans. This bacillus is virulent to rodents (rabbits), producing acute septicæmic infection, and death in twenty-four hours, the blood of the general circulation containing copiously the microbe; so strong is the gas-forming power of this microbe that the viscera of the dead (experimental) animal are found permeated by gas bubbles. My colleague, Dr. Kanthack, has also obtained this bacillus from the human dead body; it completely coincided with that described by Welch and Nutall and by Fraenkel, and after carefully investigating its morphological and cultural characters Dr. Kanthack came to the conclusion that this bacillus is a virulent variety of bacillus coli. In shape, size, in its flagella; in plate, streak, and stab culture in gelatine; in its forming copiously gas bubbles in gelatine shake culture; in its power of curdling milk and of forming indol in broth—it completely coincides with the bacillus coli, the difference being, as stated above, that the bacillus gasoformans is at first very virulent and forms copiously gas, but in continued subcultures assumes the character, both as to virulence and formation of gas bubbles, of the typical bacillus coli.

Bacillus of typhoid fever in man (Eberth-Gaffky).—In all cases of typhoid fever, if the spleen or the mesenteric glands are examined by film specimens or by culture, bacilli will be found in numbers which in morphological and cultural respects belong to the group of colon-like species that we have been describing hitherto, *viz.* they are cylindrical motile bacilli which do not liquefy gelatine, which do not form spores, and which in gelatine plates, in gelatine streak and gelatine stab, on Agar and broth, show similar characters as those of the above bacilli, but, as we shall presently show, possess, on careful analysis,

sufficiently well-marked differential characters, enabling us to say that the typhoid bacillus is a well-defined species, and to identify and distinguish it from bacillus coli and its nearest allied varieties.

The true typhoid bacillus is constantly present in the

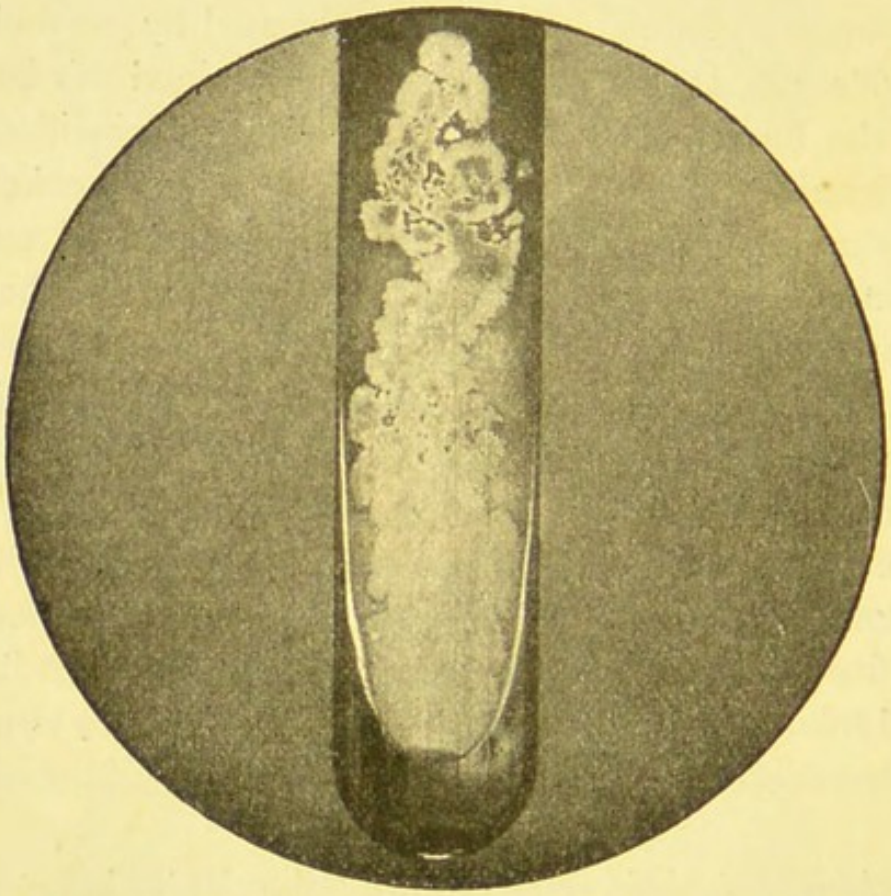

FIG. 83.—A CULTURE ON THE SURFACE OF NUTRIENT GELATINE, OBTAINED BY RUBBING A PARTICLE OF SPLEEN PULP (TYPHOID FEVER) OVER THE SURFACE OF THE GELATINE; SHOWING A CONFLUENT MASS OF COLONIES OF THE TYPHOID BACILLUS.

Natural size.

tissue of the spleen and the mesenteric glands in this disease, and in this disease only; in the spleen it occurs as a rule in larger or smaller groups, though it is also found here and there in small numbers and isolated; the same is the case with the mesenteric glands. In the wall of the ileum, in and around the swollen or ulcerated Peyer's glands

—whenever such an examination is made by microscopic or cultural specimens—this bacillus occurs in numbers. In the intestinal contents and in the typhoid stools the true typhoid bacillus can be also identified, although this examination is only in comparatively few cases successful, on account of the great number of bacillus coli present, and it is for the same reason that if the typhoid bacillus is so

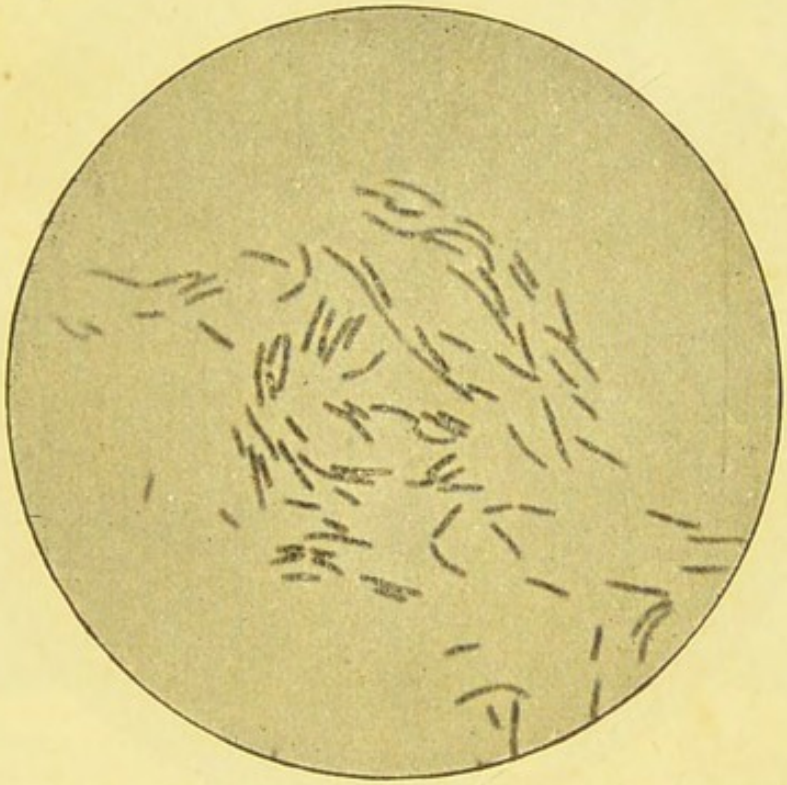

FIG. 84.—IMPRESSION SPECIMEN OF A VERY YOUNG COLONY OF THE TYPHOID BACILLUS ON GELATINE.
× 1000.

demonstrable it is in cases in which the Peyer's glands have already begun to ulcerate, *i.e.* in the second and third week of the disease, that is when their number passed from the tissue of the mucous membrane is sufficiently great. In the blood of the general circulation in typhoid fever the bacillus is not demonstrable, except in very rare instances, and then only after the second week. From this the conclusion

is drawn that typhoid fever is not a blood disease; that is to say, the blood is not the proper soil for the growth and multiplication of the microbe, but the wall of the ileum, the spleen, and mesenteric glands (and possibly other lymph glands) represent the localities wherein the bacillus grows and

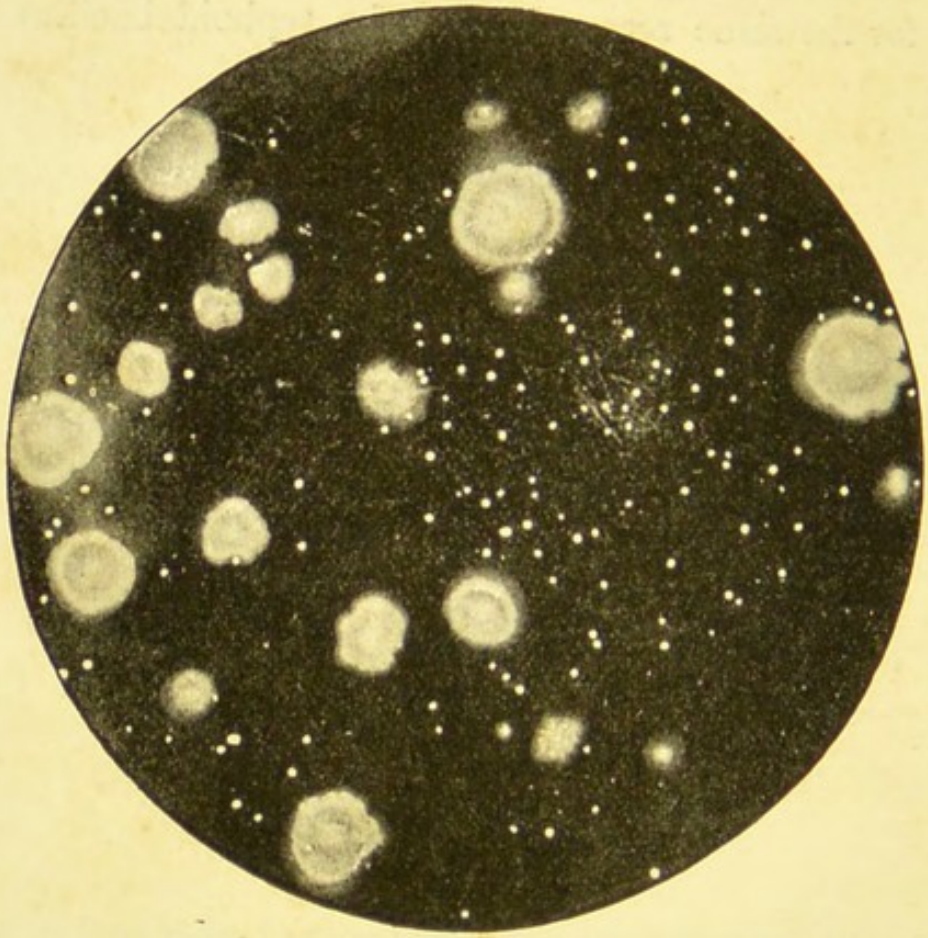

FIG. 85.—A TYPICAL GELATINE-PLATE CULTIVATION OF THE TYPHOID BACILLUS; THE SMALL DOTS ARE DEEP COLONIES, THE PATCHES ARE COLONIES ON THE SURFACE; THE CULTURE IS ABOUT 6–7 DAYS OLD AND SHOWS THE CONCENTRIC MARKINGS OF SUPERFICIAL COLONIES.

Natural size.

multiplies and produces the toxin (typhotoxin) which causes the symptoms of the disease. Thus typhoid fever would in reality be the result of intoxication in its chief clinical symptoms. Owing to the fact that the demonstration of the typhoid bacillus in the typhoid stools, because of our

at present imperfect methods, is in many cases negative, Sanarelli has come to the conclusion that the pathological changes of the intestine are as much a result of the toxin action of the bacillus distributed in the blood and viscera as

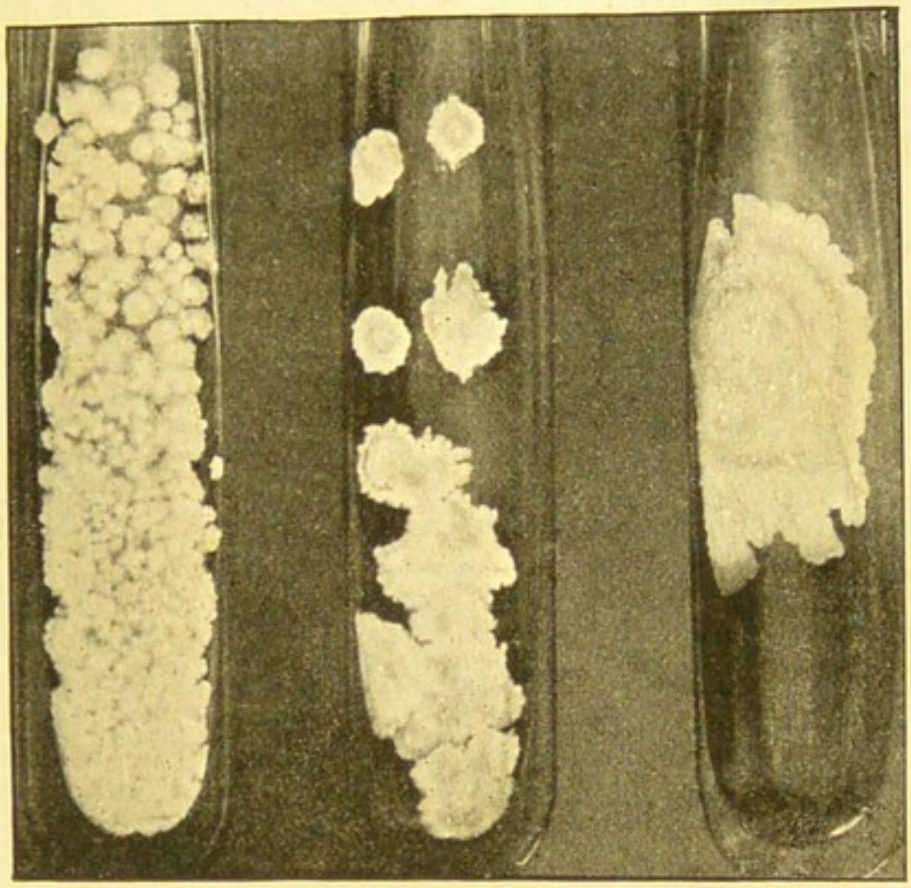

FIG. 86.—THREE TUBE-PLATE CULTIVATIONS OF THE TYPHOID BACILLUS: COLONIES ON THE SURFACE OF GELATINE. IN THE LEFT TUBE THE COLONIES ARE VERY NUMEROUS, SMALL, AND IN THE LOWER PART CONFLUENT; IN THE MIDDLE TUBE THE COLONIES ARE FEWER AND LARGER; AND IN THE RIGHT TUBE ONLY ONE COLONY OF GREAT SIZE, AND SHOWING THE CONCENTRIC ASPECT.

Compare this figure with Fig. 60 of similar cultures of bacillus coli.

Natural size.

are the other clinical symptoms. Wright and Semple[1] have attempted to give support to this theory of typhoid fever being really a blood disease by stating that in all, or almost all, cases which they have examined—some of them early

[1] *The Lancet* for July 27, 1895.

cases—the urine excreted by the patient contained the typhoid bacillus in considerable numbers, and they conclude that contrasted with the intestinal discharges the urine is more constantly and more highly charged with the contagium and deserves, therefore, more attention for purposes of disinfection than it has hitherto received. While

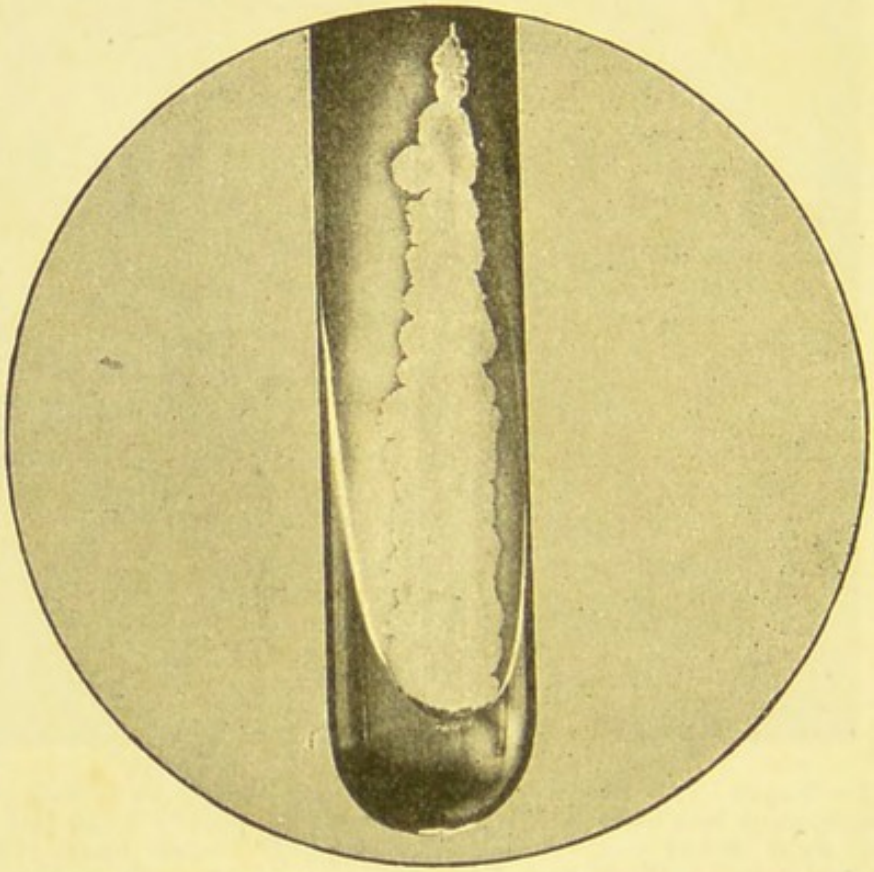

FIG. 87.—STREAK SUB-CULTURE ON GELATINE OF THE BACILLUS OF TYPHOID. Natural size.

this conclusion in a general way and to a certain extent harmonises with previous results, viz. as to the occasional occurrence of the typhoid bacillus in the kidney and in the urine, it differs in this essential respect that Wright and Semple maintain the almost constant occurrence of the typhoid bacillus in the urine, even in early cases, and for this reason they favour Sanarelli, inasmuch as they conclude

that the normal habitat of the typhoid bacillus is the circulating blood, hence its passage into the urine already in the early phases of the disease. Dr. Horton Smith, working in my laboratory, has paid special attention to this question, and his conclusions do not confirm those arrived at by Wright and Semple. The identification of the typhoid bacillus in the stools, in the urine, in the spleen, in the blood, or in the glands, &c, if it is to be considered free from criticism, must not content itself merely with the demonstration of a general similarity as to size, shape, and motility, or as to the general aspect of the plate cultivation, streak and stab cultures on Agar and in gelatine—it is precisely on account of such general observations that many of the statements made in previous years as to the occurrence of the typhoid bacilli in one or the other tissue, in one or the other locality, cannot be accepted as proven. The identification of a bacillus as typhoid bacillus must be such as to show that as regards every one and all of the following characters there is complete harmony between it and the bacillus obtainable in pure culture from the typical spleen of a typical case of typhoid fever. The characters are these:—

1. The typhoid microbe taken from the spleen of a typhoid case is a cylindrical bacillus measuring on the average 2–4 μ in length; in gelatine or Agar cultures already after twenty-four hours there are present longer forms, some filamentous; the great majority of the bacilli from a recent culture are distinctly longer and more cylindrical than those of a similar culture of the typical bacillus coli.

2. Examined in the hanging drop in sterile broth the typhoid bacillus of a recent gelatine or Agar culture (16–24 hours old) is extremely motile, contrasting markedly with a similar culture of the typical bacillus coli.

3. On staining flagella the typhoid bacillus of a recent Agar culture is seen to be possessed of a large number of long wavy or spiral flagella extending in a radial fashion on —or rather coming off vertically from—the whole length of the bacillus. From a considerable experience I am prepared to attribute to this particular distribution, and to the

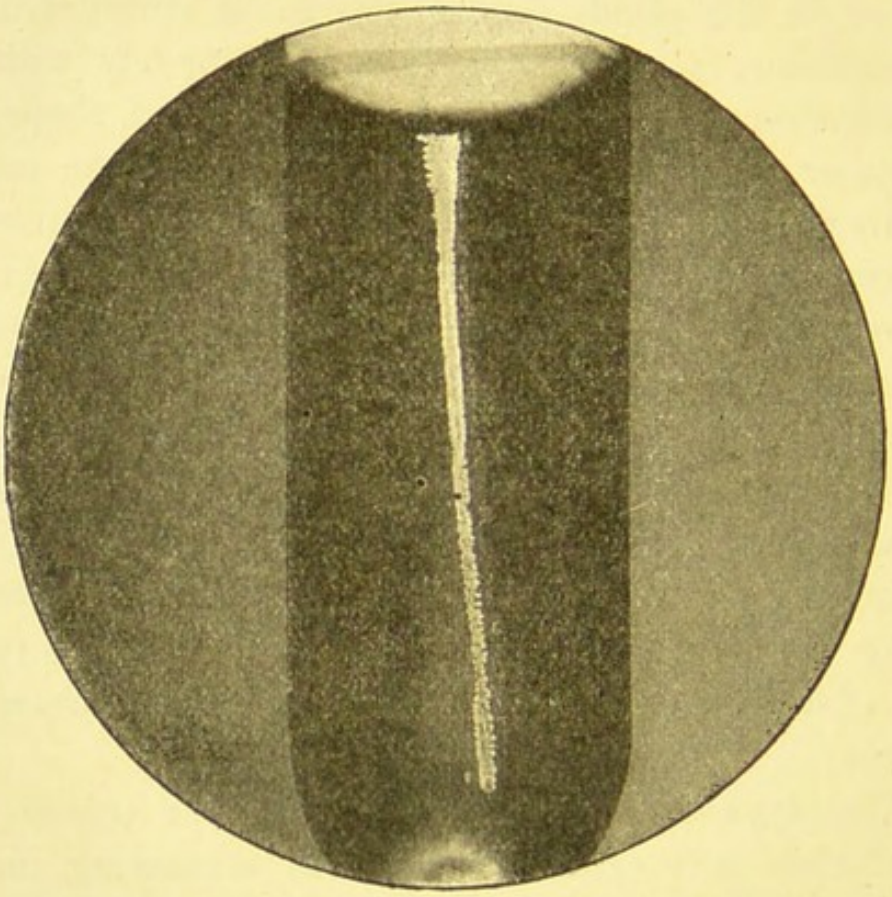

FIG. 88.—STAB SUBCULTURE OF THE TYPHOID BACILLUS.
Magnified twice.

abundance of long flagella, a very important differential value. (See illustrations of flagella in Chapter VI.)

4. In gelatine plates the typhoid bacillus grows markedly slower than the typical bacillus coli; the colonies of the former, more translucent, homogeneous, show after some days an indication of concentric layers; but their outlines are as

angular and filmy as those of bacillus coli. Compare Fig. 72, which, although of the grouse bacillus, is a good representation of a bacillus coli plate, with Fig. 85, of a plate of the typhoid bacillus. In gelatine streak and Agar streak the growth is also markedly slower and more translucent in the first few days than that of the bacillus coli, but its

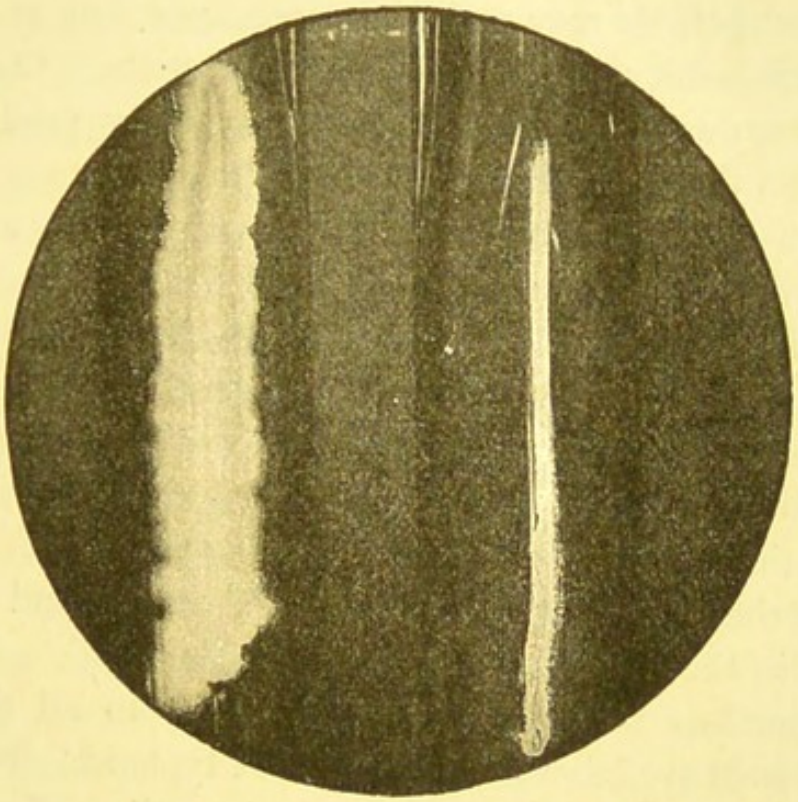

FIG. 89.—STREAK CULTURES ON NUTRIENT GELATINE AFTER 48 HOURS: ON THE LEFT, OF BACILLUS COLI; ON THE RIGHT, OF TYPHOID BACILLUS.

Natural size.

irregular filmy margin in the gelatine streak culture is the same as in bacillus coli.

5. In gelatine stab the line of inoculation is marked like that of bacillus coli, as a row or rows of droplets white in reflected, brownish in transmitted light, but on the surface of the stab in typhoid the plate-like filmy expansion is small, and is not well marked in the first few days, whereas

in those of bacillus coli this plate-like expansion is well developed.

6. Shake cultures in ordinary and in sugar gelatine do not develop gas bubbles, though the gelatine is in all layers pervaded by colonies.

7. Milk is not curdled by the typhoid bacillus.

8. Broth is made rapidly turbid, and after some days an imperfect pellicle may make its appearance, but at no time does such broth give the nitroso-indol reaction. The typhoid bacillus grown in milk, broth, or litmus-whey produces less acid than the typical bacillus coli (Petruschki).

9. On steamed potato, kept after inoculation at 37° C., the growth is a colourless transparent film.

10. In nutrient gelatine, containing gelatine to the amount of 25 per cent., the difference between bacillus coli and bacillus of typhoid on incubation at 37° C. is very striking; the (fluid) gelatine remains limpid, and its surface is covered with a thick pellicle during the first forty-eight hours in the case of the typical bacillus coli, but is strongly and uniformly turbid in the case of the typhoid bacillus.

A bacillus which does not conform to all the above points is in my laboratory rejected as typhoid. Whether or not a bacillus which in one or the other of the above points deviates and approaches the bacillus coli is or is not the typhoid bacillus, has or has not originally been derived from the typhoid bacillus, I feel neither inclined to deny nor to affirm—in the present state of our knowledge it would not be justifiable to do so; but what I maintain is that since the true typhoid bacillus taken from the spleen of a typical typhoid case possesses all and every one of the above characters it is more justifiable to reject as typhoid those which either in the number and character of the flagella, or in the manner of growth in gelatine shake culture, in milk, in broth,

on potato, and in 25 per cent. gelatine (at 37° C.) do not correspond to the above tests. In respect of the rapidity of growth of the colonies in gelatine plates and in gelatine streak a latitude may be excusable, since in these respects continued subcultures of the typhoid bacillus on artificial media show that in respect of rate of growth it does somewhat alter with age, but not in respect of greater translucency; nor have I seen any alteration, after two or three years of continued subcultivation, in the matter of flagella, of gelatine shake culture, of milk-, broth-, potato-, or 25 per cent. gelatine cultures. It may be added that bacillus coli has longer vitality, both in water and in sewage, than bacillus of typhoid, and also that, while bacillus coli requires 65° C. for five minutes to become killed, the typhoid bacillus is killed already at 62° C. in five minutes.

The identification by Dr. Horton Smith of the typhoid bacillus from the urine of cases of typhoid fever was based on the above characters, and his results are very instructive:—

(*a*) In two cases of undoubted typhoid fever—mild cases—the examination of the urine—always considerable quantities being examined by Parietti's method—commencing from the first week of illness and carried on till the temperature again became normal, revealed no typhoid bacillus.

(*b*) One case, dead from typhoid fever during the third week; the urine taken in the *post-mortem* room yielded numbers of colonies of the typhoid bacillus.

(*c*) One case, first examined on the twelfth day of illness, did not yield the typhoid bacillus, but, beginning with the fourteenth day, till the twenty-second day,—the day of fatal issue—yielded typhoid colonies.

(*d*) One case, examined first on the tenth day of illness and continued through the whole of the first attack and

right through a short relapse, failed to yield typhoid bacillus in the urine. By the thirty-ninth day, when the temperature had become almost normal again, the urine yielded abundance of typhoid bacilli, in fact the urine was quite turbid, being a pure culture of the typhoid bacillus, and, strange to say, this condition, viz. abundance of typhoid bacilli in the urine, continued until twenty-two days after the temperature had again been normal.

There is then in these observations no confirmation to be found of Wright and Semple's contention as to the early excretion of the typhoid bacillus, on the contrary they show that, as had been hitherto accepted, the general discharge of the typhoid bacillus from the system by the kidney is an occurrence belonging to the later stages and cannot therefore be taken as indicating that the typhoid bacillus is circulating in the blood in the early stages, or that therefore typhoid fever is a blood disease, a true infection like anthrax or septicæmia.

With regard to the effect of subcutaneous or intraperitoneal injection of large doses of living or sterilised culture no differentiation can be made between the typhoid bacillus and the bacillus coli, they both—in common with other species, *e.g.* bacillus prodigiosus—act in the same manner; recent gelatine cultures of either act more virulently on the mouse and guinea-pig injected subcutaneously than broth or Agarculture, producing in sufficiently large doses acute septicæmic infection. Smaller doses produce only a transitory swelling, which, however, may lead to local sloughing and necrosis of the skin.

The immunisation by injection of living culture of the typhoid bacillus and the specific action of blood-serum of immunised animals we shall have an opportunity to discuss in a later chapter.

Petruschki described (*Centralblatt f. Bakt. und Parasit.* No. 6/7, 1896) a bacillus, occurring occasionally in the typhoid stools and also other putrid materials, which coincides in most points with the typhoid bacillus. This is the *bacillus fæcalis alkaligenes*. It differs from the typhoid bacillus in forming alkali in litmus-whey, the latter being an acid former.

CHAPTER XII

PATHOGENIC BACILLI : GROUP C

As belonging to this group we consider pathogenic bacilli which in morphological and cultural respects differ from those hitherto considered. They are fine cylindrical rods of about 0·8 to 1 μ long, 0·1 to 0·2 μ thick, forming characteristic translucent, filamentous, gelatinous growths, slowly liquefying gelatine and not forming spores.

1. *Bacillus of mouse septicæmia of Koch.*—By inoculation of filthy water into mice Koch produced a fatal and acute septicæmia, which owing to the peculiar microbe has great interest. At the seat of the inoculated animals there is found slight hæmorrhage, the internal viscera are greatly congested, the spleen is not much enlarged; the animals die during the second day. In the blood of all parts are found in very large numbers exceedingly minute bacilli, some longer than others, but all very fine; many of the white blood-corpuscles are quite filled with them, being at the same time swollen up. In the lungs there is slight hæmorrhage into the alveolar tissue: everywhere one sees the swollen leucocytes completely filled with the minute bacilli, some of these also free owing to the disintegration of the leucocytes. Sections stained carefully in fuchsin and

then in methyl blue show the nuclei of the tissue and of the leucocytes blue, the bacilli bright red.

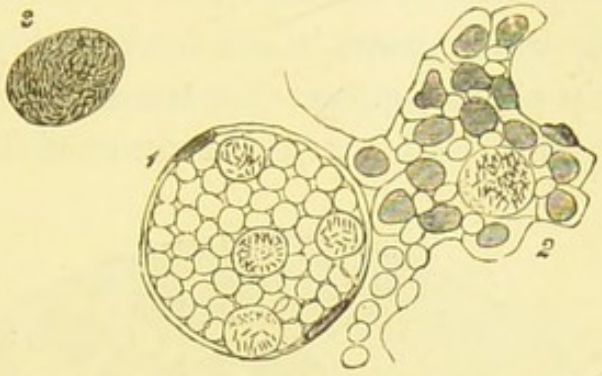

FIG. 90.—FROM A SECTION THROUGH THE LUNG OF A MOUSE DEAD OF KOCH'S SEPTICÆMIA.

1. Small vessel filled with blood ; the white blood-corpuscles are filled with very minute bacilli.
2. Interalveolar tissue : in it a white corpuscle filled with the bacilli. Magnifying power 700.
3 A white blood-corpuscle more highly magnified, 1000.

(Stained with magenta.)

Cultivations of the heart's blood or of the juice of the viscera yield numerous colonies ; pure cultivation in gelatine in test tubes can be made without difficulty directly

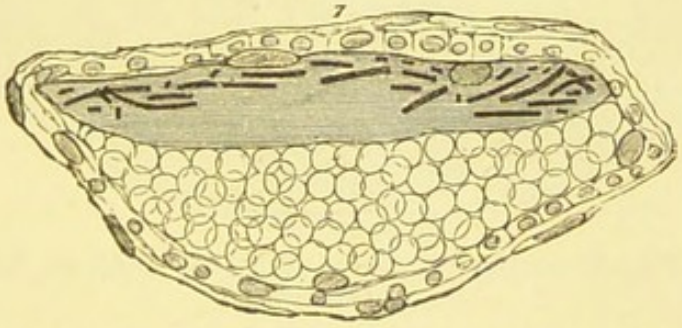

FIG. 91.—FROM A SECTION THROUGH THE SMALL INTESTINE OF A MOUSE DEAD OF SEPTICÆMIA.

The figure represents a section through a small vein in the submucous tissue, filled with blood. At 1, there is a homogeneous substance and in it numerous bacilli, but these bacilli are much larger than the bacilli of Koch's septicæmia in the mouse.

Magnifying power about 700 (Stained with methylene blue and vesuvin.)

from the heart's blood. The colonies in plate cultivations appear after two or three days as highly translucent, gela-

tinous, grey, irregularly outlined, angular, minute patches; in the stab culture in gelatine after two or three days a very characteristic growth is noticed: the stab is a translucent grey line from which branch out horizontally vast numbers of fine, closely placed, gelatinous, translucent grey threads; in the streak culture the streak becomes visible after two to

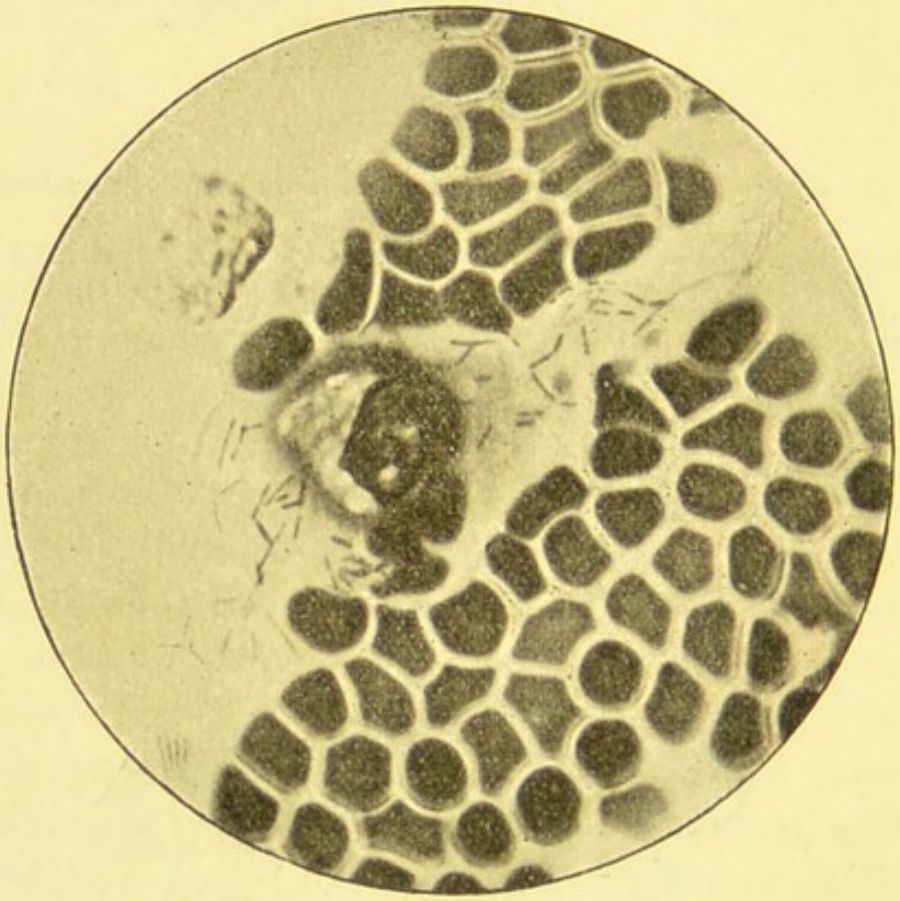

FIG. 92.—FILM SPECIMEN OF BLOOD OF MOUSE DEAD OF KOCH'S MOUSE SEPTICÆMIA.

× 1000.

three days as a gelatinous, grey, translucent, thin band from which pass out vertically numerous grey fine lines. The growth liquefies the gelatine very slowly; it takes generally some days before liquefaction commences, and it proceeds very slowly; the gelatine is thick like syrup, is fairly limpid, but contains greyish, translucent flakes. In Agar mixture

the growth is slow and very transparent. No spore formation has been observed.

Specimens made of the cultures show under the microscope, besides short bacilli, also a great many which are long threads more or less curved. Inoculation produces in mice the septicæmia with certainty.

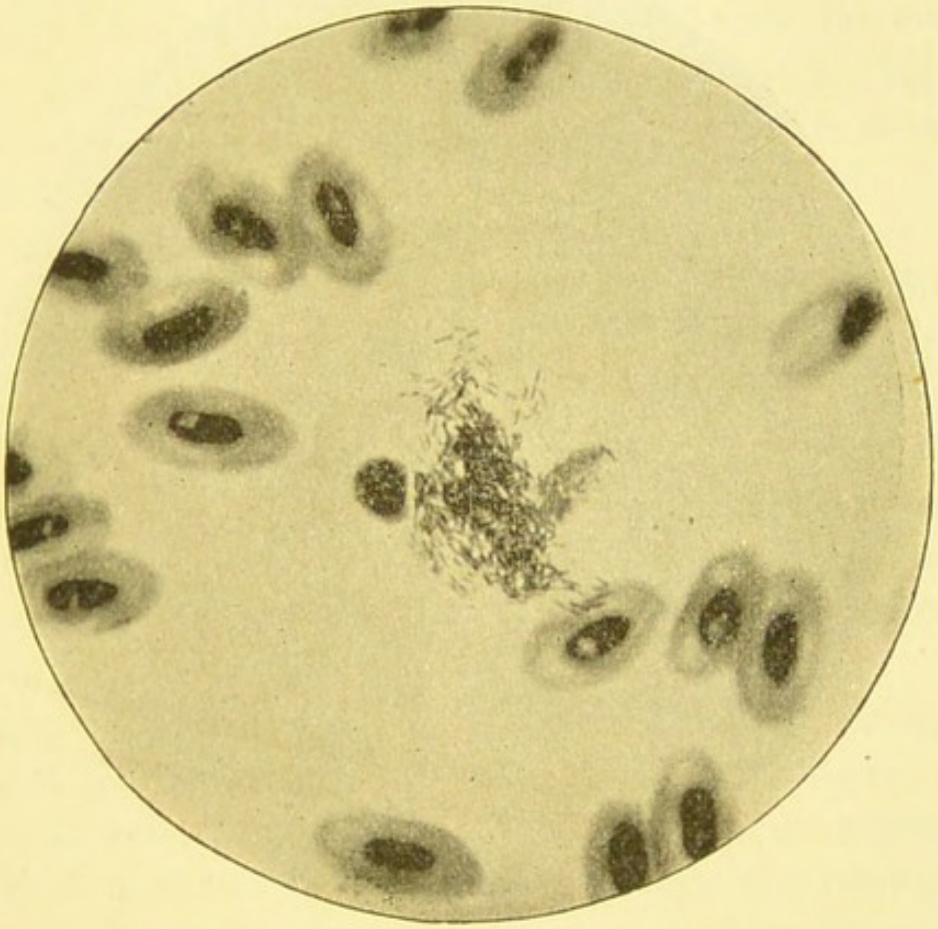

FIG. 93.—FILM SPECIMEN OF BLOOD OF PIGEON DEAD AFTER INFECTION WITH SWINE ERYSIPELAS.

× 1000.

Löffler describes a spontaneous fatal epidemic amongst white mice which occurred in his laboratory, and which was caused by this same bacillus (*Centralbl. f. Bakt. und Parasit.*, vol. xi. p. 134).

2. *Bacillus of swine erysipelas* (mal rouge, rouget, red soldier).—An acute infectious disease, to which swine are

very susceptible, and of which about 60 per cent. succumb. When affected the animals are quiet, the voice is hoarse, and the temperature is much raised; on the skin of the neck, chest, abdomen, and thighs extensive red patches of swollen œdematous skin are noticed; under convulsions—

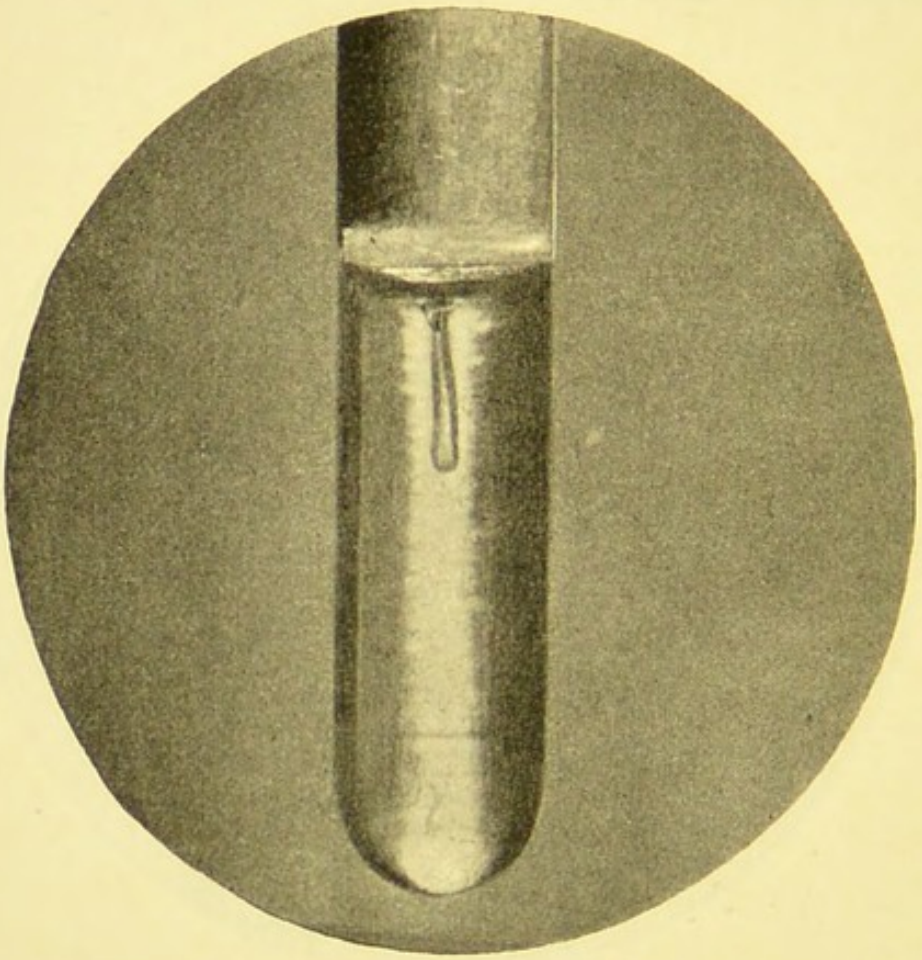

FIG. 94.—STAB CULTURE IN GELATINE OF THE BACILLUS OF SWINE ERYSIPELAS. Natural size.

occasionally paralysis of the hind extremities—the animals die from between twelve hours and three or four days since the first symptom. On post-mortem examination is found hæmorrhage in the affected patches of the skin, the lymph glands are swollen and much congested, the peritoneum is inflamed; the mucous membrane of the intestine is much

injected and œdematous; the Peyer's glands are swollen, the spleen and liver are much congested and slightly enlarged.

The blood of the heart, and particularly the juice of the lymph glands and the spleen, contain fine bacilli very similar to those of the mouse septicæmia (Schütz) (0·6-1·8 μ long). On microscopic sections through the liver, spleen, kidney, and lymph glands the bacilli are easily demonstrated in the capillary blood-vessels, either isolated between the blood corpuscles or enclosed in the swollen leucocytes. As regards cultural characters they completely resemble those of the mouse septicæmia (Koch).

Swine fed or inoculated with the blood or tissues of a pig dead of the disease become also affected. Mice[1] and pigeons are very susceptible to the disease; guinea-pigs and fowls are refractory; rabbits show generally only a local effect; mice die in two or three days, pigeons in three to four days; in both the blood of the general inoculation and of the organs contains abundantly the bacilli. In the pigeon numerous white blood-corpuscles in the vessels of the viscera are filled with the bacilli.

Pasteur has shown that the virus in its passage through a series of pigeons increases in virulence, both as regards the pigeon as well as the pig; on its passage through a series of rabbits it increases in virulence as regards the rabbit, but decreases in virulence as regards the pig. Pigs inoculated with blood of the last rabbit of the series become ill, but recover, and are then found refractory to inoculation with the virulent disease.

3. *The bacillus of Egyptian ophthalmia : catarrhal conjunctivitis* (Koch).—Koch (*Choleraberichte*, 1883) has shown

[1] Mice die with congested and enlarged spleen, greatly congested lungs; the intestines are relaxed and filled with sanguineous mucus; the kidney and liver are enlarged and congested.

that what is spoken of as "Egyptian ophthalmia" is really several kinds of infectious ophthalmiæ: one is an acute blennorrhœa or purulent ophthalmia, and does not differ from that known to occur in consequence of infection with gonorrhœal exudation. A second, the true Egyptian ophthalmia, is however of an altogether different etiological

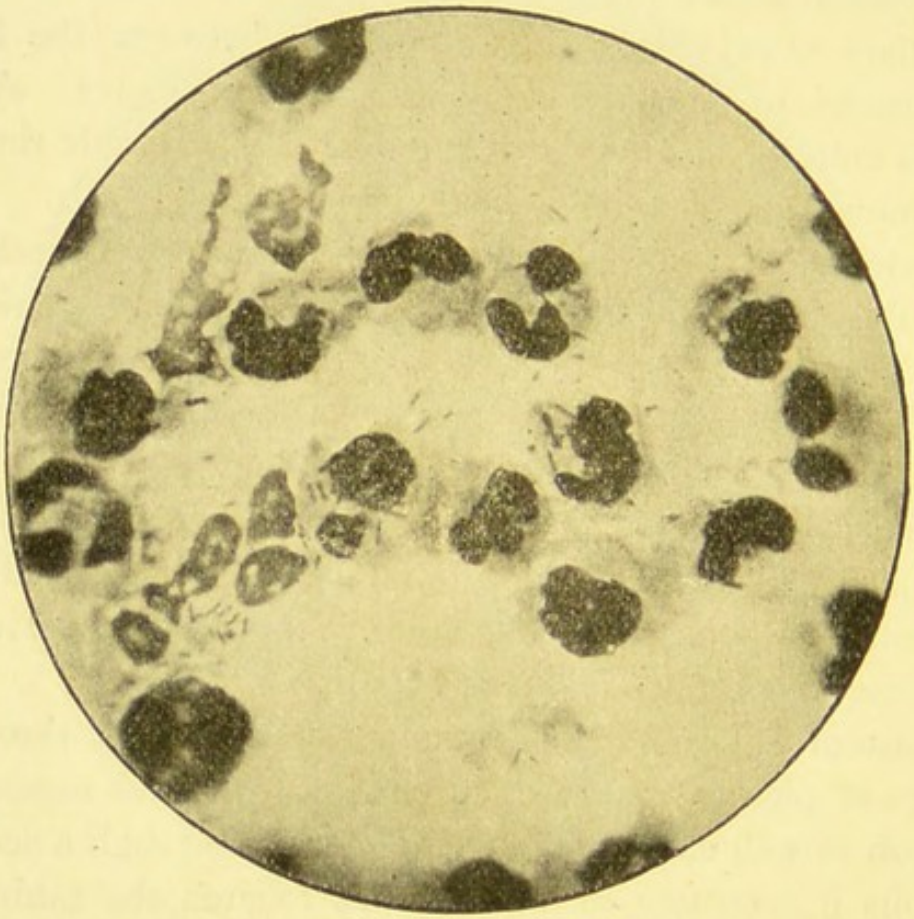

FIG. 95.—FILM SPECIMEN OF EGYPTIAN OPHTHALMIA, CATARRHAL CONJUNCTIVITIS (KOCH), SHOWING MANY PUS-CELLS CONTAINING THE SPECIFIC BACILLI IN THEIR PROTOPLASM.
× 1000.

character, though in its symptoms and pathology it is similar to, but not identical with, the blennorrhœa. This second one, the "*catarrhal conjunctivitis*," is associated, not with the gonococcus, but with a minute fine bacillus, very similar in morphological respects to the bacillus of Koch's mouse septicæmia. In this ophthalmia the bacillus

is present in the purulent exudation of the conjunctiva as isolated examples, and more commonly enclosed within the pus cells, whose protoplasm is sometimes found crowded with them (*see* Fig. 95), in the same way as we saw the

FIG. 96.—FROM A SECTION THROUGH A LYMPHATIC GLAND OF MAN DEAD OF SEPTICÆMIA.

1. A blood-vessel which at one place is distended by and filled with minute bacilli.
2. Lymph-corpuscles.
3. Degenerated lymph-corpuscles.

Magnifying power 700. (Stained with gentian violet.)

leucocytes in the mouse septicæmia crowded with the small bacilli. The cultivation of these bacilli carried out by Kartulis shows that there exists a great difference between them and the mouse septicæmia bacillus.

Kartulis, besides describing their symptoms and course

(*Centralbl. f. Bakt. und Parasit.*, Band I., No. 10, pp. 289–293), and the differential characters existing between blennorrhœa of the conjunctiva and catarrhal ophthalmia, succeeded in cultivating the bacilli of the catarrhal or true Egyptian conjunctivitis. He showed that they do not grow on peptone or gelatine; on blood-serum or on Agar they grow well between 28–36° C., forming in thirty to forty hours small white punctiform colonies, prominent over the surface of the medium; when closely sown (*e.g.*, in streak culture) they soon coalesce into a whitish-grey band of a fatty, glistening appearance; the margin of the band is wavy or crenated. Animals inoculated on the conjunctiva with the conjunctival secretion or with the culture prove refractory; but Kartulis succeeded in producing with the culture the typical catarrhal conjunctivitis in one out of six cases. The pus corpuscles resulting in this case were crowded with the characteristic bacilli. This one case was that of an individual twenty-five years old.

I append here the illustration of a *bacillus of septicæmia of man.* In several cases of human septicæmia I have found in the blood-vessels of the swollen lymphatic glands large numbers of minute bacilli, slightly thicker than those just mentioned. They form continuous masses, both in the capillaries and in the minute veins, amounting in some cases to veritable emboli. They occur isolated or in short chains, their length about 1 μ to 2·5 μ, their thickness about 0·3 μ to 0·5 μ; no cultivations having been made, the characters of these bacilli could not be ascertained.

The bacillus of influenza.[1]—R. Pfeiffer (*Deutsche Med. Wochenschrift*, No. 2, 1892) was the first who made the

[1] The following account is taken from my Report to the Medical Officer of the Local Government Board: Further Report on Influenza, 1889–92.

announcement that in all cases of influenza there are present in the characteristic grey purulent bronchial secretion enormous numbers of minute non-motile bacilli. He describes these as occurring only during the acute stages and gradually diminishing in numbers as the disease abates. The bacilli, he tells us, are very minute, about the thickness of the well-known bacilli of Koch's mouse septicæmia, but only half their length; they stain with some difficulty in anilin dyes, requiring a somewhat prolonged application of the dye. In stained specimens these bacilli have a characteristic appearance, inasmuch as their protoplasm is segregated into a stained granule at each end while the middle portion remains unstained and shows only the outline of the sheath. Thus the bacillus looks like a diplococcus, and where two such bacilli are placed end to end they look like a chain (streptococcus) of four spherical cocci. In the sputum these bacilli occur in smaller and larger masses, occasionally almost as a pure culture. In severe cases they form continuous masses in the peribronchial tissue and also in the subpleural lymphatics, and they are also met with inside the leucocytes of the sputum. As the disease passes off, so the bacilli disappear from the sputa. These bacilli are constantly present in influenza, but do not occur in the bronchial secretion of other bronchial or pulmonary affections.

Kitasato, in the same paper, gives his observations on the cultivation of these bacilli of Pfeiffer, and records that they have cultural characters by which they can be readily distinguished from other bacilli: that they are, in fact, a definite species not occurring in any disease except in influenza. They do not thrive at temperatures below 28° C., that is to say at temperatures at which nutrient gelatine still keeps its solid condition. They grow well in broth and

on glycerine Agar at 37° C. or thereabouts. The broth does not become turbid, but remains limpid. The growth in broth appears as whitish small granules and flocculi; on glycerine Agar the bacilli form minute translucent colonies like droplets, having no tendency to coalesce as growth proceeds. The cultures are also characterised by this fact that they soon die, and therefore sub-cultures cannot easily be carried on through many generations. In stained specimens grown in cultures the bacilli retain the same characters observed in the bacilli of sputum, viz., they show the characteristic bipolar staining.

These statements and observations of Pfeiffer and Kitasato are very definite, and if confirmed would afford strong reason for believing that in these bacilli we had found the special microbe of influenza. The life-history of this microbe would conform with what we believe to be the facts about the contagium of influenza, its being spread and received by the organs of respiration, and the reception of the infection by the same channel; the presence in most cases of influenza of some kind of bronchial disturbance more or less pronounced, showing itself at the outset of the disease or a few days later, and increasing after the febrile stage of the complaint had been passed.

From our own observations of a large number of cases, we find ourselves in a position to confirm the statements of Pfeiffer and Kitasato in all essential points; and accordingly we have arrived at the conclusion that the particular bacilli as described by them ought to be regarded as the specific microbe of influenza.

The bronchial expectoration was examined in twenty cases from the living patient; of these, five were cases of genuine influenza-pneumonia, that is of pneumonia setting in very soon, a few days, after the attack of influenza

commenced, and where the history showed that the pneumonia was to be regarded as a part of the disease and not as a secondary complication.

The result then of these examinations confirms fully the assertions of Pfeiffer, viz., that the characteristic influenza bacilli are constantly present in the bronchial sputum of

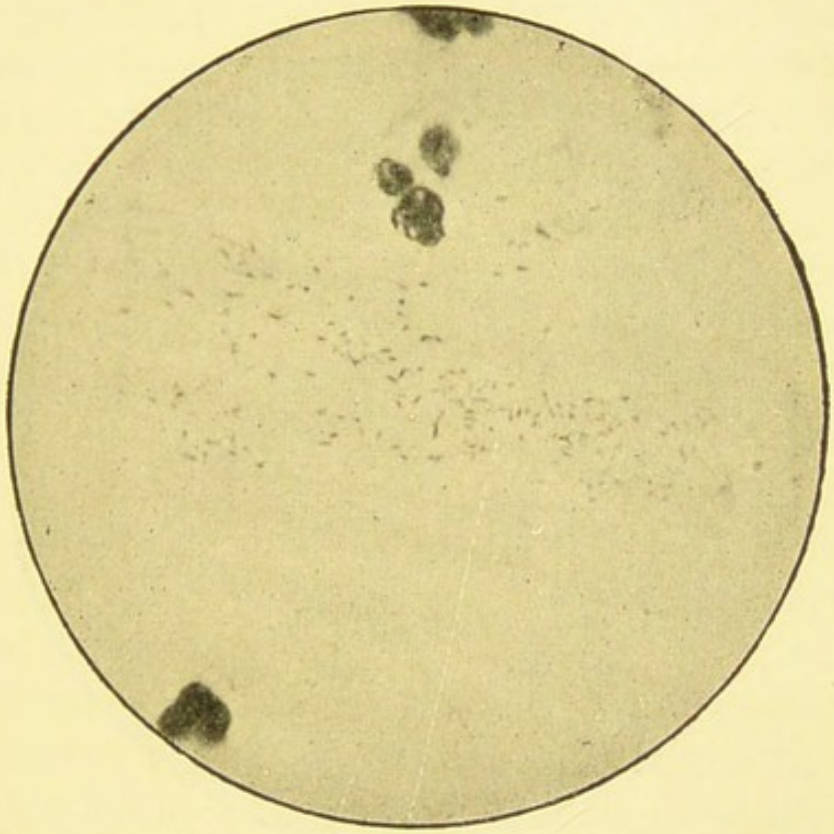

FIG. 97.—FILM SPECIMEN OF PULMONARY EXPECTORATION OF AN ACUTE CASE OF INFLUENZA PNEUMONIA; NUCLEI OF LEUCOCYTES AND THE INFLUENZA BACILLI IN PURE CONDITION.

× 1000.

influenza cases; that in well-marked cases they occur in great abundance, singly, in small groups, and in larger masses, and in some portions of the sputum almost as a pure culture. The results also go to confirm Pfeiffer's statement that as the disease abates, as the patients get better and as the sputum becomes scantier, the number of the bacilli also rapidly diminishes; this was the case in the

sputum from patients having pneumonia of influenza: before the height of the disease is passed the number of the characteristic bacilli is very great, after the height of the disease it diminishes. Also in the cases of bronchitis the number of the characteristic bacilli is found at first to be considerable, but when the disease abates and the patient improves their number becomes greatly diminished.

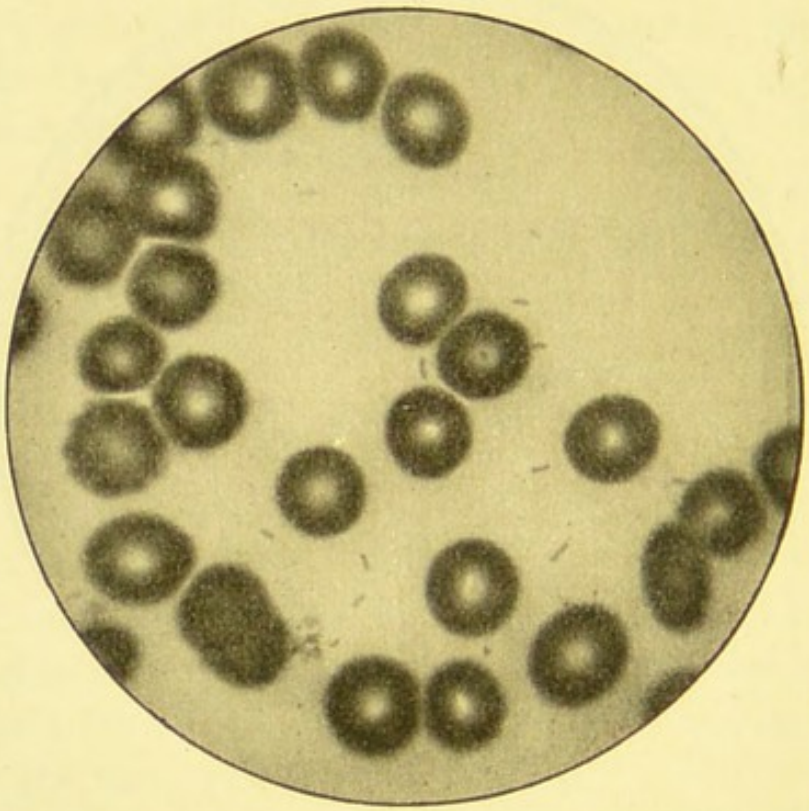

FIG. 98.—FILM SPECIMEN OF BLOOD OF A CASE OF INFLUENZA, SHOWING BLOOD DISCS AND MINUTE BACILLI.

× 1000.

It deserves notice, as a matter of no small practical importance, that in cases of acute influenza with bronchial expectoration the *fluids of the mouth* contained abundance of influenza bacilli. Thus cover-glass specimens of such bronchial expectoration that had not been washed at all (or at best not well washed) showed scaly epithelial cells,

derived from the oral cavity or fauces, literally crowded with masses of what, when duly stained, looked exactly like the typical influenza bacilli.

1. *Culture in broth.*—Broth-tubes containing a pure culture of the influenza bacillus remain quite limpid; at the bottom of the fluid there are noticed already after twenty-four hours, but better after forty-eight hours, a few whitish-grey, irregular granules or flocculi, which during the next two or three days increase in size and number and form at the bottom of the tube greyish-white nebulous fluffy masses; when shaken they break up into whitish-grey granules and flocculi, but soon again settle at the bottom of the fluid, leaving the rest of the broth perfectly limpid. In four or five days (at 37° C.) the growth has reached its maximum. Sub-cultures show the same characters, but we generally noticed that as the number of removes increases the broth has a tendency to show slight turbidity after one, two, or three days' incubation, minute granules sticking to the wall of the tube and showing themselves also in various layers of the fluid.

Furthermore, in successive sub-cultures it is noticed that the amount of growth (floccular masses) at the bottom of the fluid is not invariably the same, being decidedly less in the later than in the earlier sub-cultures.

A point of great interest is the comparatively rapid death of the bacillar elements in the broth cultures. Unless the transmission is carried on within two, three, or four, up to seven days, it will be found that the sub-cultures are sterile; broth cultures from eight to ten days old are very uncertain, broth cultures a fortnight old yield no living organisms to subsequent sub-cultures. But if the sub-cultures are set up every two or three days we did not find a limit to the number of generations to which some of our cultures could

be carried on ; although in other cases after about a dozen generations in broth no living sub-cultures could be made in broth.

2. *Culture on Agar.*—The cultivations and sub-cultivations were (*a*) on beef broth (not beef infusion), Agar (1 p.c.), peptone (1 p.c.), and salt (1 p.c.) ; and (*b*) on glycerine Agar, that is the ordinary Agar plus glycerine (6 p.c.). The growth on our ordinary Agar is rather more easily observed than on glycerine Agar, being a little more copious (the colonies being somewhat larger) and a little less translucent, and therefore more readily noticeable.

The colonies on the surface of both these media can be discerned under a glass after twenty-four hours' incubation at 37° C. They then have the appearance of extremely minute translucent flat droplets, and these during the next day or two increase somewhat in size, but even at their largest are but small—not exceeding three millimetres in breadth, and only just visible to the eye as translucent circular flat droplets—on further incubation becoming flatter (Fig. 99). Under a lens their margin is seen to be slightly crenated and their centre darker than the rest. The crenated margins show no tendency to coalesce, even when the colonies are thickly planted.

In Agar stab-culture the stab is indicated after two or more days as a grey line, this being made up of granules densely and closely placed ; viewed under a glass, minute club-shaped and pear-shaped projections are seen to extend from the dark line of inoculation. In stab-cultures, as in surface growths, the several colonies are a little more copious and less translucent when our ordinary Agar is used for the cultivations than when glycerine has been added.

The condensation water in the Agar tubes (of ordinary

as well as of glycerine Agar tubes set with slanting surface) show, in the course of one or two days, a copious floccular or granular whitish precipitate, the condensation water itself remaining limpid. The amount of this precipitate increases till about the fifth or seventh day, when it has reached its maximum.

Agar tubes inoculated with the influenza bacillus support life in the organism longer than broth tubes, particularly if the Agar tubes be inoculated by stab-culture. We have successfully carried on sub-cultures from Agar cultures through many generations, in fact we have some cases that have reached already the twentieth generation, and we see no reason why there should be any limit placed at all, provided each successive sub-culture be established within a week—after that time the result becomes uncertain.[1] But if the culture tube after five or six days' incubation at 37° C. be then kept at the ordinary temperature (capped and protected from drying) the life of the culture can be preserved for a much longer time; we have as a matter of fact found it living after two weeks; this would certainly not have been the case if any culture of the series had been kept at 37° C. for a fortnight.

3. *Culture on potato.*—No visible growth is to be obtained.

The vitality of the cultures is considerably prolonged if nutrient gelatine after inoculation is incubated at 37° C.; herein good growth occurs, and the growth remains alive for at least three to four weeks.

[1] We add here that under the above conditions we have carried on the sub-cultures on Agar from the sputum through more than thirty generations.

Microscopic Examination of the Cultures.

With the cultures above described cover-glass specimens may be made in the usual way, *i.e.* a thin film of the fluffy or floccular precipitate from the broth cultures, or of the precipitate from the Agar condensation fluid, is prepared by drying and staining; and this is found to exhibit the bacilli in long twisted chains and threads, aggregated so as to form dense networks and convolutions or frequently forming bundles (Fig. 100). Many of the threads are found to measure several millimetres in length, while some are broken up into shorter bits. The threads are formed by the individual bacilli placed end to end, the sheaths of the bacilli forming a continuous sheath for the thread; in the stained specimens each element is marked either as a minute rod or more commonly as a dumb-bell of granules, this appearance being due to the polar granules of the individual bacilli being very strongly marked: or, by staining this dried film in carbolmethyl-blue for about half to one hour, and then washing in water, drying, and mounting in balsam, the character of the bacilli in the threads may be very well seen.

In recent cultures the threads are either wholly or partially made up of bacilli which stain at the two poles; such elements as do not show this character appearing as uniform rods about 0·4 μ in thickness, 0·8 to 1·2 μ in length. Cultures several days old show many of the threads already degenerating; that is to say, shorter or longer portions being empty of protoplasm showing only the faintly stained sheath with here and there indistinct granules in it. But in all specimens made of however recent a culture there are threads, in which here and there a bacillus

is swollen up into a spherical or oval ball, many times thicker than the typical element; the number of these enlarged elements is greater in later than in recent cultures, and the largest of them often show a vacuole in their centre or at one side. From these facts it is probable that these enlarged elements are involution forms.

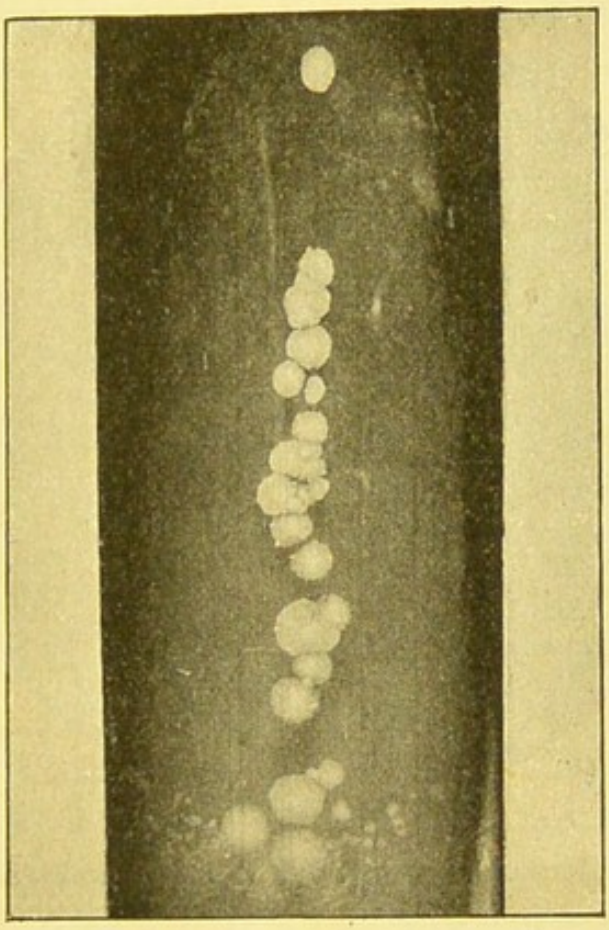

FIG. 99.—TRANSLUCENT COLONIES OF THE INFLUENZA BACILLI ON THE SURFACE OF AGAR.

Magnified twice.

Preparations made of the colonies grown on the surface of the Agar or glycerine Agar show the bacilli exactly of the same aspect and character as those grown in fluid media, namely as threads or else as large clumps; in these the bi-polarly-stained bacilli are very typical, and such clumps

resemble in every respect the clumps seen in the bronchial sputum.

By staining a cover-glass film of the young colonies first with rubin and afterwards with methyl-blue the sheath of the threads is well differentiated as of pink colour from the polar granules, or the rod-shaped protoplasm in the sheath.

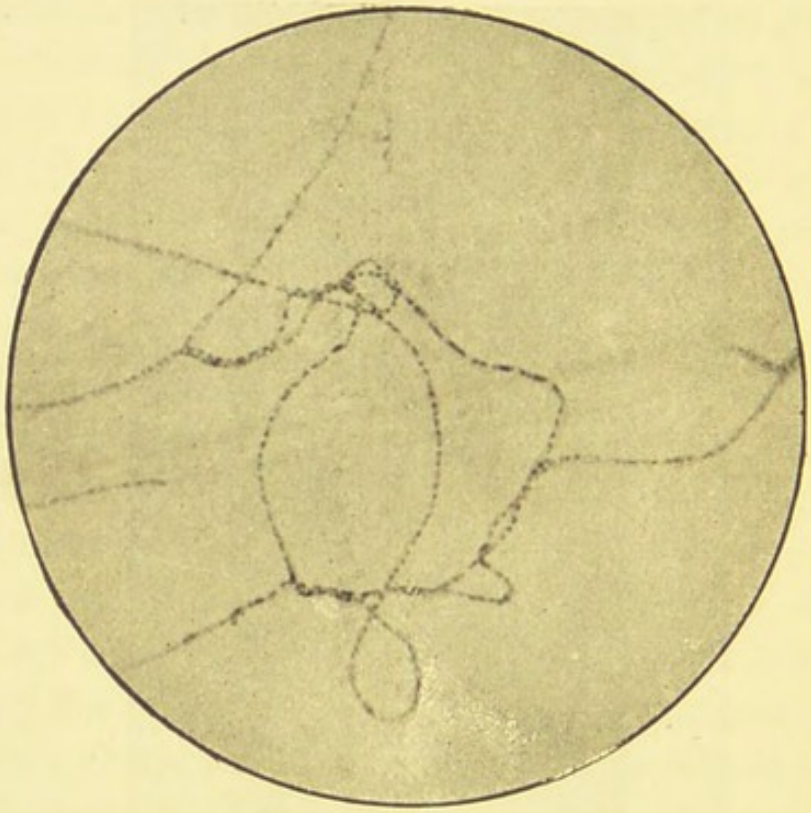

FIG. 100.—FILM SPECIMEN FROM A BROTH CULTURE OF INFLUENZA BACILLI. × 1000.

The same result is obtained from the growth in broth, but the most satisfactory specimens for microscopic observation were obtained from Agar cultures. We possess specimens from Agar cultures stained double as mentioned above, and the rod-shaped character of the elements of considerable portions of a thread is very strikingly marked by being stained blue in the pink general sheath.

On looking at the threads or clumps of any growth with

a moderately high power they are seen to resemble streptococci, but with an oil-immersion lens there is no difficulty in recognising the elements constituting the threads or clumps as really being bacilli, the protoplasm being either rod-shaped and stained uniformly, or else being segregated as a granule at each end and then receiving the stain at the two poles.

The description which we have here given of the character of the growth in the different media and of their microscopic aspect coincides in every essential with that given by Pfeiffer and Kitasato in their paper already quoted, except that in that paper sufficient prominence is not given to the thread-like nature of the growth; this, however, may be entirely owing to their communication having the character of a preliminary short account of their results.

The result of the examination of the blood of influenza cases was this :—

Of forty-three cases of blood examination, no bacterial forms could be discovered in thirty-seven. In the other six cases cover-glass specimens revealed the presence of one and the same kind of minute bacillus; in one the bacilli were numerous, in two they were fairly numerous, and in the other three they were very sparse.

In the thirty-seven cases the temperature in the majority was higher than normal, in a minority it was normal or subnormal. In some cases blood was taken at two different periods: during and after the fever; or both times during the period of raised temperature: or when the temperature had again fallen. But in these respects no definite relation as to the presence or absence of the bacilli could be made out.

It is then clear from these observations that neither during the febrile stage nor after the temperature has again

fallen do the bacilli occur in the blood with anything like constancy, considering that in thirty-seven out of forty-three cases no bacilli could be found, in each case at least four cover-glass specimens, in some six and even eight, being made, stained by the appropriate methods, that is to say by methods by which they are readily shown in the positive cases. But also the extremely varying number in which the bacilli occurred in the six positive cases indicates that their presence in the blood cannot be of the same essential value for the disease as is the case in the typical acute infectious diseases—"blood-diseases," of which the various known septicæmias, anthrax, and fowl cholera are types. This view is strongly borne out by the consideration that in all six cases in which the bacilli were found in the blood in the cover-glass specimens they could not be demonstrated in culture, the media used for these cultures being (as will presently be shown) perfectly suitable for the living bacilli of the bronchial sputum. This would appear to indicate that the bacilli found in the six affirmative cases were not living, and that any bacilli of influenza that may gain access to the circulation lose here their vitality and are present in the blood only as dead bacilli. Canon (*Deutsche Med. Wochenschr.*, No. 2, 1892) states that he found in all cover glass films of blood of influenza a particular kind of bacillus present in numbers varying from five to twenty, the bacilli and the nuclei of the white cells being stained blue, the blood discs and the body of the leucocytes pink. Now, our observations do not bear out this statement of Canon, since by the same methods as he used we stained the specimens for even a longer time than he did we failed to find bacilli in thirty-seven out of forty three cases, and we therefore, in opposition to him, do not consider the presence of these bacilli as of

pathognomonic value, or their absence as of diagnostic importance.

The same conclusion is arrived at by Pfuhl (*Centralbl. f. Bakt. und Parasit.* xi, No. 13) and by Pfeiffer and Beck (*Deutsche Med. Woch.*, May 26th, 1892).

A large number of experiments were made on rabbits and monkeys by using either bronchial sputum of influenza cases containing an abundance of the Pfeiffer influenza bacilli —the majority of the experiments—or of cultures of these bacilli, and by introducing such materials under the skin or into the trachea, or by direct injection into the vein (rabbits), but it has not been practicable to arrive at any definite production of influenza disease in monkeys or in rabbits. Only in one monkey out of eighteen was a definite disease of the lungs produced by such injection, and there (but in company with other bacilli) clumps of influenza bacilli were found; while among thirty rabbits injected with like materials there was no single instance of a disease recognisable as influenza in nature having resulted from the experiment.

Now the question has repeatedly been raised, and indeed has been repeatedly answered in the affirmative, viz., whether the disease of influenza, such as prevailed in this country, on the Continent of Europe, and in most other parts of the world in 1889–1890 and in 1891–1892, is a disease to which also the domestic and other animals are subject. It has been particularly asserted that in this country influenza was common amongst horses antecedently to and during the prevalence of influenza in man.

Though we have not made intentional experiments upon horses or other animals beyond those mentioned in these pages, we have not the less been on the watch during the time that we carried on our inquiry (February to April, 1892)

for indications of any influenza-like disease affecting the lower animals. We could not get evidence of horses being affected with any complaint identical with influenza in man, nor, as regards other animals which live amongst human habitations, are we aware of any evidence proving that amongst them influenza or any similar disease was rife during the periods of the influenza epidemic. Under these circumstances we have made inquiries at the Zoological Gardens in London, and Mr. Beddard has kindly given us the facts as to the condition of illness and deaths amongst the mammals kept there. From his record we learn that the incidence of disease and death at the Zoological Gardens was not unusually heavy during the years of the influenza epidemic in the metropolis. As regards the monkeys in particular, kept at the Zoological Gardens, we also understand from Mr. Beddard that no increased sickness was observed amongst them during these periods. The fact conforms with the results from our experimental observations on monkeys above recorded. It can hardly be supposed that if monkeys were, as a class, susceptible to the infection of human influenza the creatures living in the monkey-house in Regent's Park, frequented by many thousands of people a month while influenza was abundant in the London population, would have kept free from the complaint. And from the general experience of the Gardens of the Zoological Society it would appear that few mammalia share with the human subject a susceptibility to epidemic influenza. At all events, few of them are liable to receive the infection by the method which habitually obtains in man, through the respiratory passages.

CHAPTER XIII

THE MICROBES OF MALIGNANT ANTHRAX, OF DIPHTHERIA, AND OF GLANDERS

Bacillus anthracis.—Pollender,[1] Brauell,[2] Davaine,[3] and then Bollinger[4] recognised in the blood of animals dead of malignant anthrax the presence of stiff short and long rods, which Davaine called *bactéridie du charbon.* They were identified by Cohn[5] as bacilli in morphological respects similar to bacillus subtilis, except that the bacilli anthracis are non-motile.

Koch[6] showed the ubiquitous distribution of these bacilli in the blood of the organs, and especially of the spleen. He succeeded in cultivating the bacilli artificially, by placing a bit of such a spleen in a drop of aqueous humour, and watching the growth of the bacilli under the microscope. In this manner he ascertained that the rods multiply by division, and that they grow into long, homogeneous-looking, straight or twisted filaments in which after some time, and

[1] *Viertelj. f. Gericht. Med.*, 1855.
[2] *Virchow's Archiv*, vol. xiv. 1858.
[3] *Comptes Rendus*, lvii. 1863.
[4] *Med. Centralblatt*, June, 1872.
[5] *Beitr. z. Biol. d. Pflanzen*, vol. ii.
[6] *Ibid.*, vol. ii.

with free access of air, bright oval spores make their appearance, while the filaments become homogeneous and swollen. These spores become free, and when artificially cultivated or injected into a rodent animal germinate into the characteristic bacilli; these elongate and divide, and in artificial

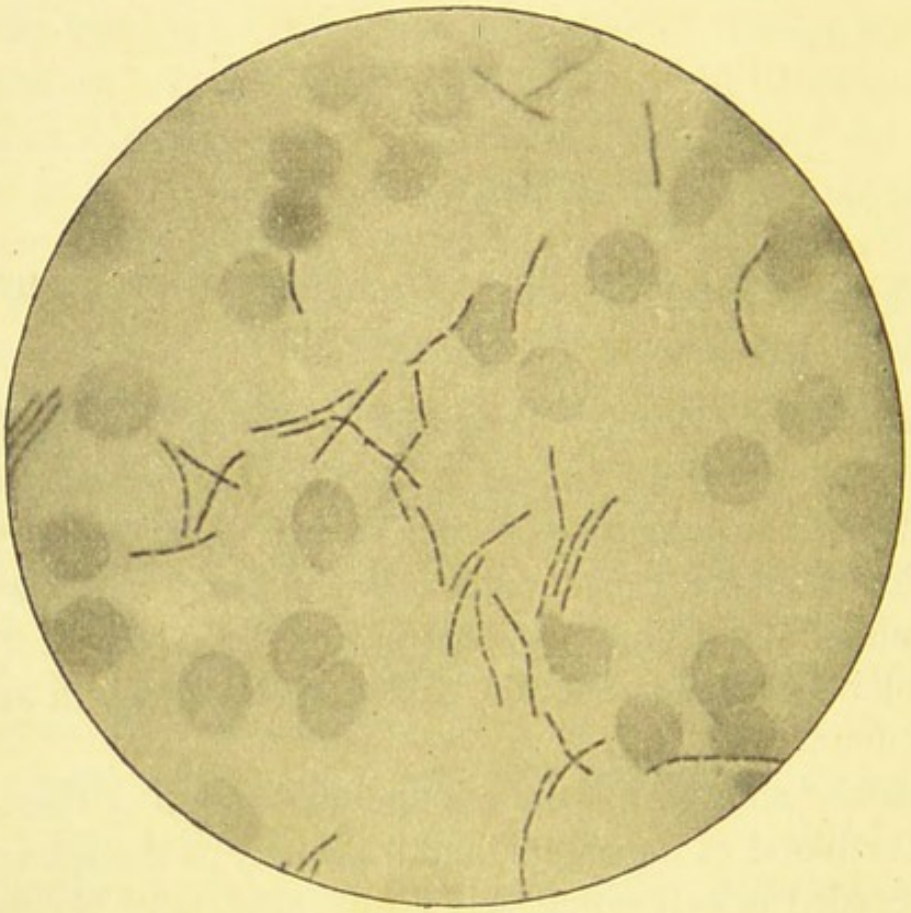

FIG. 101.—FILM SPECIMEN OF BLOOD OF GUINEA-PIG DEAD OF MALIGNANT ANTHRAX, SHOWING BLOOD DISCS AND BACILLUS ANTHRACIS IN CHAINS.

× about 700.

cultures again grow into the long leptothrix filaments, which again form spores. Koch [1] saw in preparations of aqueous humour kept at 35° C. in the incubator the spores germinating after three to four hours. The single bacilli as they present themselves in the blood measure between 0·005 and

[1] *Beitr z. Biol. d. Pflanzen*, vol. ii. part ii. p. 288.

0·02 mm. in length, and 0·001 to 0·0012 in thickness; they are truncated.[1] The spores produced by growing the bacilli with free access of air are about 0·001 mm. thick, and about 0·002 to 0·003 mm. long. They are not stained by the ordinary dyes and differ herein from the bacilli.

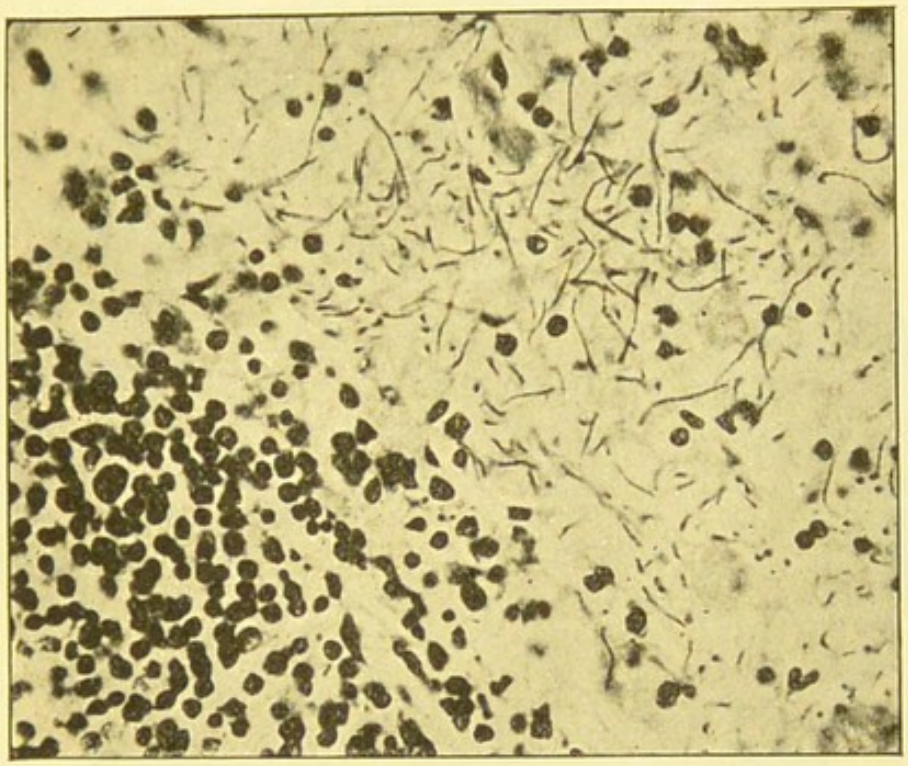

FIG. 102.—FROM A SECTION THROUGH THE SPLEEN OF A GUINEA-PIG DEAD OF MALIGNANT ANTHRAX, SHOWING NUMEROUS BACILLI ANTHRACIS IN THE SPLEEN PULP.

× about 700.

In the human subject malignant anthrax occurs as "woolsorter's disease"; for the ætiology and pathology of this malady see Spears (*Reports of the Medical Officer of the Local Government Board*, 1881 and 1882) and Greenfield (*ibid.* 1881). It occurs also in sorters of hides and rags.

All rodents and herbivorous animals are susceptible to anthrax;

[1] It is generally assumed that the bacilli are the same in all animals affected with splenic fever, but this is most undoubtedly not the case, as has been already pointed out by Huber (*Deutsche Med. Woch.* 1881); the bacilli of the guinea-pig are thicker than those of the mouse or sheep, and these again are thicker than those of the rabbit.

adult rats are, however, infected with difficulty, pigs are not very susceptible, and dogs and cats are very insusceptible. Infection of animals can be produced by inoculation into the skin and subcutaneous tissue, intraperitoneal or intravascular injections, and by inhalation and ingestion of spores. In woolsorter's disease the usual mode of infection is by inhalation of spores adhering to the wool of the fleeces of animals (sheep, goats) dead of anthrax. As in rodents infected with anthrax,

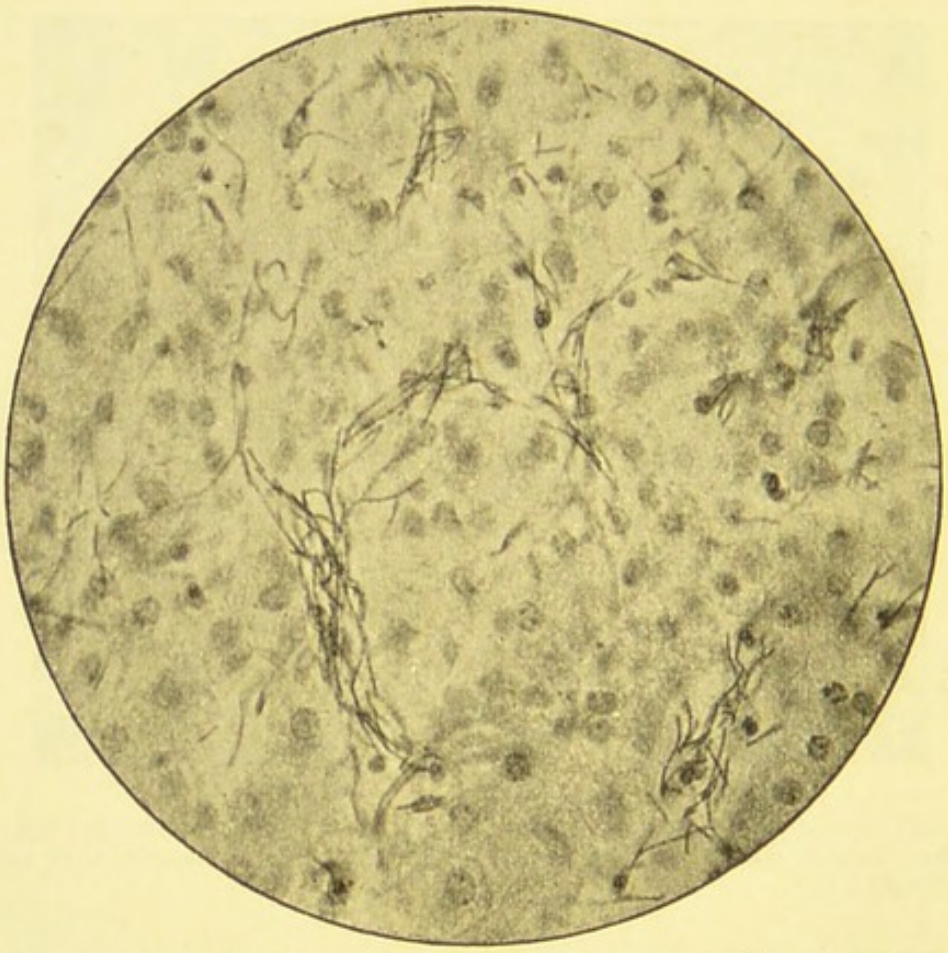

FIG. 103.—FROM A SECTION THROUGH THE LIVER OF A GUINEA-PIG DEAD OF MALIGNANT ANTHRAX. THE CAPILLARY BLOOD-VESSELS CONTAIN CHAINS OF BACILLI ANTHRACIS.

× about 700.

so also in man, the blood-vessels of all organs contain the bacilli, and extravasations of the infected blood are frequent in many parts of the body. The presence of bacilli in the extravasations into the mucous membrane of the trachea and bronchi does not necessarily mean that these parts represent the points of entrance of the bacilli into the system, as Greenfield seems to regard as self-evident (*Reports of the Medical Officer of the Local Government Board*, 1881). As a matter of fact I find in every lung of mouse, rabbit, and guinea-pig, dead after

subcutaneous inoculation with anthrax, bacilli anthracis in the alveolar cavities and in the smaller and larger bronchi. Ingestion of bacillar material is sometimes followed by anthrax, but in these cases abrasions in the mucous membrane of the mouth, pharynx, or gut may have been the real place of entrance. Mice fed with fresh anthrax material do not become infected (Klein, *ibid.* 1881). But the reported cases of intestinal mycosis (see, for the literature of this subject, Koch,

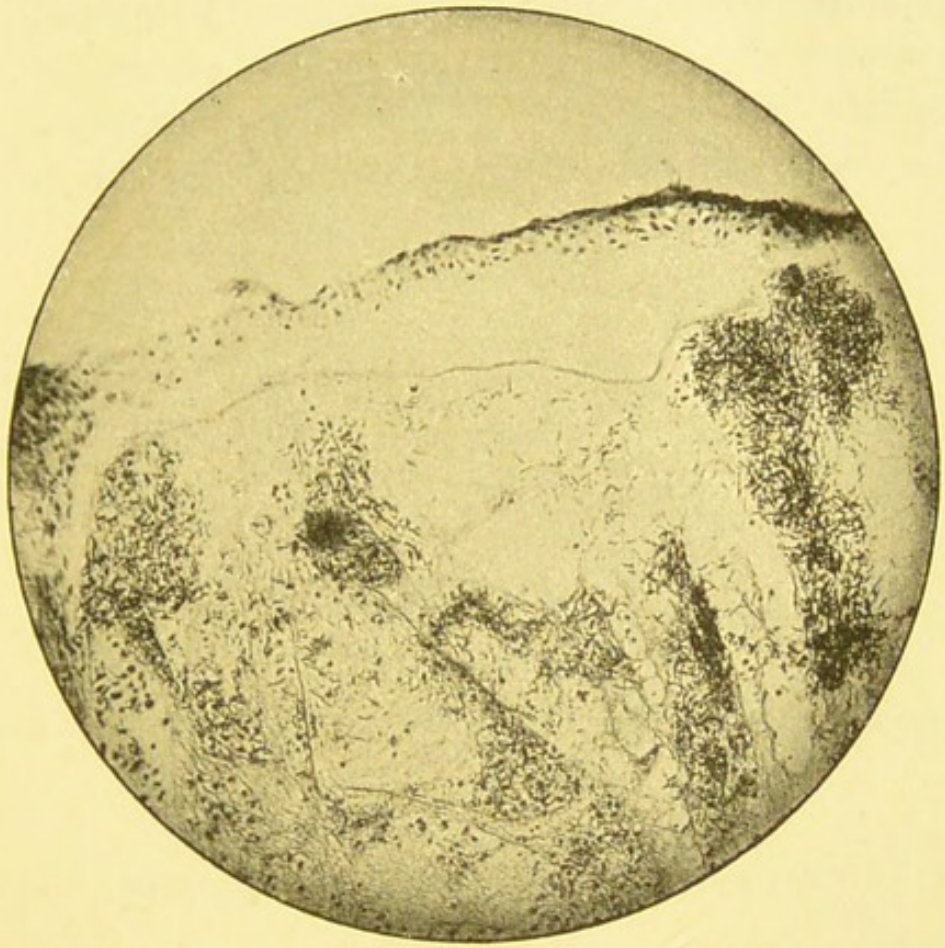

FIG. 104.—SECTION THROUGH THE PUSTULE OF MALIGNANT CARBUNCLE IN MAN. THE BLOOD-VESSELS OF THE SKIN ARE FILLED WITH BACILLI ANTHRACIS.

Low magnification.

"Ætiologie d. Milzbrandes," *Mittheil. a. d. k. Gesundheitsamte*, 1881) indicate that infection with spores by the alimentary canal is not excluded. Compare also Falk, *Virchow's Archiv*, vol. xciii. From the observations by Koch and Gaffky it has become clear that infection of sheep by the alimentary canal can be produced with spores.

Normal frogs are insusceptible to anthrax.

Frogs and adult rats are however susceptible if they are subjected to chloroform narcosis and the injection is made

during or shortly before or after narcosis (Klein and Coxwell). Petruschki has shown that by keeping frogs at the temperature of the warm-blooded animal it becomes susceptible to anthrax. Normal fowls are insusceptible, but Pasteur showed that by lowering their temperature they become susceptible.

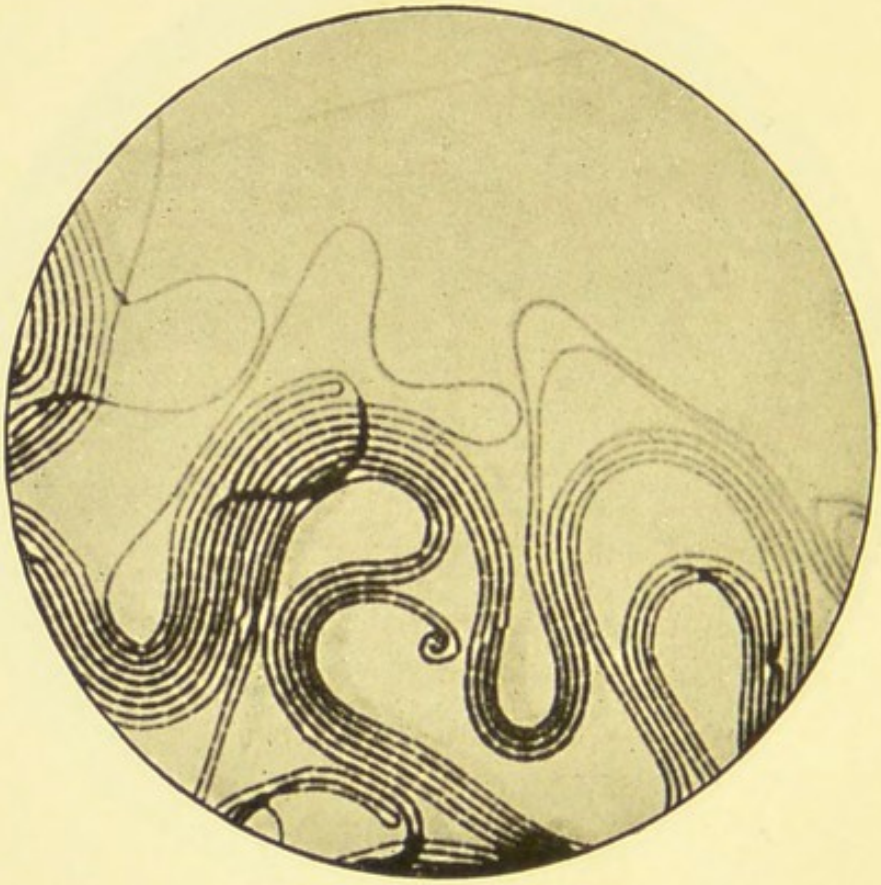

FIG. 105.—IMPRESSION SPECIMEN OF THE EDGE OF A YOUNG COLONY OF BACILLUS ANTHRACIS ON GELATINE. THREADS OF BACILLI MADE UP OF CYLINDRICAL BACILLI.

Magnified about 700.

Besides general infection of human beings by spores of anthrax (woolsorters, hidesorters, and ragsorters) they are able to contract severe local carbuncle by inoculation (through a cutaneous abrasion or wound) with anthrax blood of an animal (sheep, cattle, or horses).

Rodents inoculated with the bacillus of the blood or

spleen of an animal dead of anthrax, or with the bacillus or spores of an artificial culture, die generally within forty-eight hours; in some instances in twenty-four to thirty hours, in other instances after forty-eight to sixty hours. The blood in all instances contains the bacilli, the spleen is large and full of bacilli, and so are the blood-vessels of most other organs, the exudations, and the urine. In the placenta of a pregnant guinea-pig dead in consequence of inoculated anthrax, I have seen that the bacilli kept strictly as a rule

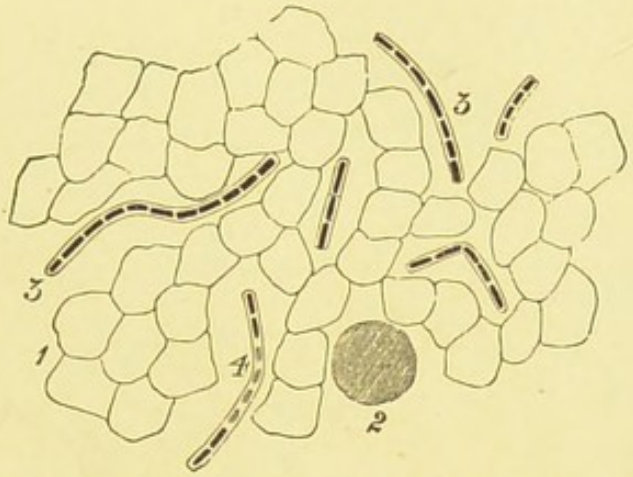

FIG. 106.—FROM A PREPARATION OF HEART'S BLOOD OF A GUINEA-PIG DEAD OF ANTHRAX.

1. Red blood discs.
2. White corpuscle.
3. Bacilli anthracis, showing well their sheath.

Magnifying power 700. (Stained with Spiller's purple.)

within the maternal blood-vessels, and are wholly absent in the blood of the vessels of the fœtus. Subcutaneous inoculation or injection into the cutis of minute quantities of bacillus-containing material (blood or virulent culture) invariably produces death. Subcutaneous injection of bacillus-containing material in the guinea-pig almost always produces a characteristic œdema, spreading sometimes over a large area. The œdematous fluid is clear and contains only a few bacilli.

Any neutral or faintly alkaline material containing pro-

teids is a suitable nutrient medium for the bacilli; they grow abundantly at all temperatures between 15° and 43° C., best between 25° and 40° C. They elongate and divide rapidly, and the bacilli grow out into long curved and peculiarly twisted filaments which often form bundles, the

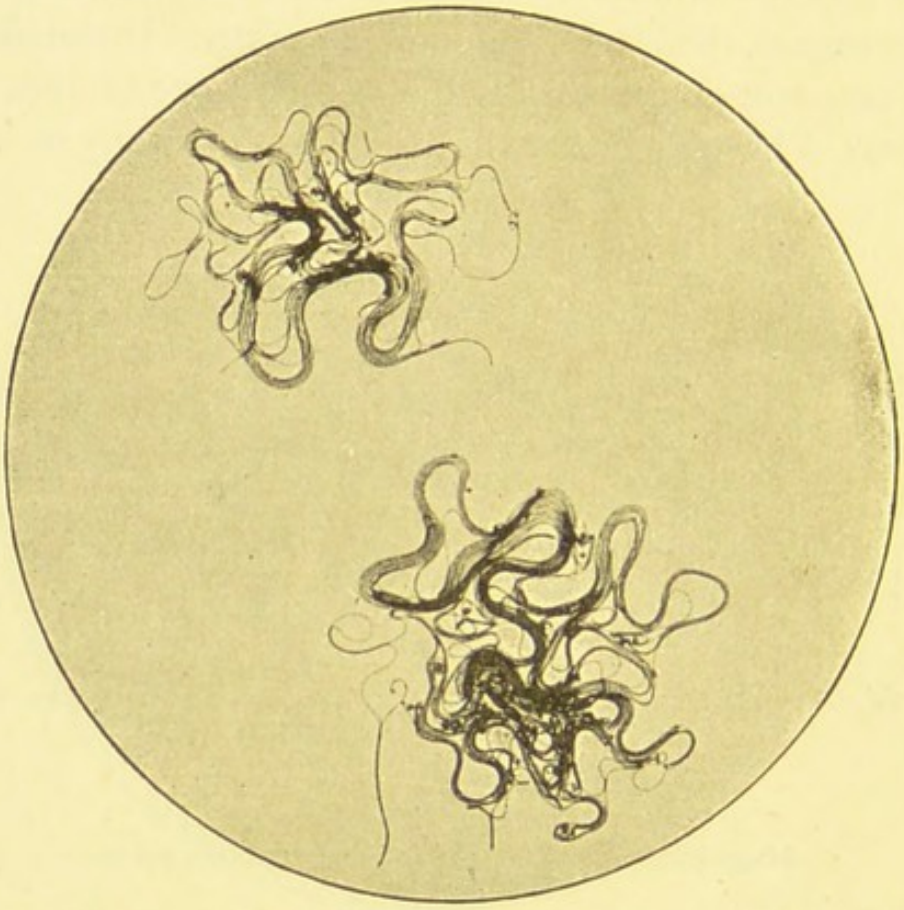

FIG. 107.—IMPRESSION SPECIMENS OF YOUNG COLONIES OF BACILLUS ANTHRACIS ON GELATINE.
Low magnification.

individual filaments being twisted round one another like the strands of a cable.

The bacillus anthracis offers some very characteristic features in cultivations. In gelatine plate cultivations made of the blood (previously well diluted with neutral salt solution or broth, on account of the large number of bacilli present in the blood) already after twenty-four to

thirty-six hours the first signs of colonies can be made out in the form of translucent, grey, angular, dots; after forty-eight hours to three days they are conspicuous by their size, and by their margin being distinctly made up, to the naked eye, of filaments, either straight or bending like loops. Under the microscope the filamentous nature of the

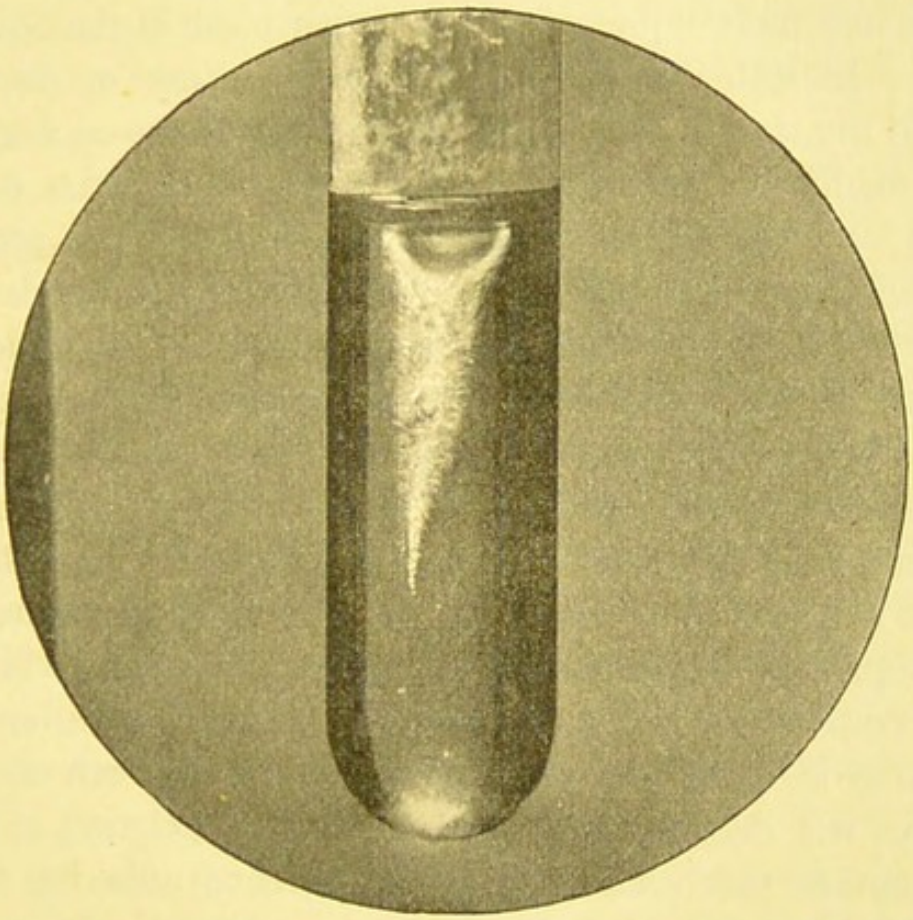

FIG. 108.—STAB CULTURE IN GELATINE OF BACILLUS ANTHRACIS. LIQUEFACTION ON THE SURFACE HAS ALREADY COMMENCED.

colonies is distinctly seen; the filaments looked at under a magnifying glass are more or less in bundles twisted like cables, and extending sometimes like radii from a centre; at the margin this is particularly conspicuous. At the same time the colony is seen to be sunk in the middle, being situated in a slight depression of the

gelatine due to commencing liquefaction. Looked at obliquely, the gelatine looks pitted by the colonies. As growth proceeds the colony enlarges; the marginal loops and bundles of twisted filaments project more or less irregularly; some project for longer, others for shorter distances, sometimes not much beyond the margin of the colony, and the gelatine surrounding the colony becomes more and more liquefied, but remains clear in the liquefied part. In stab cultures made from a culture or from the blood the stab is noticeable after a day or two as a whitish line made up of closely placed dots; in another day or two, from each dot a lot of fine whitish filaments are seen extending, often like rays from a centre. When the dots are closely placed in linear series the white filaments projecting mostly in horizontal direction from them give to the stab a characteristic appearance, like the vane of a grey feather, the stab being the middle rib; liquefaction has by this time set in on the surface, *i.e.* on the upper end of the stab, and there is here a more compact plate-like mass of filaments; the liquefaction gradually proceeds into the depth while the surface patch of the growth increases in bulk; the liquefied gelatine is clear, and the original surface growth occupies always the deepest part of the liquefied gelatine. When the surface patch while spreading remains adhering to the glass wall of the test-tube, spore formation is observed in the threads of the bacilli, but when the growth is in the depth of the liquefied gelatine no spore formation ever takes place. After ten to fourteen days at 19–20° C. the upper half of the gelatine in the tube is quite liquefied, the liquefied gelatine is clear, and the whole growth is at the bottom of the liquefied part in the form of whitish-grey fluffy masses; when shaken the mass breaks up into whitish nebulous flocculi.

In streak culture on gelatine the streak of inoculation is marked after twenty-four to forty-eight hours as a whitish-grey line; then a number of whitish fine threads shoot out horizontally from this line, liquefaction at the same time commencing and proceeding slowly and gradually; the line thickens and broadens, and after a week is made up of masses of threads twisted and convoluted, and forming a thick, white, filmy patch, which as liquefaction proceeds sinks to the bottom of the liquefied gelatine, forming here a whitish grey fluffy mass.

In neutral or faintly alkaline broth kept at 36–38° C. there is, if the broth be thin, uniform slight turbidity after thirty-six to forty-eight hours: flakes small and large then appear at the bottom of the fluid, while this latter remains fairly clear. As growth proceeds, about the end of the week, there are contained at the bottom of the fluid characteristic greyish, fluffy, loose, nebulous masses, which are masses of anthrax threads matted together; these masses increase in bulk and extend as it were from the bottom of the fluid towards the upper parts. If during the first few days some of the flakes remain adhering to the glass at the surface of the fluid, these flakes enlarge and form on the glass, on a level with the surface of the fluid, a sort of whitish ring, somewhat like a pellicle; in this copious spore formation takes place; but in the tubes, in which all the growth is limited to the deeper parts of the fluid, no spore formation occurs at any time, since for the formation of spores a free and copious supply of oxygen is required.

On Agar mixture at 36–38° C. a greyish, thick film is noticed after two days along and beyond the line of inoculation. This rapidly increases in breadth till the whole surface of the Agar is covered with a sticky, pasty, greyish

layer ; this after some days shows some patches thicker than others, is light brown, and in some patches even dark brown.

On potato at 35–37° C. a thick cohesive layer like paste is formed ; this is of a brownish colour ; the growth is ex-

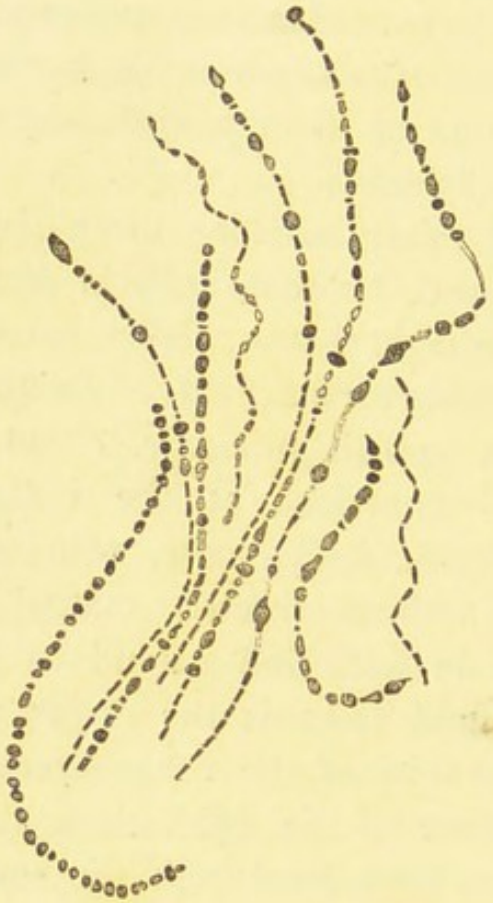

FIG. 109.—FROM AN ARTIFICIAL CULTURE OF BACILLUS ANTHRACIS, CARRIED ON AT ORDINARY TEMPERATURE AND ON SOLID (GELATINE) MATERIAL. TORULA FORM.

Magnifying power 450. (Stained with Spiller's purple.)

tensive after a few days. Both on nutrient Agar and on potato the film is a mass of threads matted together, and after two to three days copious spore formation is noticed in many threads ; at the end of ten days to a fortnight the whole of the film is a mass of spores ; little of the original bacilli is recognisable

Bacilli anthracis when growing at ordinary temperatures on a solid medium (*e.g.* a mixture of gelatine and broth, or Agar-Agar and peptone) show a very peculiar modification, inasmuch as some of the elements assume a spherical, oval, or spindle shape, a torula-form, and as such they multiply by division and form clusters or arrange themselves in chains. By-and-bye each of these spherical elements

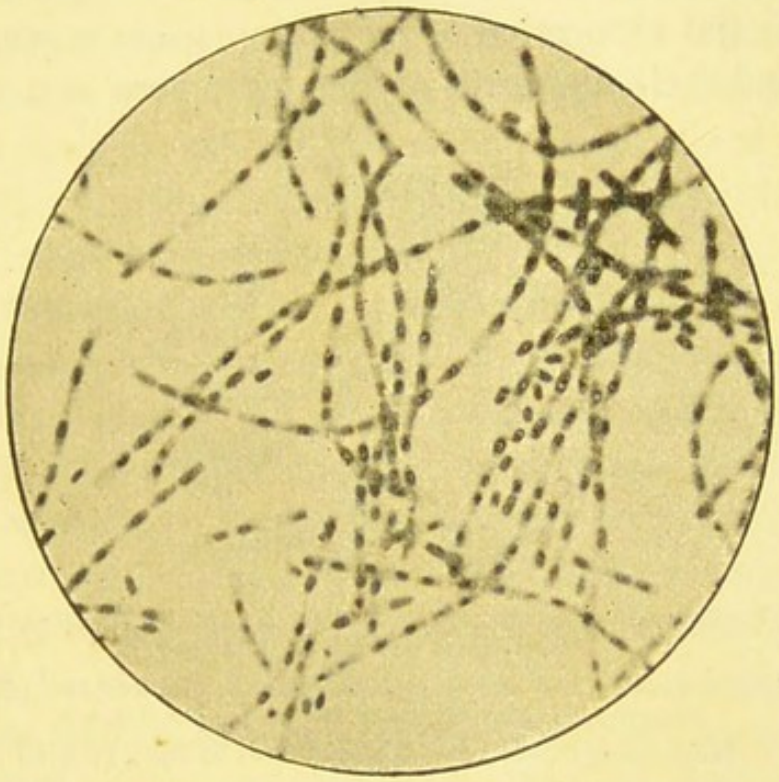

FIG. 110.—SPORES FORMING IN THREADS OF ANTHRAX.
× 700.

elongates into a rod, and when all elements have undergone this change we have the typical smooth filament of the leptothrix form. Some of the elements in such a filament remain for a long time of a spherical shape, and are much larger, looking like the sporangium of a nostoc-alga. The most interesting forms are those where an ordinary smooth filament of anthrax-bacillus at its growing ends shows itself to be composed of a chain of torula

elements. Such torula forms occur also in ordinary cultivations in fluid media at temperatures of 20° to 30° C., but not by any means so often as at ordinary temperatures and in a solid medium. Compare also the chapter on General Characters of Bacilli.

After a few days' incubation, no matter what the temperature is, many of the bacilli and their leptothrix-filaments show signs of degeneration, consisting in the granular disintegration and absorption of the protoplasmic contents of the bacilli and their filaments, at first only here and there, but

FIG 111.—FROM AN ARTIFICIAL CULTURE OF BACILLUS ANTHRACIS IN BROTH AFTER MANY DAYS' INCUBATION.

The threads are swollen and curled up, and in many places the protoplasm has disappeared, leaving the sheath and septa distinct.

Magnifying power 700. (Stained with Spiller's purple.)

by-and-bye over longer pieces. Such bacilli and leptothrix-filaments appear in such places as if empty. This is also noticed in the bacilli of the blood and spleen of an animal inoculated with anthrax, even at the point of death or soon after death, if the number of bacilli is great.

Another form of degeneration consists in the filaments of bacilli becoming much curled and swollen, and finally disintegrated into an amorphous débris.

As long as the bacilli grow in the depth of a fluid they never form spores, but when grown on the surface with free

access of air, or on solid media (*e.g.* serum gelatine, gelatine broth, Agar-Agar, potato, &c.), the bacilli, having developed into filaments, proceed to form spores. But they may form spores even in fluid media if by some accident, either by sticking to the glass vessel containing the fluid or by means of a cotton-wool fibre, some of the bacilli remain on the surface of the fluid. This formation of spores is not due to exhaustion of the nourishing medium, as has been already discussed on a former page, but represents the last stage in the life-history of the bacilli, provided they have an ample supply of oxygen. If this latter condition is not fulfilled, as when they are grown at the bottom of a fluid, the bacilli gradually degenerate as mentioned above.

Spore-formation occurs, *cæteris paribus*, at all temperatures between 18° and 45° C. Koch found 15° C. the lower limit. Under the most favourable conditions, each cubical or rod-shaped mass of protoplasm includes one spore, in which case the bacillar filament contains an almost unbroken row of spores; but in other cases only an elementary mass here and there contains a spore, the rest breaking down and becoming absorbed. In the first case, also, the protoplasm of the elements almost entirely disappears, the sheath swelling up and becoming hyaline, and only the bright spores remaining. Their linear arrangement, however, still indicates that they were formerly contained in one filament.

If bacilli grow in the depth of a fluid medium, they do not form spores, as has been stated above; and, as we have also seen, as new bacilli appear, or the old filaments increase in length, degeneration sets in. This degeneration gradually affects greater and greater numbers, and when the fluid is exhausted for the formation of new bacilli it necessarily follows that the whole growth gradually becomes involved in

the process of degeneration, the whole mass becoming smaller, and finally only débris is left. Such cultures, namely those in which the degeneration involves the whole mass of the bacilli, are quite innocuous when inoculated into animals or into fresh nourishing media. But as long as

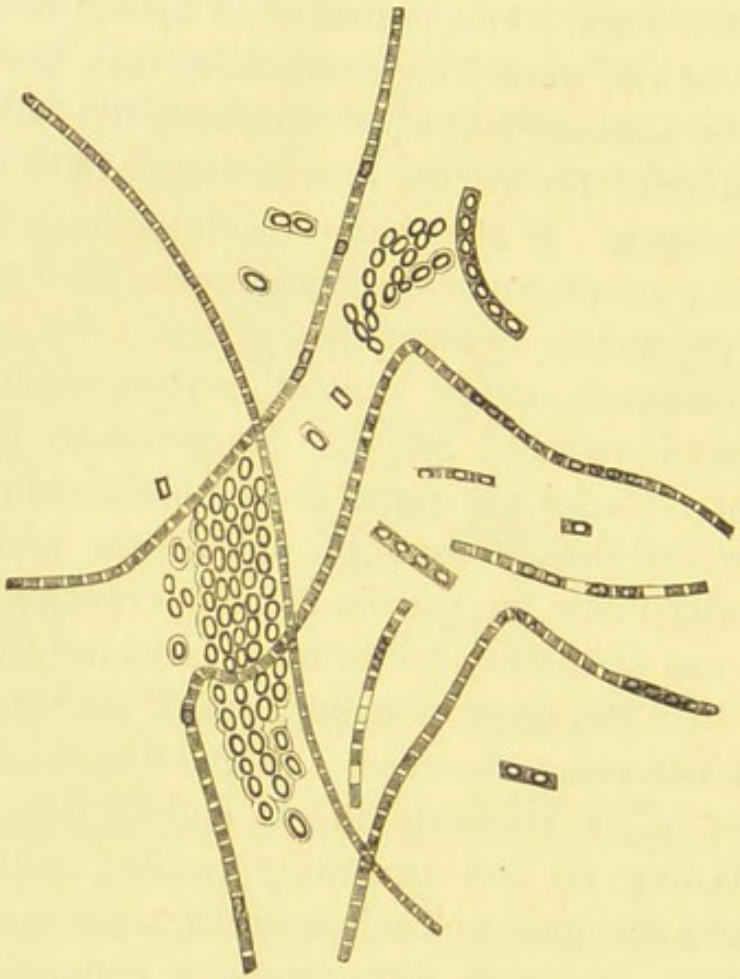

FIG. 112.—FROM AN ARTIFICIAL CULTURE IN NEUTRAL PORK-BROTH OF BACILLUS ANTHRACIS, WITH COPIOUS FORMATION OF SPORES.

Magnifying power 700. (Stained with Spiller's purple.)

there are any good protoplasmic elements of the bacilli left the culture is virulent to rodents, with the exception of mice, as will be referred to presently; and it is capable, when transferred to new suitable nourishing media, of starting new cultures that prove virulent to all rodents and sheep.

The same holds good of the bacilli in the blood and organs of an animal dead of anthrax, provided the animal be not opened, and its organs, exudations, or urine be not exposed to the free air; for the bacilli not exposed to the air gradually degenerate, and the blood and organs of such an animal, although at first deadly poison to other susceptible animals, become at length quite innocuous. Systematic observation has shown me that small animals, such as mice and guinea-pigs, when kept unopened or buried in earth, become quite innocuous after five to eight days, the anthrax-bacilli having by this time, by degeneration, altogether disappeared from the blood, spleen, and other organs. Pasteur's statement that in animals dead of anthrax and buried the bacilli form spores, and that these spores are taken up by earthworms and carried to the surface of the soil, where they are deposited with their castings and thus are capable of infecting animals grazing or sojourning on this soil, is not borne out by the above observations. And, further, Koch has proved [1] by direct experiment that spores of anthrax-bacilli when mixed with earth in which worms are present are not taken up by these creatures.

Drying bacilli of the blood or of a culture in a thin layer invariably kills them, but the spores remain unaffected.

The bacilli of the blood of a rodent dead of anthrax are always thinner than the bacilli cultivated in a neutral fluid medium.

Cultivation of the blood-bacilli at temperatures varying between 20° and 40° C. in any suitable nourishing material, solid or fluid, however many transferences (new cultivations or so-called new generations) be made, always yields a crop of virulent bacilli. It is quite incorrect to say, as Buchner [2]

[1] *Mittheil. a. d. k. Gesundheitsamte*, 1881.
[2] *Ueber d. Erzeug. des Milzbrandes*, Munich, 1880.

and Greenfield [1] maintain, that continued transference weakens and ultimately destroys the action of the bacilli; as long as the cultures remain pure, not contaminated and finally suppressed by accidental innocuous bacilli, the anthrax-bacilli retain their virulence.

Cultures of the blood-bacilli at 20° to 38° C. in neutral broth, during the first or second week, are virulent to mice, guinea-pigs, and rabbits; but after that they lose their power on mice, provided the growth takes place only in the depth and no spores are formed; but they retain it as regards guinea-pigs and rabbits, as long as they contain good bacilli at all.[2] But fresh cultures made of such bacilli invariably produce a growth which is fatal to all rodents during the first or second week.

The first observations that bacillus anthracis can become attenuated in its action without losing its morphological and biological characters were recorded by Toussaint, who found that heating anthrax blood up to 55° C. for a few minutes incapacitates such blood from producing anthrax on inoculation. Chauveau then found that the same attenuation and destruction of virulence occur when the virulent bacillus anthracis, *e.g.* the blood, is subjected to the action of 5 per cent. carbolic acid for a few minutes. Pasteur was the first who showed that when bacillus anthracis is cultivated in broth at high temperature (42·5° C.) it gradually loses its full virulence, and when such cultures are inoculated into sheep and cattle a mild and transitory form of anthrax is produced; animals so treated withstand successfully the further inoculation of virulent materials, and are therefore protected by the inoculation with the attenuated cultures.

[1] *Proceedings of the Royal Society*, June 17, 1880.

[2] Klein, *Reports of the Medical Officer of the Local Government Board*, 1881.

Pasteur has shown by a large number of experiments carried out in France and elsewhere that, by inoculation of such attenuated cultures, protective inoculation can be effected on sheep and cattle. He used two kinds of culture,

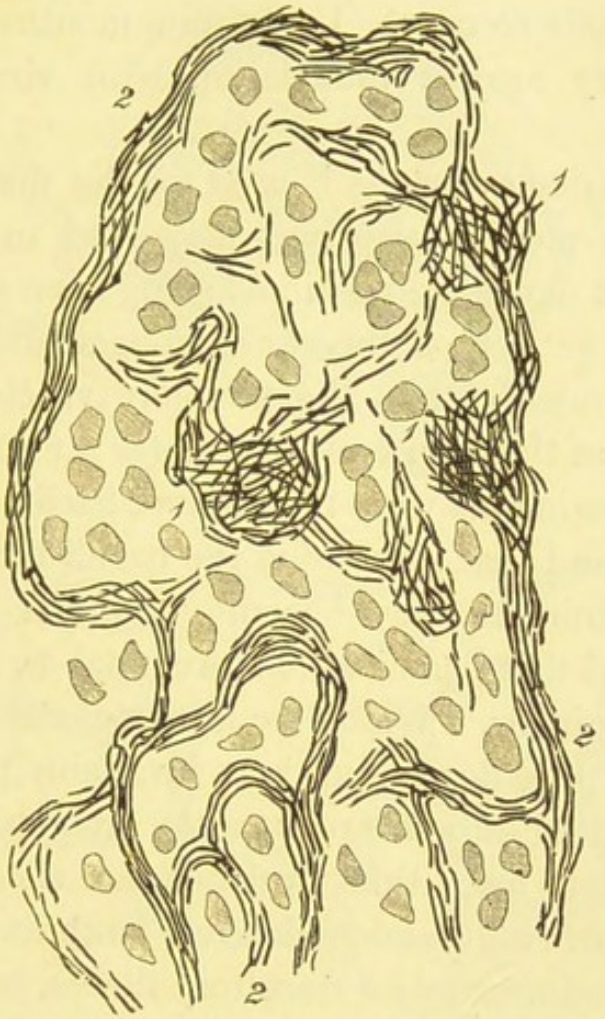

FIG. 113.—NETWORK OF CAPILLARIES FILLED WITH BACILLUS ANTHRACIS; FROM THE OMENTUM OF A RABBIT DEAD OF ANTHRAX.

1. Extravasation of the bacilli.
2. Capillaries filled with the bacilli.

Magnifying power 350.

for protective inoculation: (*a*) première vaccine: this is a culture of anthrax bacillus in chicken broth kept at 42·5° C. for fourteen days; when inoculated into sheep or cattle it produces only a slight local tumour; after about twelve days

the animals are inoculated with (*b*) deuxième vaccine : this is chicken broth culture kept at 42·3° C. for a week only. This culture produces also a local effect with slight constitutional disturbance, more pronounced than after the inoculation of the première vaccine ; but the disturbance is only transitory and the animals recover. Up to nine months such animals are refractory against inoculation with virulent anthrax blood.

If the deuxième vaccine is used for the first inoculation, the effect is more severe and may lead to fatal general anthrax ; this deuxième vaccine having been grown for one week only at 42·5° C. is therefore stronger, and is of a higher degree of virulence than the première vaccine, which had been grown at the high temperature for a fortnight.

In all experiments with the anthrax bacilli it is necessary to bear in mind that by passing the bacilli through different species of animals they become endowed with different qualities, and that bacilli which are fatal to some are not fatal to all animals. While, for instance, the blood-bacillus of sheep or cattle dead of anthrax invariably produces death when inoculated into sheep or cattle, after passing through white mice[1] it loses this virulence for sheep and cattle. The blood of white mice dead of anthrax does not kill sheep ; it produces only a transitory illness, and the animals are, for a time at least, protected against virulent anthrax. The blood of guinea-pigs dead of anthrax produces illness, sometimes death, in cattle, but as a rule does not kill (Sanderson and Duguid), and the blood of the biscachia of South America does not kill cattle, while it gives them a transitory illness, and after this immunity for a time.[2] Again

[1] Klein, *Reports of the Medical Officer of the Local Government Board*, 1882.

[2] Roy, *Nature*, December, 1883.

Pasteur's "vaccine," which does not kill sheep or cattle, is fatal to rodents.[1] From all this it follows that as regards

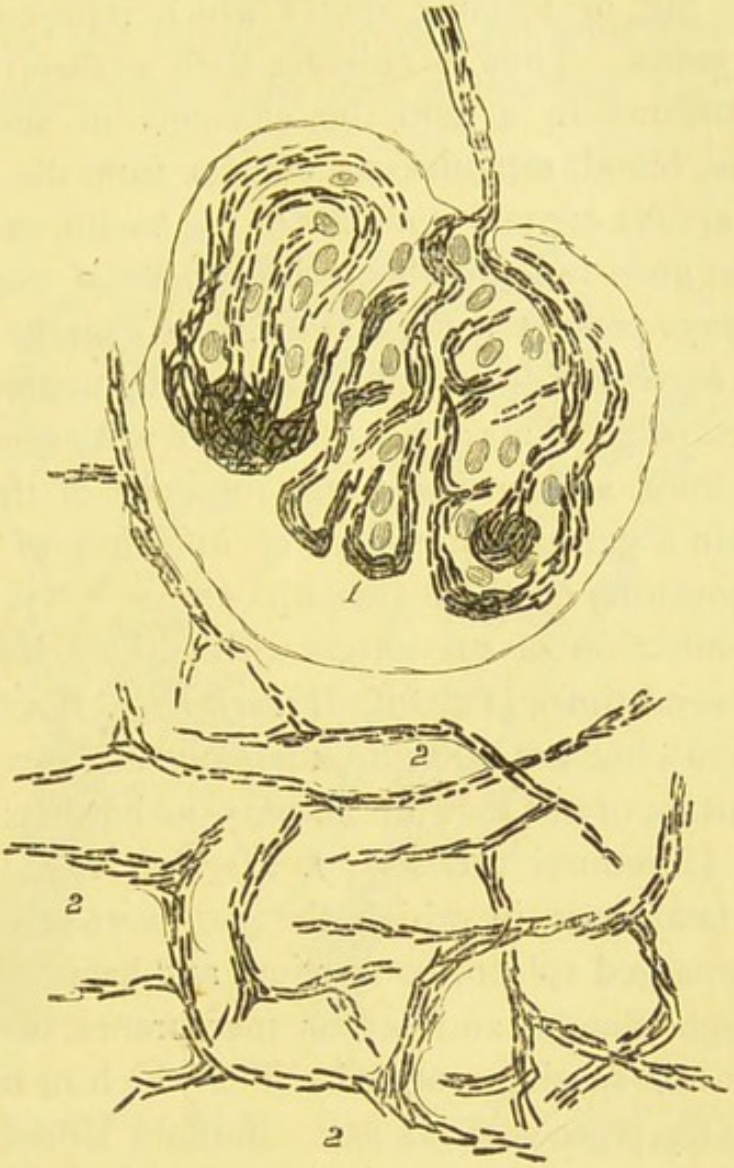

FIG. 114.—FROM A SECTION THROUGH THE KIDNEY OF A RABBIT DEAD OF ANTHRAX.

The capillaries of the cortex are naturally injected with the Bacillus anthracis.
1. A glomerulus.
2. Capillaries surrounding the convoluted uriniferous tubules not shown here.
Magnifying power 450. (Spiller's purple.)

virulence the bacilli anthracis differ in the different species of animals, and in them acquire different qualities.

[1] Klein, *Reports of the Medical Officer of the Local Government Board*, 1882. Similar results have been obtained by Gaffky (*Mittheil. a. d. k. Gesundheitsamte*, 1882).

Bacillus anthracis is capable, as we have seen, of growing well outside the body, and, when well supplied with oxygen from the air, of forming spores which represent the permanent seeds. Thus if animals, such as sheep and cattle, die of anthrax in a field the effusions of such animals (*e.g.* urine, blood, sanguineous effluvia from the mouth and nostrils) always contain numbers of the bacilli, and these will be able to grow indefinitely on the surface of the soil, there being always present a large amount of suitable nourishing material, as vegetable and animal decaying matter, and since free access of air is always ensured they will eventually form spores. Such soils, owing to the presence of these spores, will remain a permanent source of infection to sheep and cattle sojourning on them (Koch).

Acute infection of rag-sorters with anthrax has been observed several times (Paltauf, *Wiener Klin. Wochens.*, 1888, Nos. 18–26), but not all acute infectious diseases contracted by the sorters of old rags are anthrax, as has been shown by Bordoni Uffreduzzi (*Zeitschr. f. Hygiene, III.*, 2, p. 333). From a fatal case, in which the *post-mortem* examination showed enlarged spleen, congestion, and hæmorrhage of the lung, lymph glands, and serous membranes, this observer isolated a non-sporing motile bacillus which in many points resembles the proteus of Hauser. Bordoni Uffreduzzi calls it *proteus hominis capsulatus ;* it does not liquefy gelatine and acts virulently on dogs and mice, rabbits and guinea-pigs being less susceptible.

Bacillus of ulcerative stomatitis in the calf.—In the *Lancet* of May, 1883, A. Lingard and E. Batt described peculiar bacilli in ulcerations occurring on the tongue and buccal mucous membrane of the calf. "The typical ulcer in advanced cases consists of a sore with free overhanging edges. On section through the sore the tongue is found

necrosed to a considerable depth." "Whenever the sore touches any other part of the mouth or cheek, the disease is communicated and rapidly spreads. In some cases similar necrotic changes had taken place in the lung. The line of

FIG. 115.—FROM A SECTION THROUGH NECROSED AND ADJOINING INFLAMED PARTS OF THE EAR OF A RABBIT, INOCULATED WITH MATTER TAKEN FROM ULCERATIVE STOMATITIS OF THE CALF.

1. Necrosed part.
2. Inflamed tissue.
3. Bundles of bacilli.

Magnifying power 700. (Stained with magenta.)

junction of the necrotic with the healthy tissues was found to be occupied by a dense mass of bacilli having the appearance of a dense phalanx advancing upon the healthy tissues. The disease has been proved capable of transmission (to the rabbit and mouse) by injection of the bacilli in question,

which are equally numerous and virulent after passing through several generations by inoculation."

The disease often ends fatally in calves.

The best method of staining the bacilli was found to be this: The sections, both those prepared from the ulcerations

FIG. 116.—FROM A SECTION THROUGH TONGUE OF CALF, ULCERATIVE STOMATITIS.

1. Muscular fibres.
2. Inflamed tissue.
3. Bundles of the bacilli.

Magnifying power 700. (Stained with magenta.)

of the calf's tongue and from the inoculated tissues of the rabbit, are immersed in a mixture of magenta and methyl-blue, then washed in spirit, and after clarifying in clove-oil are mounted in Canada-balsam solution. The bacilli are stained deep pink, the inflamed tissue blue. The bacilli

appear as thin rods in rows, thus forming a leptothrix-like growth. In some of the long filaments the individual bacilli are not well shown. The filaments are either straight or more or less curved. The length of the single bacilli varies from 4 μ or less to 8 μ or more; the thickness is about 1 μ.

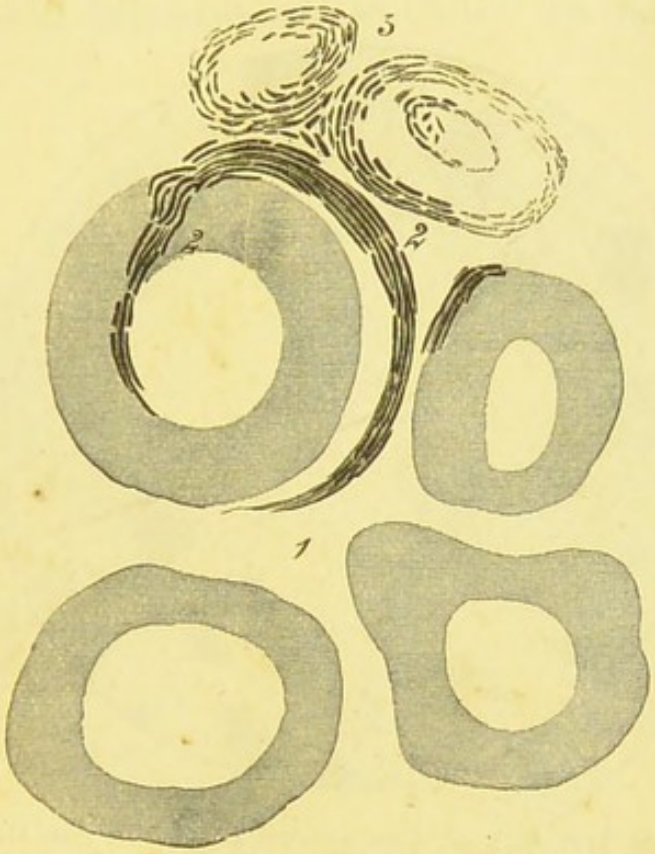

FIG. 117.—FROM A SECTION THROUGH THE CARTILAGE OF RABBIT'S EAR IN WHICH ULCERATION HAD BEEN PRODUCED BY INOCULATION WITH NECROSED MATTER OF CALF'S TONGUE.

1. Cartilage capsules.
2. Bundles of good bacilli.
3. Bundles of degenerating bacilli.

Magnifying power 700. (Stained with magenta.)

Many of them contain spores. In the ear of the rabbit they invade the connective tissue as well as the cartilage over the whole extent of the ulceration and its neighbourhood. Lingard found the same bacilli, having the same arrangement, in a case of noma in the human subject.

Bacillus diphtheriæ.[1]—This acute infectious disease, to which children and young individuals are particularly prone, shows itself in most instances as a severe inflammation and fibrinous infiltration of the mucous membrane of the fauces and pharynx, or also the larynx and trachea, leading to, and early in the disease consisting in, a necrosis

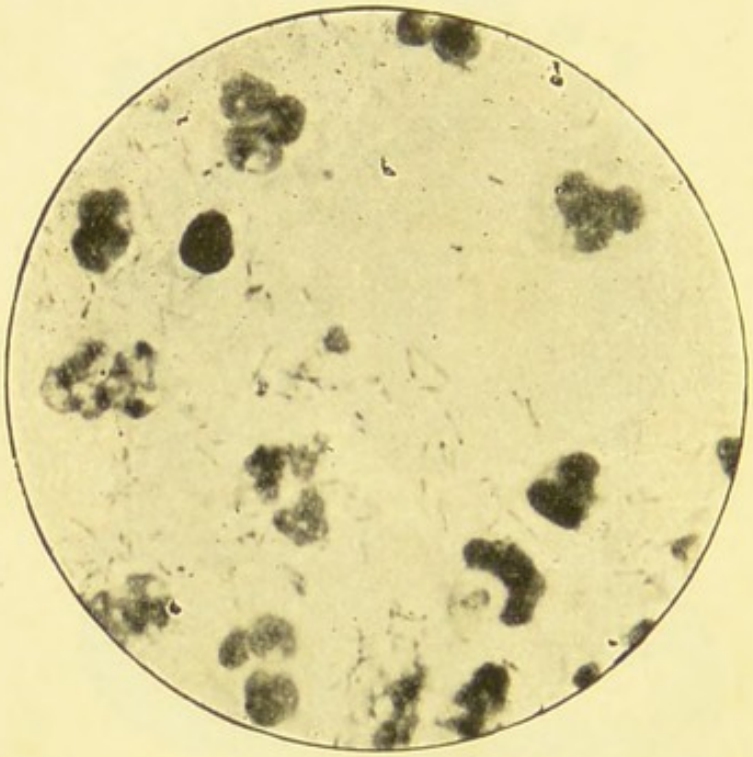

FIG. 118.—FILM SPECIMEN OF THE DEEPER LAYER OF THE DIPHTHERITIC MEMBRANE, SHOWING NUMEROUS LEUCOCYTES AND THE DIPHTHERIA BACILLI.

× 1000.

of the superficial part of the mucous membrane, and thereby changing this into a tenacious, whitish pseudo-membrane, the "diphtheritic membrane." In most cases only the mucosa of the fauces (tonsils, palatine arches, velum palati and uvula, upper part of pharynx) shows this change, *i.e.* into whitish-grey "diphtheritic membranes"; in other

[1] Part of the following account is copied from Klein's Etiology and Pathology of Infectious Diseases in Stevenson and Murphy's Treatise on Hygiene, vol. ii.

cases this necrotic change extends over the whole of the pharynx into the larynx, and even the trachea; in still other cases it starts in the larynx and invades this and the trachea —croup. In some cases a similar inflammation and the formation of diphtheritic membranes are observed in the stomach, in the intestines, in the urinary organs, and independently and primarily on wounds. In addition is to

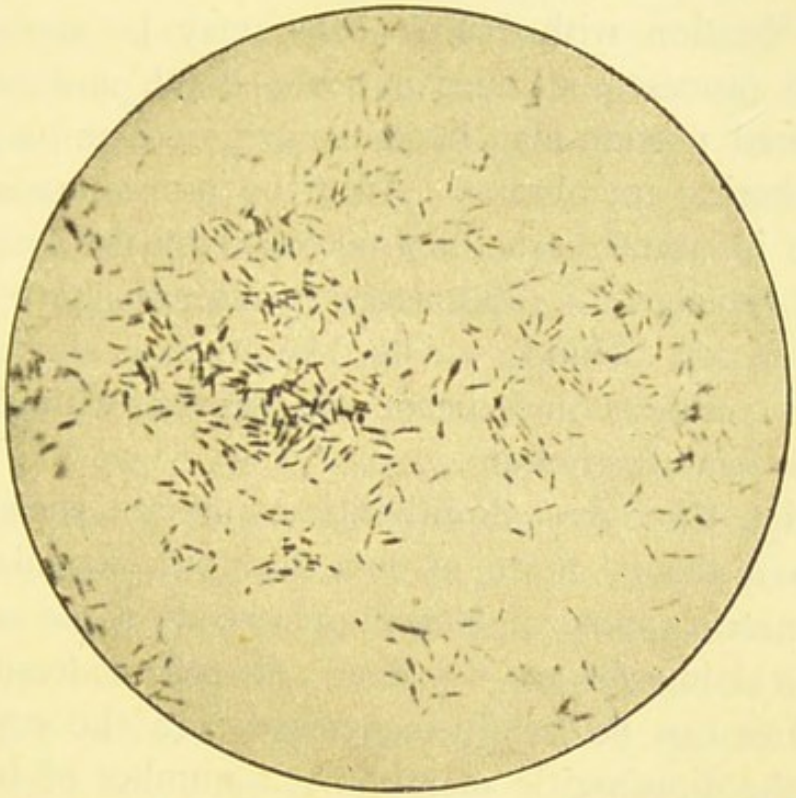

FIG. 119.—FILM SPECIMEN FROM THE SUPERFICIAL LAYERS OF THE DIPHTHERITIC MEMBRANE, SHOWING THE DIPHTHERIA BACILLI IN PURE CULTURE.
× 1000.

be mentioned myocarditis diphtheritica. The microscopic character of typical or membranous diphtheria is generally this, that the mucous membrane is the seat of a severe inflammation and necrosis: engorgement of, and extravasation from, the superficial capillaries and veins, with stasis of blood in them, and swelling due to infiltration of the mucosa with fibrine and round cells; the epithelium as

a whole is lost; the affected mucosa itself becomes necrosed and changed into a whitish-grey coagulated mass, in which fibrin, a close network of threads and septa, and in the superficial parts lymph cells, may be recognised, this necrosed or coagulated portion forming the diphtheritic membrane; close to that part which comprises the necrosed mucosa the outlines of blood-vessels filled with stagnated and coagulated blood, and extravasated blood, as also dense infiltration with lymph cells, may be recognised. When the process continues into the depth and breadth, this inflamed portion also becomes necrosed, and a part of the diphtheritic membrane. After the process passes the acme, the inflamed tissue, not necrosed by the exudation, gradually detaches the diphtheritic membrane above it, and an ulcer is left behind, which, like other healing ulcers, gradually contracts and becomes covered with healthy membrane and epithelium.

A section through a diphtheritic membrane shows a few nuclei in a dense, more or less fibrinous, reticulated or hyaline matrix, more or less ill-preserved; some of these take the staining, *i.e.* are not dead; in others already dead the outlines can be barely recognised. In the superficial parts of the diphtheritic membranes a number of larger or smaller loculi are always seen, which are filled with clumps of bacteria (see illustration). These clumps of bacteria are of various kinds: generally staphylococci and at least two kinds of streptococci, thick and long septic bacilli, and groups of minute bacilli which we will call the diphtheria bacilli. These latter are found in larger and smaller masses on the surface, forming sometimes a continuous layer; in some cases sections show that in the middle, and occasionally, but rarely, even in the deep parts, they are the only bacteria present; here they are in small clusters, or they

form large masses (see Fig. 121). In the mucous membrane next to, but not part of, the diphtheritic membrane the writer has found them occasionally in small numbers; in the inflamed mucous membrane of the depth these diphtheria bacilli are, as a rule, rarely to be found. In the blood and in the viscera the bacilli are generally absent; nor are other micro-organisms to be found as constant inhabitants. In cases of diphtheria ending fatally, even if the disease only lasted a few days, the lungs are the seat of severe bronchial catarrh, lobular or broncho-pneumonia, with numerous diphtheria bacilli; the kidney is congested and shows distinct parenchymatous nephritis: the epithelium of many convoluted tubes of the cortex is granular, disintegrating, and fatty; in the liver fatty degeneration of the liver cells is generally present. The one species of bacteria that is constant and can be easily isolated in many cases in almost pure cultivation from the superficial and even middle layers of the fresh diphtheritic membrane consists of non-motile minute bacilli: some are curved, most are straight, some slightly swollen at each end or knob-shaped at one end, many of them pointed at one end; in fact, this latter may be regarded as the typical bacillus. These bacilli occur either singly or in dumb-bells, or aggregated in continuous masses; many show a segregation of their protoplasm into granules or rods of unequal size; amongst these "granular" forms one or both terminal granules are occasionally club-shaped. Some of the single bacilli in well-stained specimens show a deeply stained granule at each end. The bacilli of Agar cultures show the same appearances as those in the diphtheritic membrane; in gelatine culture the bacilli are shorter, thicker, and many are conical (see Fig. 123). These bacilli were first seen by Klebs, and by Löffler were regarded, owing to their constancy, as pathognomonic and

pathogenic for diphtheria; Löffler had first isolated them by culture on blood-serum, but he, and then Hoffmann, found a morphologically similar bacillus in the normal discharges of the fauces. Now Löffler has shown that, while the former or the "diphtheria bacillus" is pathogenic for animals, the latter or pseudo-diphtheria bacillus is not so; but this, although not accepted by all, nevertheless corresponds to the facts.

Roux and Yersin (*Annales de l'Institut Pasteur*, iv., p. 409) state that from simple sore throat, as also from normal throat, the pseudo-diphtheria bacillus was isolated by them, which in morphological and cultural respects is identical with the true diphtheria bacillus, but which is not pathogenic to guinea-pigs. They further conclude that this pseudo-diphtheria bacillus is really the diphtheria bacillus after it has lost its virulence.

As to the virulence of the diphtheria cultures directly derived from the human diphtheritic secretion or membrane and tested on the guinea-pig (see below), this does not stand in any definite relation to the severity of the human case, for extremely virulent (for the guinea-pig) bacilli may be obtained from mild cases, while from severe or fatal cases bacilli are cultivated which are less virulent for the guinea-pig, inasmuch as of the former the subcutaneous injection of less culture material will produce a fatal result in the guinea-pig than of the latter. Similarly the length of the diphtheria bacilli in the membrane and in the cultures obtained from this is no index of their virulence; as a rule when the membrane contains the diphtheria bacilli in almost pure culture the great majority are relatively short rods.

Besides, in true diphtheria of the fauces the diphtheria bacilli can be demonstrated in many cases of fibrinous rhinitis, fibrinous croup, and in diphtheria *following* scarlatina, but

not in so-called scarlatinal diphtheria, that is in necrotic change in the fauces occurring simultaneously with scarlatina. (Löffler, Kolisko and Paltauf, Tangl, Klein.)

As a result of recent investigations the opinion is well

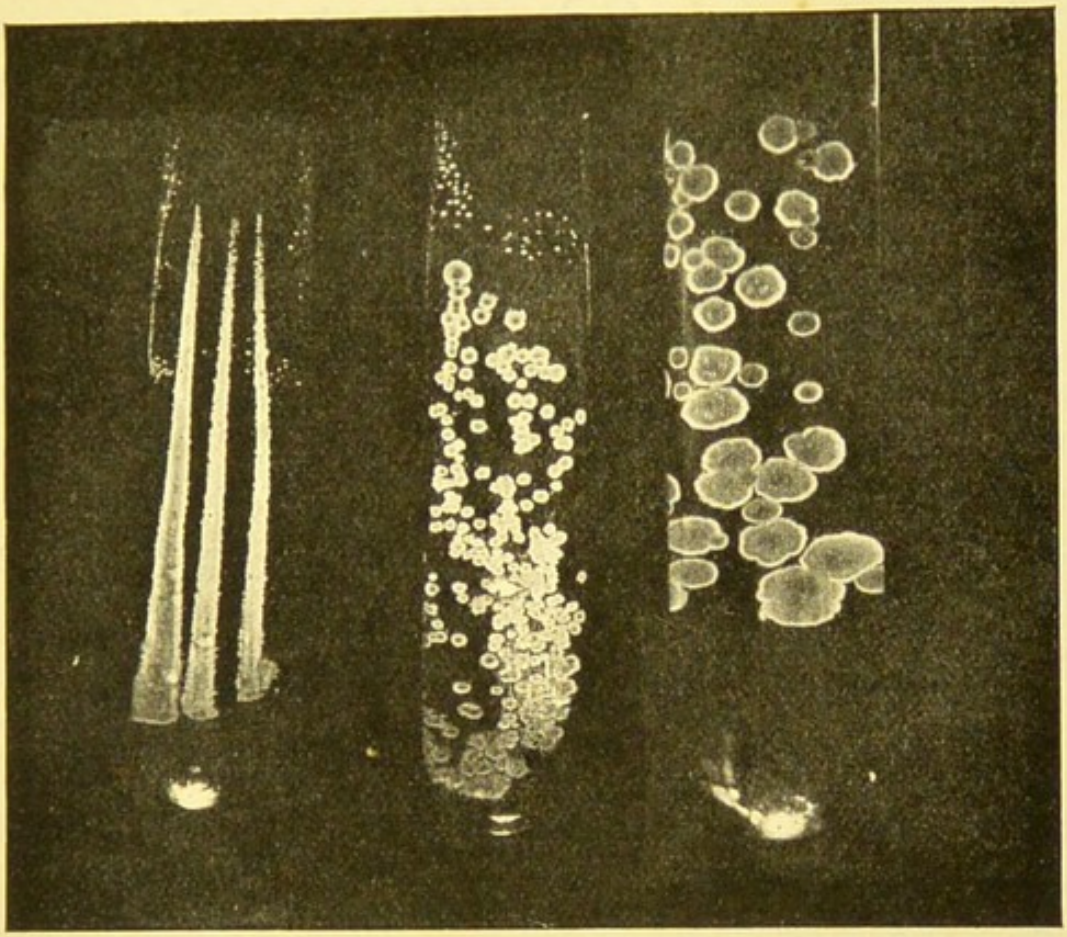

FIG. 120.—CULTIVATIONS OF THE BACILLUS DIPHTHERIÆ ON THE SLANTING SURFACE OF NUTRIENT GELATINE: ON THE LEFT, STREAK CULTURE; IN THE MIDDLE, A TUBE-PLATE CULTURE WITH NUMEROUS MINUTE COLONIES; ON THE RIGHT, A TUBE-PLATE CULTURE WITH A LIMITED NUMBER OF DIPHTHERIA COLONIES; IN ALL, THE CENTRE THICKER, LESS TRANSPARENT, THE PERIPHERAL PART MORE FILMY.

Natural size.

founded that also in cases of "simple sore throat," if the presence of the true diphtheria bacilli can be demonstrated in the secretion, those cases are diphtheria; and conversely, if in any case of sore throat, no matter whether it is or is not associated with membranous exudation, the true diphtheria

bacillus cannot be demonstrated, such case cannot be considered as diphtheria. As a matter of fact it has now been amply shown that the after-events prove the correctness of these statements, for it has been shown that the former cases, apart from their being the centre of an outbreak of cases of true membranous diphtheria, develop occasion-

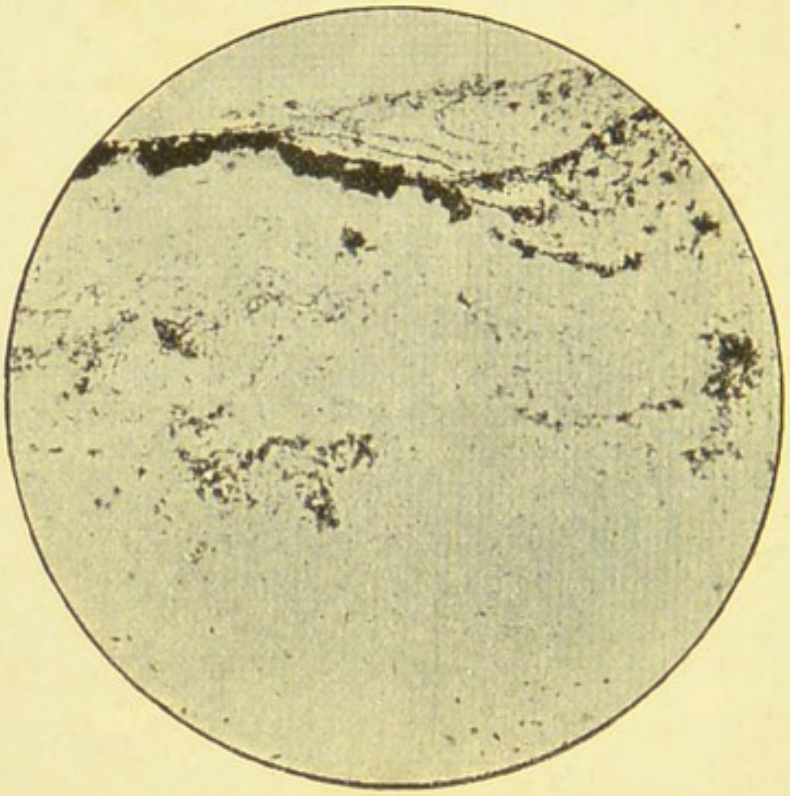

FIG. 121.—SECTION THROUGH A DIPHTHERITIC MEMBRANE SHOWING CONNECTED MASSES OF THE DIPHTHERIA BACILLI EXTENDING FROM THE SURFACE OF THE MEMBRANE INTO ITS DEEPER LAYERS. THE TISSUE OF THE MEMBRANE IS NOT SHOWN; AS CULTURES PROVED, ALL THE MASSES (BLACK) ARE MASSES OF PURE DIPHTHERIA BACILLI.

Low magnification.

ally post-diphtheritic paralysis, while the latter (non-diphtheritic) cases do not lead to post-diphtheritic sequelæ. These cases of faucial inflammation not associated with the true diphtheria bacilli, and therefore not true diphtheria, are associated with, and probably caused by, either staphylococci (staph. aureus) or streptococci, and are therefore regarded as

"cocco-diphtheria." The streptococci are at least of two kinds: the same as are occasionally also found as complicating severe cases of true faucial diphtheria. Non-diphtheritic membranous exudations of the fauces are brittle and composed of leucocytes, whereas the diphtheritic membrane is tough, coherent and poor in leucocytes, containing principally the above-mentioned reticulated mass.

In some epidemic sore throats thrush fungus or saccharomyces is present in large numbers.

As a further result of recent investigations it is admitted that the true diphtheria bacilli occur in the fauces of persons who, themselves free of diphtheria, have however been in contact with diphtheria cases, and further that, even weeks after in a diphtheria case recovery had taken place, the mucous membrane of the fauces may still harbour true diphtheria bacilli. In the majority of cases of faucial diphtheria, however, the bacilli disappear two or three weeks, or even earlier, after the mucous membrane had assumed its normal condition.

The bacillus of diphtheria isolated by Löffler forms colonies of definite characters on serum and Agar plates kept at 35–37° C.: round white colonies, thickest in the middle and gradually assuming here a yellowish-brown tint. According to Löffler it does not grow on gelatine, but the writer has shown that abundance of growth takes place on gelatine at 20–21° C.; on potato it shows no visible growth. Löffler found this particular bacillus in a large percentage, but not in all, of the diphtheritic membranes; Kolisko and Paltauf, Roux and Yersin, Zarniko and Escherich, found this microbe in all cases of diphtheria, and owing to its peculiar pathogenic action (see later) they definitely regarded it as the microbe of diphtheria. The writer has shown that there occur occasionally in diphtheritic mem-

branes two species of bacilli, similar in morphological respects and in the mode of growth on and in Agar plates, on serum, and on potato; but one species is not constant, and is probably the pseudo-diphtheria bacillus of Hoffmann and Löffler, while the other is present in all cases, and in some almost in pure culture; it is pathogenic on guinea-pigs. It grows abundantly on broth at 37° C., making this uniformly turbid already in 24 hours; this increases during the next day, while a whitish, powdery precipitate appears, and on the surface a filmy membranous-like pellicle.

On gelatine the colonies are at first rounded, white, prominent dots, which enlarging in breadth thicken in the middle and become here slightly yellowish, dark brown in transmitted light, the peripheral part being thin, plate-like, and angular (Fig. 120). In the streak cultivation on gelatine the streak becomes marked as a white band, at first made up of droplets, but soon becoming confluent into a uniform band; at the margin the droplets and knob-like expansions can still be recognised; the middle is thick and prominent; in stab culture in gelatine the stab becomes indicated by a line of droplets, white in reflected, brownish in transmitted light; the upper point of the stab is occupied by a crenate, convex, white plate. Of course on gelatine, at 19–21° C., the growth is much slower than on Agar-Agar at 35–37° C. In milk kept at 20° C. our bacillus grows luxuriantly and produces already after three days, or even less, slight curdling of the milk, minute flakes of coagulated casein; at 37° C. the growth is curiously less abundant in the same space of time, and the curdling far less. The diphtheria bacilli are killed by heating to 60° C. for five minutes; they do not form spores. The diphtheria bacilli when transmitted through several subcultures acquire the power to grow more and more rapidly on gelatine at 20–21°C.,

as also they appear to form longer rods and chains than at first. The club-shaped forms and the chains of granules and rods with spindle-shaped and clubbed ends also appear sooner in the cultures; these forms have nothing to do with involution forms, as they can be demonstrated already in the active and early phases of the development of the colonies.

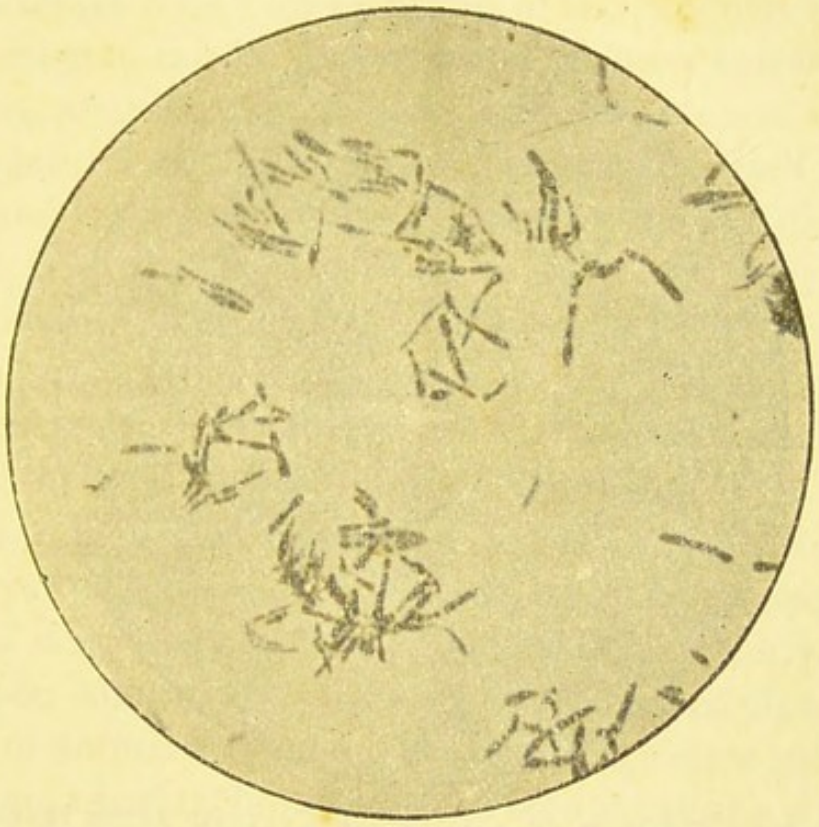

FIG. 122.—FILM SPECIMEN OF AN AGAR CULTURE OF BACILLUS DIPHTHERIÆ AFTER A FEW DAYS' GROWTH; CHAINS AND CLUBS ARE WELL SHOWN.

× 1000.

For the isolation of the diphtheria bacillus, serum (pure blood-serum or, better, Löffler's serum) or nutrient Agar is used, for then the colonies if present can be recognised already after twenty-four hours. As stated above, in some cases of membranous diphtheria numerous colonies, occasionally in pure culture, of the diphtheria bacilli can be easily obtained either by rubbing a particle of the membrane over the slanting surface

of the solid medium or by first shaking up a particle of the membrane in sterile salt solution and rubbing a droplet of this over the culture surface. But, unfortunately, in a large percentage of doubtful cases the diphtheria bacilli are mixed up in the exudation with numerous cocci: in such cases it is necessary to use serum cultures. On this medium the diphtheria bacillus grows better than the cocci, and therefore

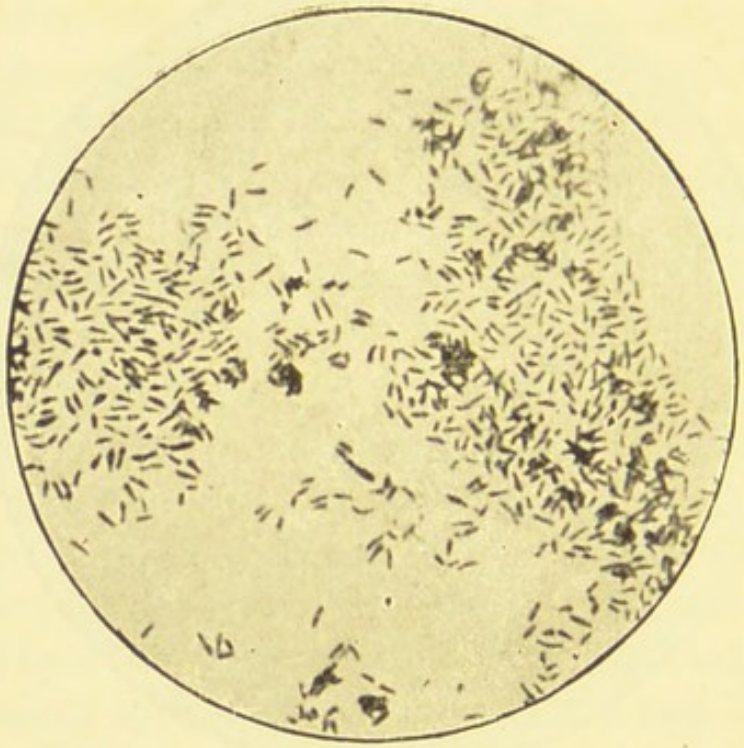

FIG. 123.—FILM SPECIMEN OF A GELATINE CULTURE AFTER SEVERAL DAYS GROWTH.
× 1000.

after 24–36 hours its colonies can be recognised. Another plan which I found useful is to melt over the flame sterile nutrient Agar or Glycerine Agar and to pour it out into sterile plate dishes; after it has set herein a particle of the suspected secretion or membrane is rubbed over the whole surface of the solid Agar, and the plate is incubated at 37° C. After twenty-four hours, by means of a magnifying glass or simple microscope, the colonies are carefully examined, and

those which resemble diphtheria colonies are subjected to microscopic examination in stained film specimens and to subcultures. I have thus succeeded in finding a few diphtheria colonies amongst crowds of colonies of cocci, whereas serum tubes have failed to give a positive result. But by far the best method is the Ascites Agar fluid set with slanting surface, which was mentioned in a former chapter as Kanthack's serum Agar; for by means of this medium the diphtheria colonies can be demonstrated far more certainly than with any other medium. I have seen this in cases in which the diphtheria bacilli were scanty and much mixed up with cocci, and yet a particle of the secretion on the membrane rubbed over the slanting surface of Kanthack's serum Agar, and incubated at 37° C., produced in twenty-four hours a pure crop of diphtheria colonies.

Recent cultures of the diphtheria bacillus on Agar, on gelatine, on serum, and in broth prove virulent on guinea-pigs, but this virulence decreases with the age of the culture. A broth culture of which after forty-eight hours' incubation at 37° C. 0·25 to 0·3 cc. is capable of killing in thirty to forty hours one kilogramme body-weight of guinea-pigs is considered of normal virulence (Behring). Of gelatine subculture made from a normal broth culture (three streaks on a slanting surface six centimetres by two centimetres) incubated at 20–21° C. for seven to ten days, the growth being then scraped down and suspended in sterile broth, one-sixth of the total growth is sufficient to kill one kilo. guinea-pig in thirty to forty hours. In an Agar culture made in the same way, incubated at 37° C., the same amount of virulence is found during the first three or four days; later the virulence decreases, as does also often that of a broth culture after the first six or seven days.

Löffler has shown that with cultures of the diphtheria

bacillus definite pathological results—inflammation, with something like diphtheritic necrotic membrane—can be obtained by rubbing them into an abraded surface of the mucous membrane (mouth, trachea) of rabbits, fowls, or pigeons, and Roux and Yersin found the same; but such results are not easily and constantly obtainable either with human diphtheritic membranes or with the cultures of the diphtheria bacillus. By subcutaneous inoculation of guinea-pigs with diphtheritic membrane, and particularly with cultures of the bacillus diphtheriæ, definite results are obtained. After subcutaneous inoculation with cultures a few days old the result is very rapid and more striking than with diphtheritic membrane; for obtaining very acute results only a small particle, not more than what can be removed from a colony with the end of a platinum loop, often suffices. In the severe cases produced by injecting several minims of a recent broth culture (forty-eight hours old) the animals are very quiet already after twelve or sixteen hours; a soft, painful swelling is found at the seat of inoculation. During the second day the hair is erect, the eyes are small, the temperature is raised; the animals are tremulous and refuse food; the condition grows rapidly worse, movement ceases, the body temperature rapidly falls, and they are found dead before thirty to forty hours are over. In other cases the illness lasts two to three days; in still others as long as five days, or even more. The younger the culture the more active it is, and the more bacilli are injected the shorter the illness. On *post-mortem* examination we find hæmorrhage and œdema in and about the place of inoculation, in the subcutaneous and muscular tissue, extending sometimes over considerable areas; when inoculation is made into the groin the changes (hæmorrhage and œdema) extend over the thigh, abdomen, and even

chest of the inoculated side; the inguinal glands of the inoculated side are deeply congested. The lungs are congested, sometimes more, sometimes less; sometimes the greater part of one lobe or another is deep purple; pleuritis and pericarditis are often found; the liver is slightly or not at all congested, is even pale; the spleen is not enlarged; the serous covering of the stomach and intestines is congested; the suprarenals are deep red; the kidney is congested in the medullary part. Neither from the heart's blood nor from the lung, liver, spleen, or kidney can as a rule any organisms be cultivated, but occasionally the lungs and the omentum yield positive results; from the subcutaneous tissue of the inoculated part, particularly from the congested inguinal glands, the bacilli can be obtained in pure cultivations, some tubes showing a limited number of colonies, others showing them abundantly; but not in all animals is the culture test successful, though in most it is so.

While guinea-pigs are very susceptible to subcutaneous inoculation, they show considerable resistance to intraperitoneal injection. It has been mentioned in a former chapter (Chapter vii.) that, while a number of species of bacteria possess in their protoplasm substances which act poisonously on the animal body (protein poisons, intracellular poisons) when introduced in sufficient doses as bacterial bodies, living or sterilised, into the peritoneal cavity—*e.g.*, vibrio of Finkler and cholera, bacillus prodigiosus, bacillus coli and typhosus, proteus vulgaris, &c.—causing acute fatal peritonitis, and while further some notoriously pathogenic bacilli—*e.g.*, anthrax, fowl cholera, and diphtheria—do not contain these intracellular poisons, at any rate large doses of the bacterial bodies (sterilised) can be injected intraperitoneally without producing the acute fatal peritonitis. Now it is a strange fact

that the diphtheria bacillus does not cause this peritonitis even if injected into the peritoneal cavity in a living state. If from an active gelatine culture (slanting surface) the growth is scraped off and distributed in sterile bouillon, and of this suspension one-sixth is injected subcutaneously into a guinea-pig of 500–700 grammes weight, the typical tumour is produced, and death occurs in thirty or thirty-six hours with certainty, but the same dose of the same culture injected into the peritoneal cavity of a guinea-pig half that weight does not cause fatal illness. If from the peritoneal fluid a little is withdrawn two, three, four, and six hours after the intraperitoneal injection of the large dose of living diphtheria bacilli, and examined, it will be found that most of the bacilli are dead already after two hours, and that no living bacilli (no successful subculture) can be established after four to six hours. Such guinea-pigs as had been once intraperitoneally injected with more than about a double, otherwise fatal, dose of living gelatine culture can repeatedly at intervals be injected with increasing amounts —at the fifth injection as much as one-third of a living gelatine culture can be introduced intraperitoneally—that is to say, an otherwise fourfold fatal dose—without producing any illness. Moreover, such guinea-pigs appear also immunised against an otherwise fatal dose of living diphtheria bacilli subcutaneously injected, no tumour and no disease is hereby produced. By these and other similar experiments to be mentioned in the chapter on Immunity, I have been able to show that the specific immunising or germicidal power against a bacterial species which the blood-serum of repeatedly intraperitoneally injected (immunised) guinea-pigs acquires (R. Pfeiffer) is related to substances derived from the bacilli themselves that had been introduced into the peritoneum, and had been used for the immunisation.

Roux and Yersin [1] have separated certain chemical products (toxins) from broth cultures, and shown that those products themselves act poisonously in the proportion in which they are injected. Roux and Yersin have also observed in experimental animals, after inoculation with small doses of broth culture or of the diphtheria toxin separated by filtration from broth cultures, the same kind of paralysis as occurs also in human diphtheria in the later stages, that is after the acute symptoms have passed away. Sidney Martin has published an account of the chemical nature of the poisons occurring in the human diphtheritic membrane; these same poisonous principles (ferment, organic acid, albumoses) were also obtained from albumen cultures of the diphtheria bacilli. Dr. Martin shows that with the chemical products the same diphtheritic paralysis can be produced, and he further shows that this paralysis is due to degeneration of the peripheral nerves. (Reports of the Medical Officer of the Local Government Board, 1891–1892.) According to Roux and Yersin [2] the toxin of broth cultures is a ferment and when injected into guinea-pigs produces the same œdematous hæmorrhagic tumour and death as the living culture. Roux and Yersin [3] have further shown that by growing the diphtheria bacilli in broth under constant supply with fresh oxygen a toxin can be obtained of high degree of virulence, 0·2 gramme being capable of producing a tumour and fatal result in forty-eight hours in one kilo. of guinea-pig. Löffler, Roux and Yersin, and others have therefore justly concluded that in diphtheria we have to deal with a chemical poisoning, the chemical poison being produced by the living bacilli in the diphtheritic membrane

[1] *Annales de l'Institut Pasteur*, December, 1888.
[2] *Ibid.*, June, 1889.
[3] *Ibid.*, vol. iv., p. 421.

of the human mucous membrane, and in the case of the experimental guinea-pigs, at the seat of inoculation, and absorbed by the system, produces the whole set of general disease symptoms in the lung, liver, kidney, and nervous system, associated with and characterising diphtheria; the absence generally of the bacilli from the circulation and all affected organs, and their localised presence in the diphtheritic membrane, suggests this already. From this it follows that if the growth and multiplication of the bacilli in the diphtheritic membrane could sufficiently early be prevented or checked—by cautery or otherwise—the amount of the poison would be small, and the disease would cease. Diphtheria is then not a real infection but more of the nature of intoxication.

It has been asserted by various authors that a necrotic, chronic, infective process observed in the mucous membrane of the mouth and pharynx in fowls, calves, and pigeons is intimately connected with human diphtheria; but Löffler[1] has shown this is not the case, since these necrotic processes are both as to the pathology and the microbe altogether different diseases.

Cats, however, have unquestionably been observed[2] to suffer in connection with human diphtheria; in houses where human diphtheria obtained, cats have been known either antecedently, or coincidently, or subsequently to become ill; they appear to have some kind of throat illness and cannot swallow; as a rule, bronchial mischief is already noticed early, and if the disease is protracted through several weeks, as it generally is, they become much emaciated and die. On *post-mortem* examination the lung is found to be full of

[1] *Mittheil. aus d. k. Gesundh.*, vol. ii., p. 482.

[2] Dr. George Turner, Dr. Bruce Low, Dr. C. T. Renshaw, Dr. A. Downes, Dr. Thursfield; *see* the writer's Report in the Volume of the Medical Officer of the Local Government Board, 1889, p. 162.

grey, consolidated, lobular patches, and the kidneys are always *enlarged and white;* on a section the whole cortex is found to be fatty degenerated, while the medulla shows congestion. Further, I have ascertained that an infectious disease with the same symptoms and leading to the same result exists naturally amongst cats; the animals have severe lung trouble, emaciate, and die with the same pathological appearances, notably on the part of the kidney. In one case I have seen such a cat after several weeks' illness showing paresis of the hind extremities.

When cats are inoculated subcutaneously in the groin with a particle of human diphtheritic membrane they become very ill, show already after twenty-four hours a painful swelling in the groin, have high temperature, and refuse food. In the severe cases these symptoms increase in intensity during the next days, and the animals die before the end of the week. On *post-mortem* examination the subcutaneous and muscular tissue at and near the seat of inoculation are found to contain hæmorrhage and œdema, and the tissue is separated into layers, which are more or less necrotic. The viscera show much congestion, particularly the lungs, also the serous covering of the stomach and intestine as well as the peritoneum; the kidney is *large and white*, the medulla congested, while the cortex is more or less uniformly fatty. This condition is more marked the longer the illness; when the animals die in three to four weeks, or later, the condition of the kidney is very striking, and then also the lungs show lobular patches of grey consolidation. Still more striking is the result when a small quantity, 1 cc., of a virulent culture of our bacillus diphtheriæ is subcutaneously inoculated. If a fresh culture—one twenty-four to forty-eight hours old—is used, the animals are very ill already after twenty-four hours: they

are quiet, refuse food, the temperature is raised, and at the seat of inoculation is a painful swelling; some animals die after two, three, or four days, others live to the end of the week. On *post-mortem* examination the same appearances of the viscera, notably of the lungs and kidney, are found; and here also the fatty white kidney and the pneumonia are the more marked the longer the duration of the disease; in animals that die forty-eight to seventy-two hours after inoculation with culture the subcutaneous and muscular tissues about the seat of inoculation show much hæmorrhage, in many parts the tissues are almost gangrenous. On the death of the animal, the bacillus diphtheriæ can be recovered by cultivation in numerous colonies, but no bacilli can be demonstrated in the lungs, liver, or kidney.

The dog is similarly affected by subcutaneous injection of virulent diphtheria culture.

Different animals offer, however, different degrees of resistance to infection with living culture or with toxin produced by Roux and Yersin's method in broth culture and separated by filtration with a Chamberland filter. Thus the sheep and goat, the ass and the horse, offer different degrees of susceptibility; the sheep and goat react well (Behring), the ass better, and the horse as a rule least (Roux); in the latter animal the relative dose of living culture or toxin can be taken greater than in the ass in order to produce a positive result, but also amongst horses the resistance varies in different animals. On subcutaneous injection of a non-fatal dose a tumour is formed at the seat of inoculation, the body temperature is raised next day (by 0·5–2° C. according to the dose and virulence of the material), the animals are quiet and do not feed quite in the normal manner. But they soon again recover their normal temperature, feed again well, the local tumour becomes

smaller and in a few days has almost entirely disappeared. By reinjection after the lapse of a week to a fortnight the dose of culture or toxin can be made a little larger or the virus a little more potent without again producing more than the former transitory result. In this way Behring[1] was the first to show that the resistance of the animal can be gradually more and more increased, inasmuch as after repeated injections it is capable of resisting (except for the transitory tumour and rise of temperature) larger and larger and more potent doses of the virus, doses which at a former stage would have at once produced fatal results. Behring has thus succeeded in "immunising" sheep and goats to a very high degree, that is to say that after many injections with increasing amounts and potency the animals are capable of resisting a dose of virus many times the former fatal dose. Roux[2] uses for this purpose the horse, and he succeeds after many injections (over thirty, extending over nearly three months) in enabling this animal to at last resist the intravenous injection of the prodigious amount of 250 cc. of the most potent toxin.

As is well established, diphtheria is a highly contagious disease, transmissible from person to person, its contagium belonging to the group called fixed contagia. But it is likewise well established that milk infected from a human source has, in several epidemics, been the means of producing diphtheria in the consumers (Ballard). It is further known that a room in which a diphtheria case has once existed may for years harbour the contagium of diphtheria, so that any new-comer or inhabitant may contract the disease; moreover, it is known that in a locality in which diphtheria has once been rife the disease may at any time reappear, and in these instances the transmission of the

[1] Behring, *Deutsche Med. Wochenschrift*, 1890, No. 50.
[2] Roux, *Annales de l'Institut Pasteur*, September, 1894.

contagium from sewers is maintained by some sanitarians. Lastly, it has been shown by Mr. Power, Dr. Mason, and Dr. Philpott that in certain epidemics of diphtheria (Yorktown and Camberley, Barking, Croydon), while the milk was the vehicle of infection, the milk did not receive its infective power from a human source.

Several epidemics of milk diphtheria, in which fouling of the milk with human diphtheritic material could not be demonstrated, but, on the other hand, could be excluded, have of late years become known, and in these cases the suspicion attached itself to the cows, for it could be shown that there existed on the farms concerned no other condition which in any way could account for the infectivity of the milk; besides, this infectivity was inherent to the milk over a certain period. In the case of the Yorktown and Camberley epidemic (*see* Mr. Power's Report in the volume of the Medical Officer of the Local Government Board for 1886) the cows were certified by a veterinary surgeon to have been in good health, though even several days after the human diphtheria cases had ceased to occur two of the cows showed some slight signs of "chaps" on their teats. Mr. Power saw at the farm one cow which had suffered from chapped teats in October, 1886 (the month in which the epidemic occurred), and which still had at the beginning of November a scab or crust at the site of a "chap." At Barking the cows whose milk produced the diphtheria (in 1888) suffered from a distinctly contagious eruptive disease on the teats and udder, showing itself in sores covered with brown black crusts. The same was noticed in connection with an outbreak of diphtheria (through milk) at Croydon, November, 1890. The question which was therefore considered important to decide was this: Can cows be infected with the bacillus diphtheriæ? During the years 1889, 1890,

and 1891 I made experiments on eight milch cows (which had calved some weeks previously), which strikingly showed that this is really the case. The results of some of these experiments are so definite and so important in connection with milk derived from such cows being charged with the diphtheria contagium that we may be excused for giving two of these experiments somewhat in detail.[1]

A broth culture was made of the bacillus diphtheriæ derived from a human diphtheritic membrane, but passed through several gelatine subcultures; the broth culture had been growing for two to three days at 37° C., and was very virulent on the guinea-pigs.

One cubic centimetre of the culture was injected under the skin into the subcutaneous tissue of the left shoulder in each of two cows. These animals were, at the time of the experiment, in very fine condition (teats and udder quite clean, copious milk secretion), and had been so during eight to ten days, during which they had been under observation. During the second and third days after inoculation the body temperature showed a slight rise (to 40·6°), and they did not feed well on those two days; but afterwards the temperature went down to the normal state, and the animals became all right again otherwise. But at the seat of the inoculation there was a painful large soft tumour to be felt and seen. On the fifth to the sixth day, for the first time, there was noticed on the udder and on one teat in one cow an eruption of about half a dozen firm papules: red and injected, projecting above the surface of the skin, the subcutaneous tissue indurated with a nodule. In addition to the papules about half a dozen vesicles and two round patches covered with brown crusts could be seen on the udder.

[1] *Report of the Medical Officer of the Local Government Board for* 1889, p. 168.

Some of the vesicles contained clear lymph, others were pustular, *i.e.* purulent.

On the seventh day new papules and vesicles were found; those of the previous day had already become changed into dark brown crusts. On the eighth day a new crop of vesicles could be noticed on this cow's udder, and on that day for the first time about half a dozen were also seen on the udder of the second cow. Some were vesicular, others pustular, and still others covered with brown-black crusts; the vesicles and pustules were round and prominent, with a narrow margin of injected skin, the crusted places irregular. The whole thickness of the skin and subcutaneous tissue felt hard, nodular. For two or three days (ninth to twelfth day) did this go on in the first cow; that is, new vesicles appeared: those that were vesicles with clear lymph one day were pustular the next, and crusted the following day. The crusts did not remain long; after two or three days they became loose, and left a dry healing sore behind, but when recent, on removal, showed a bleeding sore of the corium underneath.

We have, then, here a new eruptive disease on the teats and udder of the cow: a disease marked by papule, vesicle, pustule, sore and crust, but of a very rapid progress, since the crusts fell off and the sore healed in less than seven to nine days since its first appearance, the skin being at the same time much indurated. This eruptive disease on the udder, be it well observed, was produced by inoculating the animals subcutaneously in the region of the left shoulder with a culture of the bacillus diphtheriæ.

As stated above, in both animals on the second and third days there was a painful soft tumour to be felt at the seat of inoculation. From day to day the tumour became larger; about the end of the week it was as large as a man's fist; after this time it gradually became firm; but about the

fifth and sixth days it was still soft, felt like œdema, and on pressure a quantity of clear serum could be squeezed out from it. After the death of the animals (one died after a fortnight, the other was killed on the twenty-fifth day) the tumour was examined, and it was found to be located in the subcutaneous tissue, but was firmly connected both to the skin above and the muscular tissue below, and was surrounded by œdematous tissue. It was streaked white, was firm, but on section clear serum could be pressed out from it. Under the microscope the tissue of the tumour was found to be of the same nature as diphtheritic material: a general matrix of reticulated necrotic tissue in which remnants of nuclei, outlines of blood-vessels, and remnants of extravasated blood could be recognised; this tissue shaded gradually both into the cutis and into the surrounding muscles.

Both animals showed normal temperature to the end, but they both coughed and gradually fell off from feeding and did not take any water. One of them by the end of the fortnight suddenly became worse: it took no food or water, its milk failed, its evacuations became scanty and dry, its breathing became very rapid, and after a sudden collapse it died. The other animal after twenty-four days (since inoculation) grew much worse, and was therefore killed.

In both animals the lymph glands nearest the left shoulder, *i.e.* close to the tumour, were much enlarged, very œdematous, and contained hæmorrhage; no change in the organs of the throat; both lungs showed extensive congestion, in fact almost amounting to red hepatisation of the upper lobes and the upper portion of the middle lobe, petechiæ, and hæmorrhagic patches under the pleura; the pleural lymphatics were everywhere in the congested portions conspicuous and distended, either with clear lymph, or, as was the case in the second cow, tinged with blood. Cutting into

the congested portions, the lung was seen to be highly œdematous, a large quantity of blood-tinged serum flowing from and accumulating at the cut end; the lobules were well mapped out, and there was also sharp demarcation by œdematous connective tissue between the normal lung tissue and the deeply congested lobules, as also between groups of lobules and individual lobules in the congested areas; hæmorrhage appeared as spots and patches on the parietal and visceral pericardium. The liver showed yellow-grey necrotic patches, the spleen showed grey, necrotic streaks in the capsule; both kidneys showed congestion of the medulla, and fatty patches in the cortex. We have, then, in both these animals a striking result, completely coinciding with the disease in the cat.

The next important point ascertained in these cows had reference to the distribution of the diphtheria bacilli inoculated. In the tissue of the tumour in both animals after death, *i.e.* after fourteen and twenty-four days respectively, the diphtheria bacilli could be demonstrated without any difficulty under the microscope in the sections and by culture. On sections the necrotic tissue of the tumour contained great numbers of the bacilli in clumps; culture experiments on gelatine and on Agar with a particle of the tissue of the firm tumour produced innumerable colonies of the diphtheria bacillus; when examined under the microscope they resembled the human diphtheria bacillus in all respects. They were also tested on guinea-pigs and found to act extremely virulently, causing death of the animals under the typical appearances in thirty to fifty hours. But neither in the heart's blood nor in the lung or liver of these cows could any microbes be demonstrated in microscopic specimens or by culture. So far, then, there is complete analogy between the cows, guinea-pigs, and cats, that is to say the

diphtheria bacilli introduced in the subcutaneous tissue produce here by growth and multiplication the chemical poison, setting up the general disease in the viscera. The presence of the diphtheria bacillus in the eruption of the cow could be demonstrated both microscopically and by culture during the vesicular and pustular stages; in the latter also numerous pus cocci.

That in the cow the diphtheria bacillus as such passed into the system of the animal and appeared, though not in the viscera, but on the udder, was demonstrated conclusively by the fact that before the end of five days after inoculation, in the milk of the cow collected under all precautions, the presence of the diphtheria bacillus could be demonstrated with certainty by microscopic and culture observation; the number of bacilli present on that day in the milk amounted to thirty-two per cubic centimetre. It need hardly be added that these results throw a great deal of light in understanding certain epidemics of milk diphtherias, such as at Camberley and Yorktown, Enfield, Barking, and Croydon.

This positive result of udder eruption was also obtained on two of further six experimental milch cows, and in one of two cases the bacillus was demonstrated in the milk about the end of the first week after inoculation. In all cases, however, the culture used was very virulent broth culture.

With cultures not of the virulent character—*e.g.* Agar cultures or broth cultures of some standing, inoculation produces a transitory tumour and smaller in extent without the visceral disease, and the animals soon recover. Such was the case in some of my own milch cows and in those experimented upon by Abbott (*Journal of Pathology and Bacteriology*, vol. ii., 1893, p. 35).

Von Emmerich isolated short thick rods from diphtheritic membranes, with which he produced a fatal disease in pigeons, rabbits, and mice. He found that, inoculated into the mucous membrane of the trachea of rabbits, the microbe produces death in sixty hours, with grey fibrinous membranes on the mucous membrane; the bacilli are present in the mucous membrane, blood, and viscera.

Löffler[1] showed that the so-called diphtheritic deposits in the mucous membrane of the fauces, larynx, and conjunctiva of fowls and pigeons is not the same as human diphtheria; in the pigeon it is different from that of fowls, while in the former it is caused by minute bacilli, thinner and a little longer than those of rabbit's septicæmia (Davaine, Koch); he also showed that the so-called diphtheria of calves is not the same as human diphtheria, since it is caused by long bacillary threads. Lingard and Batt have found previously the same bacilli in the necrotic masses in the mouth in calves; they have described the disease as a chronic ulcerative necrotic stomatitis. Dr. Lingard has shown that it is transmissible to the rabbit's ear, wherein the characteristic bacilli produce the same chronic necrotic ulcerative process.

As to the necrotic deposits in the fauces and mouth of fowls, not at all rare amongst poultry, and regarded by some as identical with human diphtheria, Löffler has already pointed out that it is different from the similar disease in the pigeon; it certainly is not due to the same bacteria as those shown by Löffler to be the cause of the pigeon's disease. The writer has cultivated from the caseous yellow-white deposits in the pharynx and mouth of such a fowl an organism which was present in almost pure culture. The yellow-white deposits are dry and brittle, and are made up of epithelial cells and débris. There are present various species of microbes in the superficial layers; but in the deeper parts was present predominantly one species of minute more or less constricted rods, of the same size as those of fowl cholera, but differing from these latter by the fact that on potato they form rapidly a characteristic deep yellow growth; on gelatine they form already after twenty-four to forty-eight hours white, round, prominent dots, which become more yellowish and project over the surface like little buttons, and are easily lifted off bodily; they are very tenacious, and do not break up when shaken in fluid.

Bacilli resembling the diphtheria bacilli in some of the morphological characters have been obtained from various materials. Besides the non-pathogenic-pseudo-diphtheria bacillus of Hoffmann, Löffler, Klein,

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[1] *Mittheil aus dem k. Gesundh.*, vol. ii.

Roux and Yersin, isolated from the fauces of the healthy as also of inflamed throat, there occur bacilli which form very pronounced clubs and threads with segregated protoplasm with terminal knobs, but which in cultural respects differ from the pseudo-diphtheria and the true diphtheria bacilli.

Thus, from milk taken directly from the teats of the cow I have

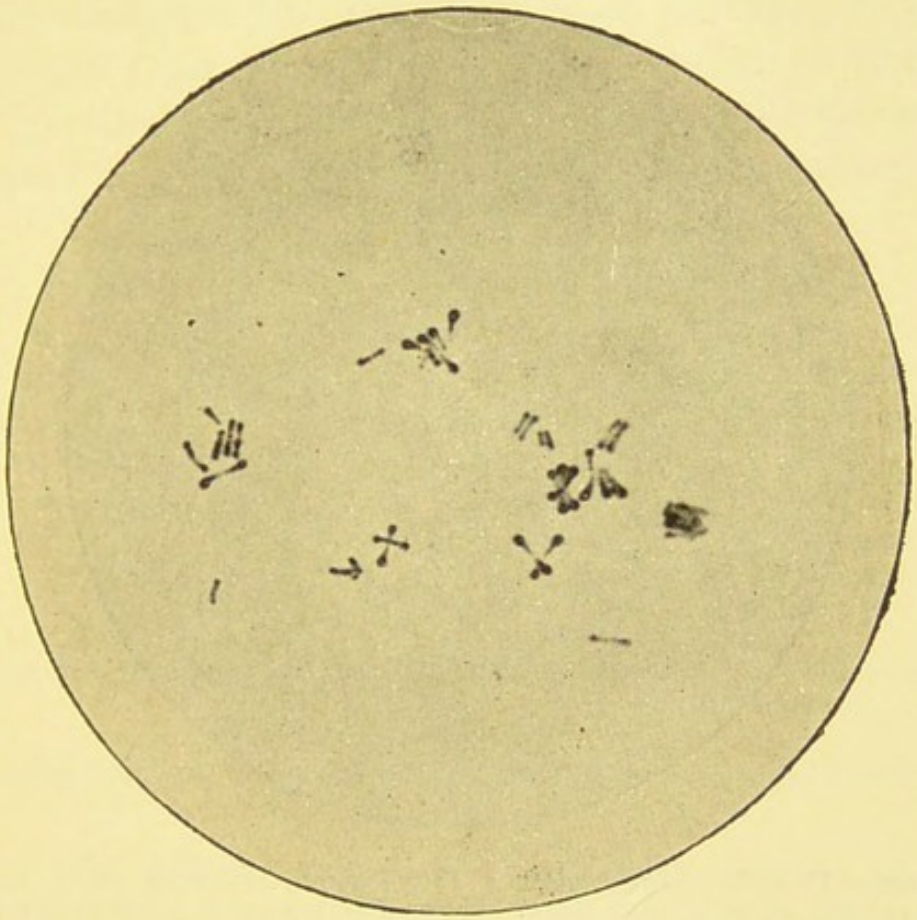


FIG. 124.—FILM SPECIMEN OF A COLONY ON AGAR OF A BACILLUS OBTAINED FROM MILK OF A COW; THE COLONY WAS DISTINCTLY YELLOW. MARKEDLY CLUB-SHAPED BACILLI.

× 1000.

isolated a bacillus forming exquisite clubs (*see* Fig. 124), but which in cultural respects markedly differs from the diphtheria bacillus; it forms on Agar *yellow* round colonies and grows much faster than the diphtheria bacillus, besides being non-pathogenic. Similarly from putrid beef I have isolated bacilli which in morphological respects bear a great resemblance (*see* Fig. 125) to the diphtheria bacillus but do not grow at 37° C.

The Bacillus of Glanders.—In 1882 Schütz and Löffler[1] demonstrated the constant occurrence of definite bacilli in the characteristic deposits and nodules of the nasal mucous membrane and internal organs, such as the lung, spleen, and liver of horses dead or dying from glanders. This bacillus is called bacillus mallei or glanders bacillus. The

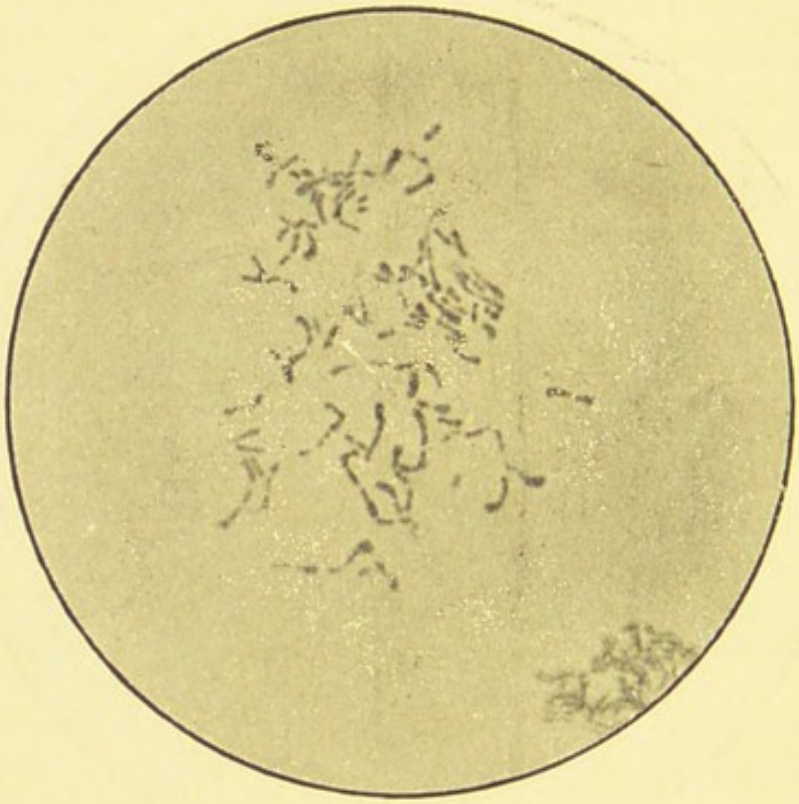


FIG. 125.—FILM SPECIMEN FROM A COLONY ON GELATINE OF A BACILLUS OBTAINED FROM PUTRID BEEF. MANY BACILLI SHOW SEGREGATED PROTOPLASM AND CLUBS SIMILAR TO THE DIPHTHERIA BACILLUS.
× 1000.

bacilli occur generally isolated, and in small groups between, and also enclosed in, the cells of the nodules: they are more numerous in the nodules which have not become purulent; after the nodules have become purulent the number of the bacilli in them diminishes. The bacilli are non-motile rods, of about 1·5 to 3·5 μ in length, that is the

[1] *Deutsche med. Wochenschrift*, 52, 1882.

same size as tubercle bacilli, but a little thicker, rounded at their ends, straight or sometimes more or less curved; this latter is especially noticed when they lie in groups; their substance is either homogeneous or, like that of the tubercle bacilli, shows segregation of the protoplasm into granules within the sheath. The bacilli stain best in alkaline methylene-blue and then washed in acidulated water (acetic acid 1 per cent.); also in alkaline fuchsin of Ehrlich, or in gentian violet aniline water. The bacilli are easily cultivated at 35–38° C. on blood serum, Agar mixture, and

FIG. 126.—PUS OF A PULMONARY ABSCESS IN A HORSE DEAD OF GLANDERS.
1. The nuclei of pus cells.
2. The glanders-bacilli.
Magnifying power 700. (The preparation has been stained with methylene-blue.)

potato. On boiled potato at 35° C., they form a characteristic yellow-brownish amber-coloured sticky film. On solid blood-serum at 37° C., after three days, one notices small translucent droplets slightly projecting over the general surface. These are the youngest colonies. On Agar culture the colonies are also translucent greyish droplets, gradually flattening and becoming dark in the centre.

According to Raskina the glanders bacilli grow also at 18–20° C., on gelatine, milk, serum, and white of egg. Kranzfeldt grew them also on glycerine Agar mixture.

There is no difficulty in obtaining good cultures in the ordinary beef broth peptone gelatine kept at 20-21° C., as also on potato at this temperature. They form on ordinary gelatine whitish-grey, flat, round, disc-shaped colonies. The gelatine is only very slowly liquefied.

Löffler and Schütz proved that the artificial cultivations inoculated into horses and asses produced typical glanders. On most white mice the bacilli do not act, nor does fresh glanders material directly taken from the horse[1]; wild mice (field mice), however, are very susceptible to inoculation with the cultures; they die within eight days, and their spleen and liver are riddled with yellowish-grey minute nodules containing numerously the glanders bacilli. In the rabbit subcutaneous inoculation produces generally a positive result; in most cases, however, only a local abscess is formed which leads to a sore rapidly healing. In guinea-pigs both the fresh glanders material, as also the culture, produce a characteristic disease: on the third or fourth day a sore is found at the seat of inoculation, which soon involves the nearest lymphatics, these being found swollen and congested; further the testis or ovary become much swollen, congested, and the seat of minute glanders nodules, so does the skin and the nasal mucous membrane, leading to purulent infiltration and, after the discharge of the pus, to ulceration. The spleen contains white nodules. The glanders bacilli are present everywhere in the deposits.

Glanders bacilli of cultures are killed by prolonged drying (in about fourteen days); the glanders material directly from the horse becomes innocuous after a few days' drying, which facts seem to indicate that the bacilli do not form

[1] H. Leo (*Zeitschrift f. Hygiene*, VII. 3) succeeded in giving glanders to white mice after feeding them for days with phloridzine, whereby their tissues contained much sugar.

spores. With this agrees also the observation of Löffler, that the cultures of the bacilli die after a few months, and Cardéal and Malet found that putrefaction destroys the bacilli, though only in many days. Löffler studied also the resistance of the bacilli to heat, and he found that, for instance, ten minutes' heating to 55° C. completely killed

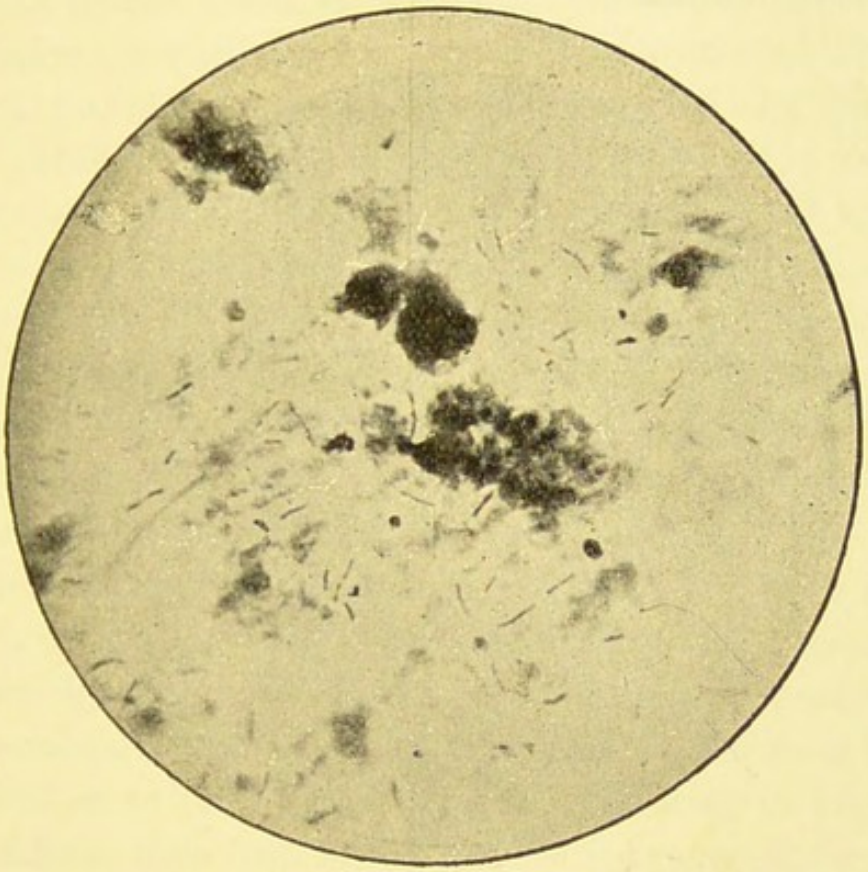


FIG. 127.—FILM SPECIMEN OF A PULMONARY NODULE OF A HORSE DEAD OF GLANDERS; NUMEROUS GLANDERS BACILLI ARE SHOWN.
× 1000.

the bacilli of the cultures; in this respect the glanders bacilli are even less resistant than many other non-spore-bearing bacilli. Further Löffler found that perchloride of mercury 1 : 5,000 kills the bacilli in two minutes, carbolic acid (3 to 5 per cent.) in five minutes. All these facts strongly point that no spore formation took place.

Under natural conditions the general mode of infection

seems to be that of inoculation. It appears to be doubtful whether the direct transmission of the glanders material on to the intact nasal mucous membrane can produce infection, since such a mode yields experimentally no result; but cutaneous and subcutaneous inoculation in horses and asses is always followed by the characteristic disease of the nasal mucous membrane.

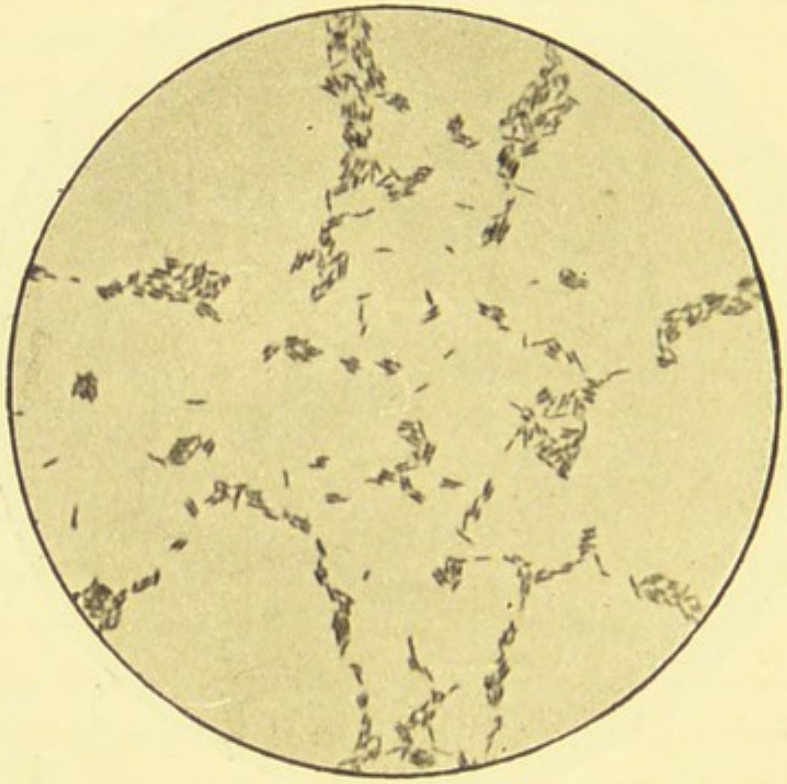


FIG. 128.—FILM SPECIMEN OF THE GLANDERS BACILLI FROM A POTATO CULTURE. × 1000.

Horses and asses are very susceptible; of carnivorous animals glanders has been observed in feline animals (lions and tigers fed on flesh of glandered horses); cats, dogs, and sheep are only very slightly susceptible, but in goats glanders has been observed; in cattle glanders is unknown. Rodents are easily infected by inoculation (*see* the experiments of Löffler and Schütz).

In man glanders occurs after infection from the horse,

generally through a cutaneous wound; it generally runs an acute course, characterised by the appearance of purulent infiltration about the seat of infection of the skin, particularly the muscular tissue, further the lung and respiratory mucous membrane; metastatic purulent infiltration occurs also in the joints, the liver, spleen, kidneys, and testis.

Within recent times it has been shown by a series of observations, carried out by a number of workers,[1] that the chemical products in the artificial cultures of the glanders bacilli (Malleïn) injected into horses produces a definite reaction—viz., a decided rise of temperature, if the animals are affected with glanders; but no reaction follows in healthy horses. So that in doubtful cases the injection of the Malleïn determines the diagnosis. The Malleïn is prepared in the same way as Koch's tuberculin (*see* below), and is a further instance of the vast importance of the study of the chemical products of pathogenic bacteria.

Malleïn at present used is either an extract of old potato cultures of bacillus malli with dilute glycerine, filtered and sterilised by steam (potato culture extract, Preusse); or Roux establishes cultures of virulent glanders bacilli in broth, incubated at 37° C. for four weeks; by heating to 110° they are sterilised, then inspissated at low temperatures to one-tenth bulk, filtered, and finally before use diluted with ten times its bulk of 0·5 per cent. carbolic (Bouillon malleïn).

Foth[2] obtains Malleïn in the form of a powder (dry Malleïn): virulent bacilli of glanders are grown in broth to which 4·5 per cent. glycerine is added and incubated for

[1] Helman, *Veterinary Society*, St. Petersburg, April, 1890; Kalning, *Archiv f. Veterinärwiss* I. 1891, St. Petersburg; Preusse, *Berliner Thierärztl. Wochenschr.*, No. 29, 1891; Heyne, *Berl. Thierärztl. Wochenschr.*, 1891, Nos. 33 and 39; Pearson, *Zeitschr. f. Veterinärk.*, No. 5, 1891; Sohne, *Sächs. Vet. Jahresbericht*, 1891, p. 56.

[2] *Fortschritte der Medizin*, No. 16, 1895, p. 639.

twenty days at 37·5° C. The culture is then inspissated at 80° C. to one-tenth its volume and filtered. From this filtrate by addition of a thirty-fold volume of 99 per cent. alcohol a white voluminous precipitate is produced, which dried *in vacuo* over calcium chloride yields a white powder, easily soluble in water. 0·04 to 0·05 grain of the powder is a dose for a horse; of the fluid preparation above mentioned (Preusse's potato culture extract) and (Roux's broth culture Malleïn) 1 cc.

If horses are injected subcutaneously with the Malleïn those affected with glanders react with great swelling and rise of temperature from 1°–2·5° C. or more; those without glanders do not react as a rule, but 1° C. rise of temperature may occur also in normal horses. The enormous number of observations on the diagnostic value of Malleïn in all countries leave no doubt that although not infallible in all cases, it has, nevertheless, in an overwhelming number of trials proved of the greatest value.

Bacillus of Syphilis.—Lustgarten described (*Med. Jahrb. der k. k. Gesellsch. d. Aerzte*, Vienna, 1885) peculiar bacilli as occurring in syphilitic products. They resemble in size and aspect very much the tubercle-bacilli; their ends are slightly thickened, and they often show nodosities; these bacilli are never found free between the tissue elements, but always inclosed in cells, generally singly or in couples, or rarely in groups, but their total number in a given section is always small. The peculiarities they show in their mode of staining have been mentioned in a former chapter.

Doutrelepont and Schütz (*Deutsche Med. Woch.* 1885, No. 19) have also demonstrated the occurrence of these same bacilli by simply staining sections made of syphilitic tissues in a watery 1 per cent. solution of gentian-violet with subsequent contrast staining by safranin.

On the other hand Cornil, and particularly MM. Alvarez and Tavel, state that a bacillus identical in mode of staining, size, and aspect with the one described by Lustgarten as the specific syphilis-bacillus, has been found by them in some normal secretions (*Brit. Med. Journ.*, Oct. 17, 1885). Klemperer, Zeissl, Baumgarten, and others have failed to find Lustgarten's bacilli in syphilis materials.

Bacillus of Foulbrood.—Messrs. F. Cheshire and Watson Cheyne described (*Microsc. Journ.*, August, 1885) a peculiar bacillus, *bacillus alvei*, which occurs in the tissues and juices of bees, and especially their larvæ, which sometimes in beehives become affected with, and die of, the disease known as "foulbrood." This bacillus shows certain peculiarities in its mode of growth in nutritive gelatine and Agar-Agar, and is capable of forming spores. With such cultivations the disease was reproduced in healthy bees.

Bacillus of Rhinoscleroma.—A. von Frisch [1] was the first to show that in the tissue of rhinoscleroma, particularly in the large hyaline cells, known as "Mikulicz cells," there occur small oval bacilli, either singly or as dumb-bells. He cultivated them and used them for inoculations on animals, but without result. Cornil and Alvarez [2] then showed that the rhinosclerom bacilli possess a gelatinous capsule, and therefore resemble the pneumonia bacilli of Friedländer (*see* a former chapter). Dittrich has then made extended experiments and observations on these rhinosclerom bacilli, and showed that morphologically and culturally they are distinguishable, but only with difficulty, from Friedländer's bacilli; though he maintains that in some minute details as to staining and as to appearance in gelatine cultures the two can be distinguished from each other. This is, how-

[1] *Wiener Med. Wochenschrift*, No. 32, 1882.
[2] *Archives de Physiologie normale et path.*, vi., 1885.

ever, not admitted by many observers. Alvarez, Paltauf and Von Eiselsberg, Wolkowitsch and Dittrich found these bacilli also in the lymphatics of the surrounding tissue. Paltauf and Von Eiselsberg, then Dittrich, Babes, and others, produced in guinea-pigs, mice, and rabbits a septicæmic infection similar to that producible by Friedländer's bacilli, but no chronic nodular disease.

The constant presence, then, of the capsulated rhinosclerom bacilli in the scleromatous tissue, particularly the Mikulicz cells, is a fact of which there can be no doubt, but it is equally a fact that they are identical with the bacilli of Friedländer; their causative relation to the rhinoscleromatous process is, therefore, more than doubtful, or at any rate not sufficiently supported.

CHAPTER XIV

BACILLUS TUBERCULOSIS AND BACILLUS LEPRÆ[1]

Bacillus Tuberculosis.—The first decisive experimental proof that tuberculosis is a communicable disease has been given by Klencke and Villemin, the latter showing that by inoculation of tubercular matter, such as sputum derived from a tuberculous patient, into guinea-pigs a chronic disease is produced, which had the distinct characters of disseminated tuberculosis in the lymph glands, the lungs, the serous membranes, the liver, and spleen. The deposits are at first minute and gray, not larger than a pin's head; they gradually enlarge and caseate in the centre, which caseation spreads over the whole tubercle. Chauvau, Wilson Fox, Burdon-Sanderson, Klebs, Cohnheim, and many others have repeated and confirmed these experiments. Inoculations with bovine tubercular matter were also made on guinea-pigs and rabbits, and true disseminated tuberculosis was produced. Feeding of calves, pigs, guinea-pigs, and rabbits with tubercular matter, both

[1] The greater part of the following account is taken from Klein's article in Stevenson and Murphy's *Treatise on Hygiene*, vol. ii., p. 210 *et passim*.

human and bovine, produced disseminated tuberculosis. The tubercular deposits of all such experimental animals transferred to normal animals again produced the same tuberculosis.

When inoculation into the subcutaneous tissue of the groin of guinea-pigs is carried out with a minute particle of human tubercular material, after a lapse of about twelve days, more or less, the lymph gland nearest the seat of inoculation can be easily felt, being a firm swollen nodule of the size of a pea; after a lapse of a further ten or twelve days the first gland is much enlarged (size of a bean or filbert) and may have become already changed into an abscess firmly fixed to the skin, but one or the other lymph gland near it can now be felt as a firm swollen nodule. The abscess soon opens and discharges thick creamy pus, a sore is established which persists, and though it may from time to time become covered with scab or crust, the accumulation of thick pus underneath soon causes again its being opened. The other enlarged lymph glands about the seat of inoculation also become converted into abscesses. When killing the animal after about four to six weeks, we find at the seat of inoculation an open sore discharging thick pus, and the subcutaneous connective tissue around and for some distance is hyperæmic and œdematous. In connection with the sore we find a chain or a packet of swollen firm lymph glands (from the size of a split pea to that of a bean) containing cheesy, yellow deposits. When cutting into such a gland we find it very juicy, and containing larger or smaller yellowish masses; in the largest gland some of these masses are already changed into thick creamy pus. At or about this stage, *i.e.* four to six weeks, in most instances either no tubercles visible to the unaided eye are yet found in the lungs, or only very few minute punctiform nodules; in the

spleen, which is enlarged, we find already numbers of minute granules projecting above the surface of the capsule, thus making the surface uneven and rough. In the liver there are numerous minute, gray, punctiform nodules, which in some places have a tendency to confluence; on section grayish streaks are recognised under a glass between the normal red liver tissue; the whole organ is slightly enlarged. The omentum shows also numerous minute opaque patches, which are only more numerous and larger than those normally found. The lymph glands in the porta hepatis are large and firm, so also those in the hilum of the spleen; the mesenteric glands are large and firm. In the marrow of long bones gray and even caseous tubercles can be distinguished. If the animal is allowed to live, it will be found gradually getting thinner towards the third or fourth month; it dies generally not before the end of the third or later than the end of the fifth month, the average duration being 100 to 120 days after inoculation.

Rabbits inoculated subcutaneously in the inguinal region with human tubercular sputum show very much less pronounced disseminated tuberculosis than guinea-pigs; after many weeks—twelve to sixteen or more weeks—the animal is found much emaciated; the lymph glands of the inguinal region enlarged, caseous; in the lungs few or no tubercles, in the liver a few tubercles, some gray, others yellow; the spleen is enlarged and contains many tubercular deposits; the mesenteric and other abdominal lymph glands swollen, firm, caseous; the process, on the whole, is very distinctly less intensive and extensive than in the guinea-pigs. I have seen numerous cases in which, after twelve to sixteen weeks, the only organ containing numerous tubercles was the spleen, the liver contained only few, the lungs none.

Feeding guinea-pigs and rabbits on human tubercular

matter produces tuberculosis, but with this difference, that while in the guinea-pig it leads to general disseminated tubercular deposits, it is far less so in the rabbit. In the guinea-pig, if the animal be killed after six to eight weeks, we find distinct tubercular deposits in the wall of the small intestine, the tubercles are situated in the Peyer's glands of the ileum and ileo-cæcal valve, are of various sizes, and

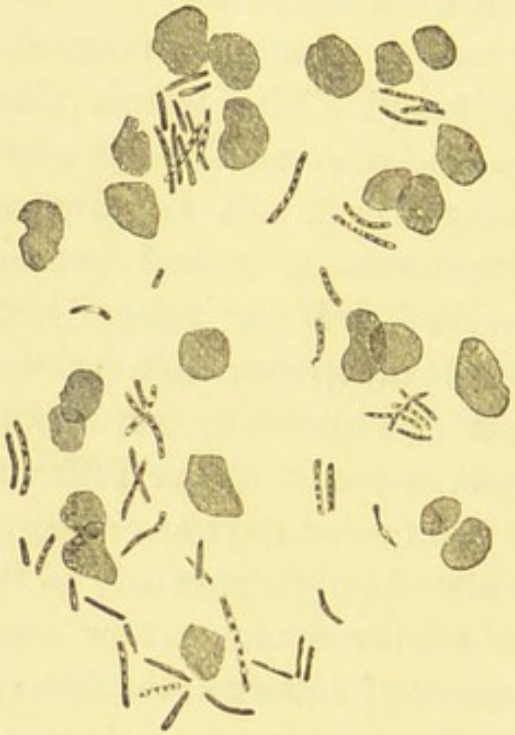


FIG. 129.—FROM A PREPARATION OF HUMAN TUBERCULOUS SPUTUM, STAINED AFTER THE EHRLICH-WEIGERT METHOD.

Nuclei and the tubercle-bacilli. Magnifying power 700.

more or less caseous in the centre; the mesenteric glands are always enlarged and contain firm caseous deposits. The liver also shows already grey tubercular nodules and streaks, the spleen is slightly enlarged and granular. The whole process, judging from the amount and progress of the changes, started in the lymphatic follicles of the ileum and spread from here into the mesenteric lymph glands, liver, and spleen. The lungs show by this time no tubercles yet.

If the disease is allowed to run its course, the animal becomes greatly emaciated and dies in about four to five months or later, and then we find tubercles in all lymph glands, in the viscera, and in the marrow of bone and serous membranes, but the changes in the abdominal viscera are the most extensive, those of the thorax considerably less.

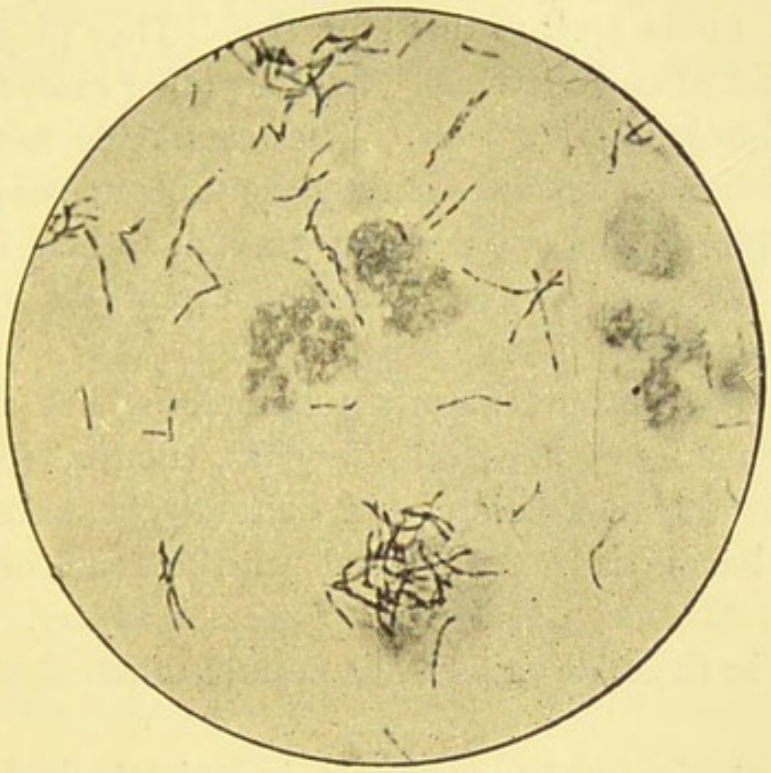


FIG. 130.—FILM SPECIMEN OF HUMAN PULMONARY TUBERCLE-SPUTUM. NUMEROUS LONG TUBERCLE BACILLI WITH SEGREGATED PROTOPLASM. (A. PRINGLE.) × 1000.

In the rabbit, on the other hand, feeding with human tubercular matter produces considerably less result; in a large percentage of cases, even after many weeks, caseous tubercles are found only in the lower ileum and mesenteric glands, the spleen, liver, and lungs appear free, only in a few cases are also these organs involved but to a small degree, viz., containing only few tubercles.

In the fowl, both by subcutaneous inoculation and by

feeding with human sputum, tuberculosis can be produced, although not all animals are equally susceptible. In most cases tubercles of the spleen, in others of the spleen and liver are the result; the intensity of the process in both these organs is striking only in a very few successful cases; in these cases we find both those organs enlarged and containing numerous spherical, firm, white nodules, from the size of a millet seed to that of a pea. They project over the capsule when superficial. In many other cases tubercles are found only in the spleen. The remarkable fact is that in most instances, notwithstanding the tuberculosis going on in their spleen, the animals are very fat; when, however, the liver becomes involved to a large extent, the animal is found emaciated.

In the fowl occurs natural tuberculosis, but as Koch has shown (*Internat. Med. Congress*, Berlin, 1890) this disease in the morphology and cultural characters of the tubercle bacilli is not identical with human or bovine tubercle. Mafucci (*Archiv f. Hygiene und Inf.* vol. xi.) has more in detail described this natural tuberculosis in the fowl.

Tuberculosis can be produced in animals (guinea-pigs, rabbits) by inhalation. By a spray producer tubercular matter finely divided can be distributed in the air in which guinea-pigs sojourn; the majority of these will become affected with general tuberculosis in the usual lapse of time, the lungs being here most advanced in the tubercular process. I have had guinea-pigs kept in their cages in the ventilating shaft at Brompton Hospital, and have thereby produced general tuberculosis in the great majority of these: caseous tubercles in the lungs, in the lymph glands, spleen, liver, pelvic glands, were the result; thus proving that the air of any place where tuberculous persons sojourn contains the

tubercle virus, and must therefore be considered as not free from danger.

In guinea-pigs, rabbits, and fowls, in all tubercular deposits giant cells are numerously met with.

Bovine Tuberculosis

Tuberculosis is a common disease of the bovine species: the number of tubercular animals is astonishingly great. In

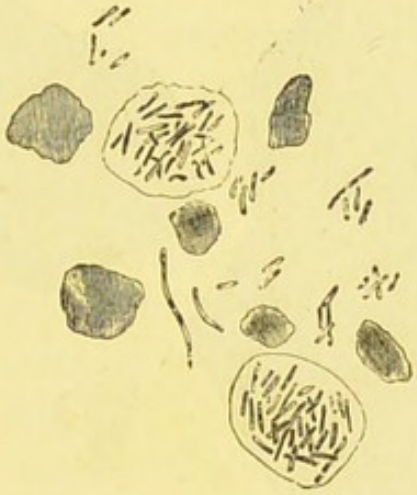


Fig. 131.—From a Preparation of Caseous Matter from Pulmonary Deposits in Bovine Tuberculosis, stained as in preceding figure.

Magnifying power 700.

many instances, on slaughtering them, only the lungs are found diseased, presenting a peculiar and characteristic appearance, viz., the surface of the lung, of the pleura and diaphragm presenting numerous oval, spherical or irregular shaped nodules, some with short broad basis, others with long thin basis or stalk fixed on to the organs, sometimes clusters of them projecting from the general surface; these appearances have caused the disease to be called "the grapes," in German "Perlsucht." Not only the surface of the lung, but also the interior contains numerous such nodular deposits. They differ considerably in size, some

not larger than a split pea, others as big as a filbert or walnut, or larger. Some of these nodules are filled with thick, creamy pus, others are yellow and caseous but firm, still others contain calcareous matter. Under the microscope the nodules contain in the periphery round cells in a fibrous matrix, amongst them very numerous giant cells of all different sizes, from one only twice or thrice the size of an ordinary leucocyte to that of a real giant, with twenty to

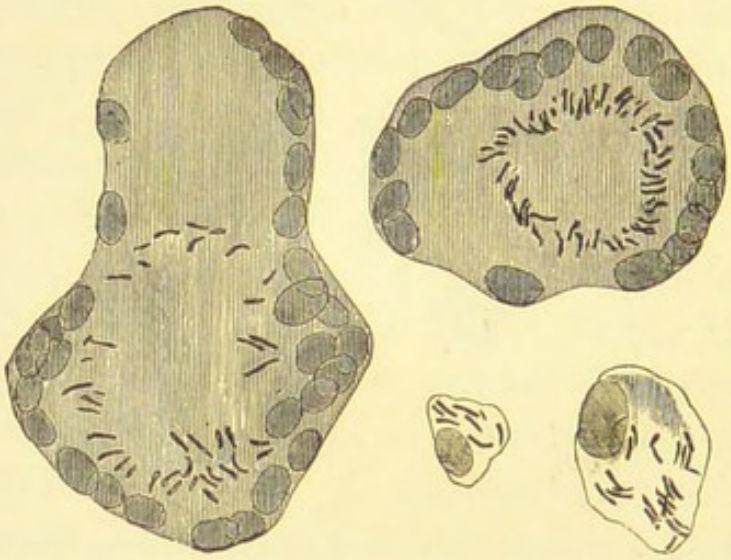


FIG. 132.—FROM A SECTION THROUGH TUBERCULOUS DEPOSITS IN THE LUNG OF A COW.

Two giant-cells and two small cells containing tubercle-bacilli.
Magnifying power 700.

thirty and more nuclei all regularly disposed near a peripheral zone of the cell. Near the caseous portion these huge giant cells are very conspicuous ; the caseous part may still show the outline of the giant cells, but their nuclei do not take the stain, and the whole tissue of the caseous portion is a granular débris. In pronounced and advanced cases, freely projecting nodules, as also nodules within the substance, having a great tendency to suppurate, are met with in the lymphatic glands, in the spleen, liver, and even

in the milk gland. In this latter, the condition assumes an important practical aspect, since the udder of a cow may contain tubercular nodules without these being easily diagnosed, and which may give to the milk infective properties. Although in light cases the milk gland is found free of tubercles, yet in many advanced cases purulent tubercular deposits have been demonstrated in the udder.

In the lymph glands, spleen, and liver, the character of the nodules is the same as in the lung, and giant cells form a very conspicuous feature.

Infection with general tuberculosis of guinea-pigs and rabbits by bovine tubercular matter, both by feeding and subcutaneous inoculation, is easily achieved ; the result is more intense and much more rapid than by infection with human tubercular matter. Guinea-pigs subcutaneously inoculated develop disseminated tuberculosis of the lymph glands, lungs, liver, spleen, serous membranes, and marrow of bone in less than half the time ; in some cases the animals die in about five to six weeks with remarkably widespread and advanced tubercular deposits. Also as regards rabbits, the process is much more rapid and more intensive ; for while these animals, as mentioned above, after inoculation with human tubercular matter, develop, as a rule, only a more or less mild form of tuberculosis, limited chiefly to some lymph glands, spleen, and perhaps the liver, after inoculation with bovine tubercular matter they show very numerous tubercular deposits in the lungs, liver, and spleen, all lymph glands, and even the kidneys. The same results are obtained by feeding rabbits and guinea-pigs with bovine tubercular matter. Here also the process starts with tubercles of the ileum, then spreads to the mesenteric glands, the pelvic glands, the omentum, spleen, and liver, and finally the lungs and sternal and bronchial lymph glands. The differ-

ence in the intensity and duration of the process is decidedly more pronounced with bovine than human tubercular matter, and also in the rabbit the difference between feeding with bovine and with human tubercular matter is striking; so that there can be no question that bovine tubercular matter acts in a conspicuous degree more virulently than human tubercular matter, both in guinea-pigs and rabbits.

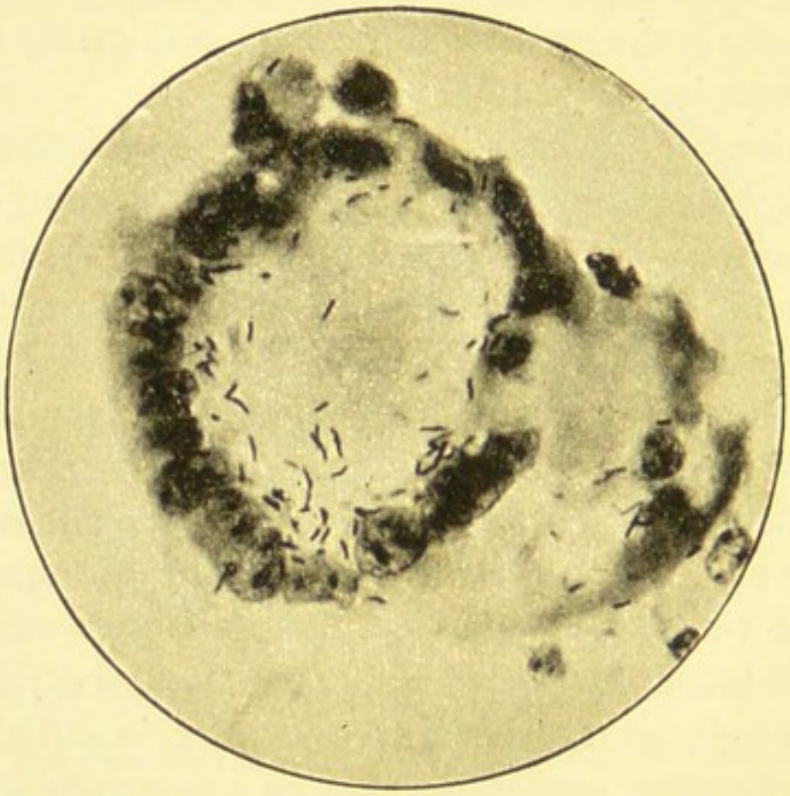


FIG. 133.—A SINGLE GIANT-CELL IN BOVINE PULMONARY TUBERCLE CONTAINING NUMEROUS TUBERCLE BACILLI; AROUND THEM THE CHARACTERISTIC RING OF THE NUCLEI. (A. PRINGLE.)

× 1000.

Feeding calves with milk derived from an udder containing tubercular deposits produced tuberculosis in these calves, but milk coming from a healthy udder (though the cow had tubercles in the lung) failed to produce tubercle.

Hirschberger (*Experim. Beiträge zur Inf. der Milch tuberculöser Thiere*, München, 1889) finds that at least five per

cent. of milch cows are tubercular, and though in many cases their milk is not different from the milk of normal cows, and no tubercle bacilli can be detected, the same milk injected into the peritoneal cavity of guinea-pigs nevertheless produced miliary tuberculosis in the peritoneum, spleen, and liver. Out of twenty series of experiments only once could the tubercle bacilli be demonstrated in the milk,

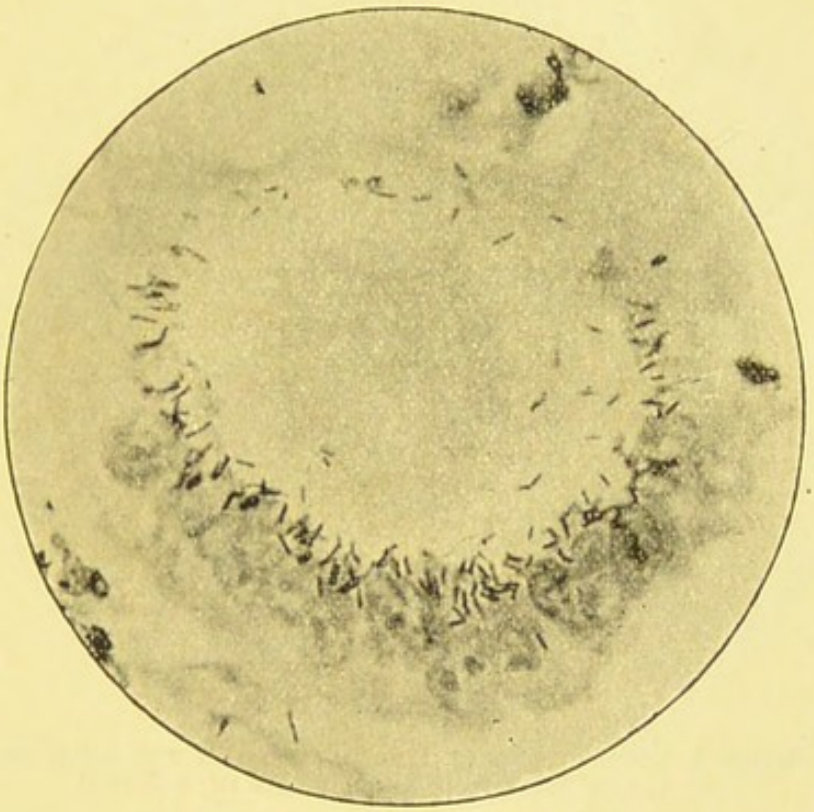


FIG. 134.—A SINGLE GIANT-CELL, FROM A SIMILAR SPECIMEN AS IN PRECEDING FIGURE.

The ring-like arrangement of the tubercle bacilli and the nuclei around them are well shown. (A. Pringle.)

× 1000.

and yet in ten such experiments in which the milk did not show tubercle bacilli, it nevertheless produced tuberculosis on intra-peritoneal injection. Hirschberger explains these results by assuming that though tubercle bacilli were not present in the milk as bacilli, their spores must have been present.

The comparatively numerous cases of miliary tuberculosis in children suggest the probability that they are due to the consumption of cows' milk containing the tubercular virus derived from a tubercular udder. Dr. Sims Woodhead's explanation that the numerous cases of tabes mesenterica (tuberculosis of the intestines and mesenteric glands) of

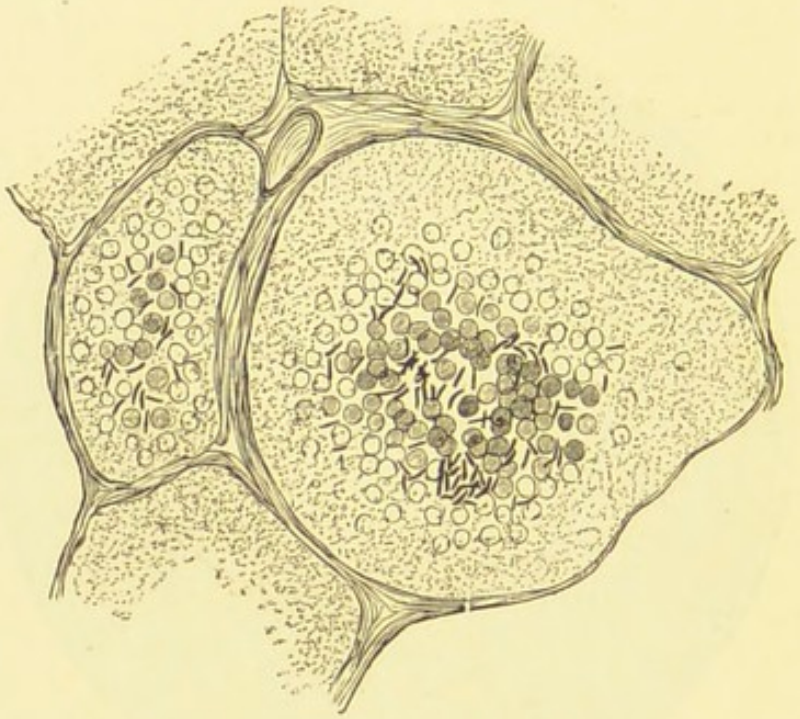


FIG. 135.—FROM A SECTION THROUGH A TUBERCLE OF THE LUNG FROM A CASE OF ACUTE MILIARY TUBERCULOSIS IN A CHILD.

Several alveoli are seen filled with débris ; in the centre of this are numerous nuclei, and amongst them the tubercle-bacilli. Magnifying power about 350.

children are attributable to the consumption by these children of milk derived from the tubercular udder of the cow seems a feasible one.

Cohnheim and Salamonson were the first to show that in all tubercular material there is present a specific virus. By injecting a small particle of such matter into the anterior chamber of the eye, they noticed that after the first result due to the injury has passed off, the introduced particle gradually

undergoes diminution to almost complete disappearance, but in about a fortnight or three weeks there occur in the iris a crop of minute gray nodules which in reality are typical young tubercles; these gradually enlarge, and like all ordinary tubercles undergo caseation; while the tubercular

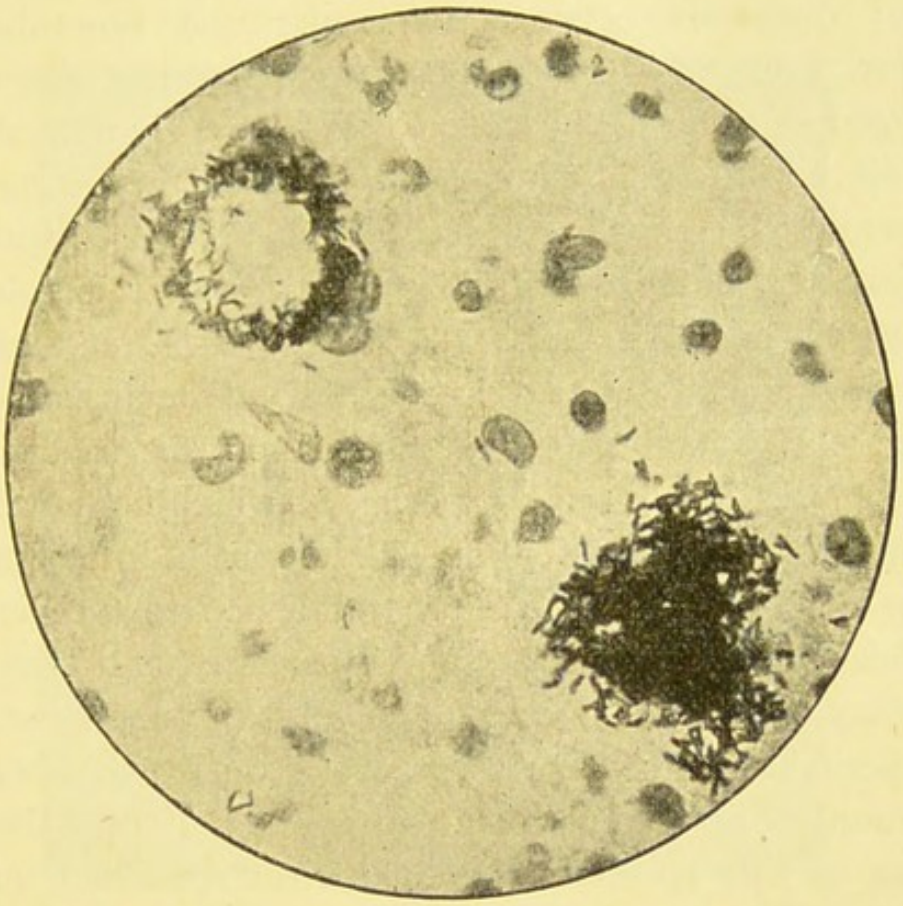


FIG. 136.—FROM A SECTION THROUGH A TUBERCLE IN THE LIVER OF A RABBIT INFECTED INTRAVENOUSLY WITH CULTURE OF TUBERCLE BACILLI.
Two giant-cells full of tubercle bacilli are seen in the tissue of the tubercle.
× 1000.

process is at first localised to the iris, it gradually spreads to the cervical lymph glands, and ultimately leads to general tuberculosis of the other lymph glands and viscera exactly as after subcutaneous inoculation. This typical production of a crop of gray tubercles on the iris by tubercular matter enabled Cohnheim and Salomonson to differentiate tuber-

cular from non-tubercular matter, and they have formulated this fact by saying that only matter that is derived from tubercle is capable of producing tubercle, and that whenever any substance is found capable of producing this iris tuberculosis, it is derived from tubercle. By this clear proof for the first time a means was offered to make a definite differential diagnosis between tubercular and non-tubercular matter, a diagnosis which those who, at former times, were engaged in work on tubercle, found extremely difficult. As is well known from clinical observation, the diagnosis of tuberculosis of the lungs is sometimes associated with difficulties: the physical examination and symptomatology do not always insure a correct diagnosis. It is true that Villemin had proved by experiment that tuberculosis is inoculable, and Wilson Fox had insisted on the specificity of tuberculosis by numerous experiments which he himself had carried out, yet there were authorities who did not draw this sharp distinction, but were rather inclined to the view that artificial tuberculosis is due to infective matter derived from a variety of sources not necessarily always tubercular. For that period, therefore, the exact proof given by Cohnheim and Salomonson marked a very important step, though the exact nature of this specific tubercular virus remained undetermined. The next discovery was that of Koch, who showed what this nature is; he demonstrated a particular species of bacilli, now familiar to all pathologists as the *tubercle-bacillus*, which he found only in tubercle and in no other disease, a bacillus so peculiar and so constant that its important diagnostic value was at once recognised. No matter whether it is a nodule in any tissue or organ that does or does not present the typical pathological (gross and minute) characters of the classical tubercle, no matter whether in man or the bovine species, in the sheep, in the monkey, dog, cat,

rat, mouse, rabbit, or guinea-pig, fowl or ostrich, if in such a nodule the bacilli characteristic of tubercle can be demonstrated, *that nodule is tubercle, and the disease tuberculosis.* The discovery by Koch of this fundamental fact marks one of the most brilliant and most practical discoveries of modern medical science; the diagnosis of tubercle,

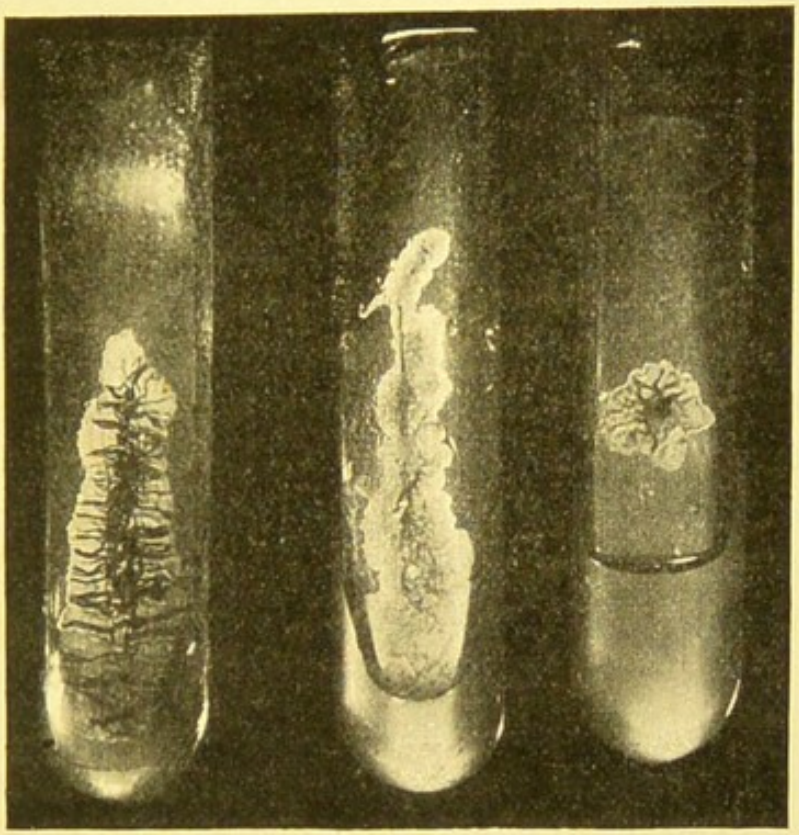


FIG. 137.—THREE TEST-TUBE CULTIVATIONS OF TUBERCLE BACILLI ON GLYCERIN AGAR AFTER SEVERAL WEEKS.

Natural size

once so difficult to make with certainty, is now, by means of the demonstration of the presence of the tubercle bacilli, one of the easiest and at the same time one of the most important helps in the formation of a correct diagnosis in many otherwise doubtful cases. Koch further proved that not only are these particular bacilli present in all and every tubercle of man and brutes, but he also showed that

these bacilli can be artificially cultivated outside the animal body, and with such cultures by inoculation typical and general tuberculosis can be produced, the tubercles thus produced again containing the same tubercle bacilli; in short *he conclusively established that these bacilli are the* vera causa *of the disease tuberculosis.* The whole problem concerning one of the most widespread, fatal, and little understood diseases of man and animals was by these researches at once cleared up, and considering the difficulties in the solution of the problem and the necessity of having had to invent special methods by which this research was carried to a successful issue, I have no hesitation in saying that this discovery of Koch marks one of the most important, if not the most important, landmark in pathology. Many are the workers who since Koch have contributed towards furthering details as to this question, but without any intention of minimising the importance of any and every contribution of facts towards a clear understanding of disease and its prevention, I am, I think, within the limits of absolute correctness in saying that Koch's publications on tuberculosis (*Deutsche med. Woch.*, 1881; and *Mitth. aus d. K. Gesundheitsamte, ii.*) contain almost the complete solution of the problem. The best method, and always used with success, is the staining of film-specimens or of sections with Ziehl's carbol fuchsin from twenty to thirty minutes at 35° to 40° C., then wash in water for a second or two, then for a few seconds (five to ten) in 30–33 per cent. nitric acid, wash again in water and place in methyl-blue aniline oil for five to ten minutes, wash and treat in the usual way, according to whether cover-glass specimen or section. I have never seen failure with this method: the tubercle bacilli are always brought out with striking clearness.

A great many modifications for staining the tubercle

bacilli have been published, which are all good to a lesser or greater degree, but the one just mentioned is as good and in many instances has proved simpler and better. The tubercle bacilli always occur in tubercular nodules, more numerously where caseation has already set in than in the earlier stages. They occur isolated or in groups between the cells constituting the tubercle, or they are found singly or in small groups within the larger cells; when present in giant cells they are found in large numbers forming a sort of zonular ring around the central portion (*see* Figs. 133 and 134). In some giant cells their number is sometimes very limited, and Koch has concluded from this fact that the tubercle bacilli suffer death in the giant cells and hence disappear from them. In human tubercle the tubercle bacilli are, as a rule, between the elements constituting the tubercle, but as just mentioned they also occur within the cells uni- and multi-nucleated. In bovine tubercle, however, the rule is that they are mostly present in the uni- and multi-nuclear cells, and only when these degenerate and break up do they become free; in the caseous matter they are present in groups in the granular débris. In tubercle of rabbit (lung and liver) produced by inoculation with bovine tubercular matter, or with artificial culture derived from bovine tubercle, the presence of tubercle bacilli within the cells—small, large, and giant cells—is very conspicuous, and yields very remarkable specimens (see Fig. 136).

The tubercle bacilli in human tubercle are delicate cylindrical rods measuring 1·5–4 μ; many are straight, with rounded ends, but others are slightly curved; in preparations (sputum, purulent matter or sections) stained in the above manner the bacilli always appear composed of granules, that is to say, within a faintly stained sheath the protoplasm is segregated into deeply stained, cubical, spheri-

cal, or rod-shaped granules; between the granules the sheath is empty, but these empty places are not to be taken for bright spores, as is done by some observers, nor is it proved that the above granules are spores. That the tubercle bacilli contain spores is proved by numerous experiments of drying and heating, to be detailed below, but what the character of these spores is, and how they appear in the bacilli, has not been satisfactorily shown. In bovine tubercular matter prepared in the same manner the tubercle bacilli are distinctly shorter and thinner, and though I do not for a moment question the fact that some tubercle bacilli of human tubercle are as short and thin as those of bovine tubercle, I am confident from numerous observations that the majority of the human tubercle bacilli of sputum are longer and thicker than those of bovine tubercle; besides, in preparations stained in the above manner alike, the segregation of the protoplasm within the sheath, though also present in many tubercle bacilli of bovine tubercle, is not so general and uniform as in those of human tubercle. But these minute differences need mean nothing more than differences due to the different soil on which the bacilli were reared. Such morphological differences in size and aspect in one and the same species of microbes are well known to occur in other instances if the microbe be cultivated in different soils. When the tubercle bacilli from whatever source (bovine, human, or from articially infected animals) are passed through the rabbit or the guinea-pig, in these animals the new crop of bacilli all appear to be morphologically the same.

Scrofula and Lupus

Koch and many other observers have shown that, both in scrofula and lupus, tubercle bacilli occur, and that with both these materials general tuberculosis can be induced in guinea-pigs. But since these two diseases are in the human subject well-marked disorders, distinct from pulmonary tuberculosis, it is necessary to assume that the tubercle bacilli in the three diseases possess some functional differences. To say that lupus is a form of tuberculosis of the skin does not cover the facts, since real tuberculosis of the skin does occur, and is totally different from lupus ; so also scrofula is not merely tuberculosis localised in the cervical lymph glands, since, in many instances, it does not lead to pulmonary and general tuberculosis, whereas the true tuberculosis of lymph glands does do so. It is quite feasible to assume that both lupus and scrofula are tuberculosis, but that in origin and virulence their tubercle bacilli are different from the bacilli, causing true tuberculosis. That the virulence of the virus of lupus and scrofula cannot be the same as that of the material of human and bovine pulmonary tubercle is proved by experiments of Dr. A. Lingard,[1] who showed that the duration and extent of the disease induced by inoculation of lupus or scrofula into guinea-pigs are quite different from that induced by pulmonary tubercular matter, and, further, that if a guinea-pig is made tubercular with scrofulous matter, and the tubercle of such an animal is again transmitted by inoculation through several generations of fresh guinea-pigs, the disease thus produced gains gradually in shortness of duration and intensity, until after

[1] *Reports of the Medical Officer of the Local Government Board*, 1888–89, p. 462.

several generations the same effect of general tuberculosis is produced as that directly by matter of pulmonary human tuberculosis.

The tubercle bacilli show definite characters in cultivation. Koch succeeded in cultivating them on solid blood-serum. Inoculating the slanting surface of the solid serum with tubercular matter, and provided no other bacteria are introduced, Koch noticed the first signs of growth in ten to fourteen days. Koch used for inoculations of the serum tubes the tubercular deposits of a swollen lymphatic gland of a guinea-pig, three to four weeks previously inoculated with tubercular matter, clean sterile instruments being used. After ten to fourteen days the first signs of the growth of the tubercle bacilli show themselves in the form of whitish points and patches, resembling dry scales. On further growth they enlarge, and where close together they coalesce into dry whitish scaly masses with irregular outline. From such primary cultures subcultures on serum were then carried out. But under a magnifying glass, or better under the microscope, the growth and multiplication of the tubercle bacilli can be seen already before the end of the first week. Peculiar curved, or convoluted, or S-shaped whitish lines, which prove to be strands of tubercle bacilli, are noticed even at this early stage. On Agar broth the growth is very limited, so also in broth. But Roux and Nocard showed that by adding six per cent. glycerine to Agar meat infusion, or to meat broth, the tubercle bacilli can be brought to rapid and extensive multiplication. On glycerine-Agar-beef broth the tubercle bacilli grow very rapidly, the growth being already visible after six to eight days, and after several weeks covers the whole surface as a whitish, peculiarly wrinkled, dry film (Fig. 137) extending as a pellicle over the condensation water at the bottom of the tube. In order to

obtain good and copious growth it is necessary to keep the tubes capped from the outset, and in this way I have obtained very copious growths in alkaline broth, to which a piece of boiled white of egg is added. In such tubes the broth kept at 37° C. remains clear for four to five days, the minute flocculi and granules appear at the bottom and along

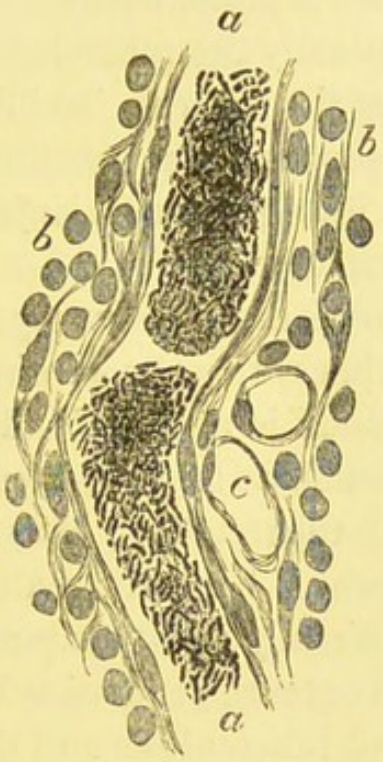


FIG. 138.—FROM A SECTION THROUGH THE KIDNEY OF A RABBIT DEAD OF ARTIFICIAL TUBERCULOSIS.

a. Blood-vessel filled with caseous matter, and in it numerous tubercle-bacilli.
b. Nuclei of cells of the tuberculous new growth.
c. Capillary vessel in cross section.

Magnifying power 700.

the wall of the tube where it is in contact with the broth ; after a fortnight the growth is abundant, and on shaking the broth is made turbid by the numerous flocculi. On potato moistened with broth it is likewise possible to get growth. Temperatures between 36° to 38° C. are most favourable for the growth ; below 30° or above 42° C. no growth can be noticed.

Koch has shown that by subcutaneous inoculation, by inhalation, inoculation into the peritoneum, the anterior chamber of the eye, &c., of artificial subcultures far removed (by many generations) from the original source, typical tuberculosis is produced in all animals susceptible to tubercle (guinea-pigs, rabbits, dogs, rats, mice), and that of course the tubercular deposits in these experimental animals again contain abundantly the tubercle bacilli; thus the final and exact proof that the tubercle bacilli are the *vera causa* of the tubercular process was definitely established. The intravascular and intra-peritoneal injection produced the most striking and rapid results.

Although the growth on glycerine Agar mixture is copious, it yet has this drawback, that by continued subculture the virulence of the bacilli is worn off. The first subcultures act virulently, inasmuch as they produce on inoculation into guinea-pigs general tuberculosis; thus even with a fourth and fifth subculture I have succeeded in producing the same results as by directly using sputum or bovine tubercle, but after the eighth or tenth generation I have not succeeded in producing general tuberculosis and death of the guinea-pigs by inoculation. I have found that if from an Agar-glycerine culture, which, owing to age or subcultures, has lost completely its virulence, new cultures are established in alkaline beef broth, to which a piece of boiled white of egg is added, these acquire rapidly again a somewhat virulent character.

Also on Agar ascites fluid with glycerine (*see* a former chapter) the original virulence can be re-established. It ought to be also stated that by continued subculture in glycerine broth or in glycerine Agar the growth becomes more abundant and makes its appearance in much shorter time.

The subcultures on glycerine Agar show after several months besides the typical forms of cylindrical and granular tubercle bacilli also some filaments made up of rods and granules. Some of these filaments are remarkable by their being undoubtedly branched like the mycelium of a hyphomycetes, and, further, that some are club-shaped at the end or beaded in their course; these club-shaped and branched filaments (see Fig. 138) are the more numerous the older the culture. Although the club-shaped and beaded condition might correspond to involution of the threads, the branched condition cannot, and therefore the club-shaped forms may well represent the growing ends of the threads of a mycelium; the two together, *i.e.*, club-shaped and branched threads, would, therefore, indicate that the typical tubercle bacillus is a phase only in the development of an organism, which under certain conditions (glycerine Agar) declares its true nature and origin, being, namely, comparable to a fungus having a mycelial stage (*see* Klein in the Reports of the Medical Officer of the Local Government Board, 1889-90, Plate XXVII., Figs. 61, 62, 63).

Later, Mafucci (*Archiv f. Hygiene und Infect.*, xi. p. 445) described the same forms in the culture of the tubercle bacilli of the fowl, and Fischel (*Fortschr. d. Med.*, Bnd. x. No. 22, p. 908) also of the human tubercle cultures; and this latter observer arrived at the same conclusion as myself—viz. that we are dealing with forms which are comparable to a mycelial fungus.

That the tubercle bacilli in one phase or another do contain spores has been shown by Koch, who found that tubercular sputum when thoroughly dried maintains its virulent character. This has been confirmed by other observers.

Now, if the tubercle bacilli had no spores, they would not in all cases survive thorough drying; no sporeless bacillus is known that can survive thorough drying; whereas all bacilli in the stage of spore-bearing survive this

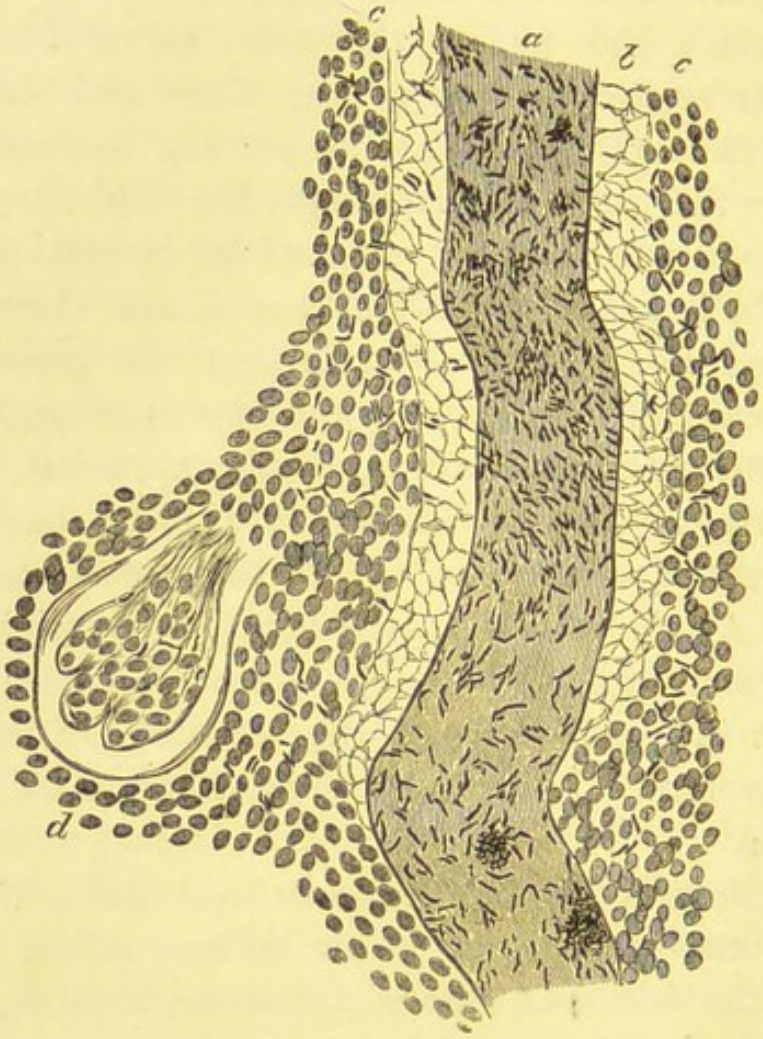


FIG. 139.—FROM THE SAME KIDNEY AS IN PRECEDING FIGURE.

a. Large artery filled with caseous matter, and in it numerous tubercle-bacilli.
b. Coat of artery.
c. Nuclei of the tuberculous new growth. *d*. A Malpighian corpuscle.
Magnifying power about 500.

process. Further, tubercular matter and cultures of tubercle bacilli survive temperatures up to 100° C. Non-spore-bearing bacilli and micrococci are killed by being exposed for five minutes to a temperature of 60–70 C., whereas

spores of other bacilli withstand much higher temperatures. Tubercular sputum distributed in salt solution does not lose in the least its virulence by being kept at 100° C. from one to two minutes. Nor does a solution of perchloride of mercury kill the tubercle bacilli in the way it does sporeless bacilli. Dr. Lingard found (Report of Medical Officer of Local Government Board for 1885–86, p. 183) that solution of perchloride of mercury, one grain of mercuric bichloride to 960 grains of water, that is to say, about one in 1,000, although it kills the bacilli in human

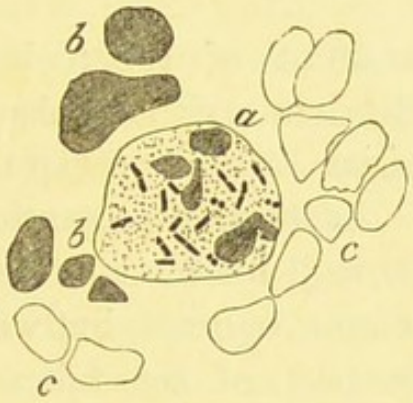


FIG. 140.—FROM THE JUICE OF LUPUS-TISSUE PREPARED AFTER THE KOCH-WEIGERT METHOD OF DRYING A THIN LAYER ON A COVER-GLASS.
Magnifying power about 700.

tubercular matter when acting on it for four hours, does not do so in the case of bovine tubercular matter, since not even eight hours' exposure to the solution is sufficient to neutralise the infective power of that material. This also shows what has been mentioned already on a former page, viz., that bovine tubercular matter is of a higher degree of virulence than human tubercular matter.

The Royal Commission on Tuberculosis issued in 1895 their Report (Part I.) and from the evidence given before them and the extensive researches made for them by

Dr. Sidney Martin and Dr. Sims Woodhead they arrived unanimously at the following conclusions (p. 20) :

"We have obtained ample evidence that food derived from tuberculous animals can produce tuberculosis in healthy animals. The proportion of animals contracting tuberculosis after experimental use of such food, is different in one and another class of animals ; both carnivora and herbivora are susceptible, and the proportion is high in pigs. In the absence of direct experiments on human subjects, we infer that man also can acquire tuberculosis by feeding upon materials derived from tuberculous food-animals.

"The actual amount of tuberculous disease among certain classes of food-animals is so large as to afford to man frequent occasions for contracting tuberculous disease through his food. As to the proportion of tuberculosis acquired by man through his food or through other means we can form no definite opinion, but we think it probable that an appreciable part of the tuberculosis that affects man is obtained through his food.

"The circumstances and conditions with regard to the tuberculosis in the food-animal which lead to the production of tuberculosis in man are, ultimately, the presence of active tuberculous matter in the food taken from the animal and consumed by the man in a raw or insufficiently cooked state.

"Tuberculous disease is observed most frequently in cattle and in swine. It is found far more frequently in cattle (full grown) than in calves, and with much greater frequency in cows kept in town cow-houses than in cattle bred for the express purpose of slaughter. Tuberculous matter is but seldom found in the meat substance of the carcase, it is principally found in the organs, membranes,

and glands. There is reason to believe that tuberculous matter, when present in meat sold to the public, is more commonly due to contamination of the surface of the meat with material derived from other diseased parts, than to disease of the meat itself. The same matter is found in the milk of cows when the udder has become invaded by tuberculous disease, and seldom or never when the udder is not diseased. Tuberculous matter in milk is exceptionally active in its operation upon animals fed either with the milk or with dairy produce derived from it. No doubt the largest part of the tuberculosis which man obtains through his food is by means of milk containing tuberculous matter.

"The recognition of tuberculous disease during the life of an animal is not wholly unattended with difficulty. Happily, however, it can, in most cases, be detected with certainty in the udders of milch cows.

"Provided every part that is the seat of tuberculous matter be avoided and destroyed, and provided care be taken to save from contamination by such matter the actual meat substance of a tuberculous animal, a great deal of meat from animals affected by tuberculosis may be eaten without risk to the consumer.

"Ordinary processes of cooking applied to meat which has got contaminated on its surface are probably sufficient to destroy the harmful quality. They would not avail to render wholesome any piece of meat that contained tuberculous matter in its deeper parts. In regard to milk we are aware of the preference by English people for drinking cows' milk raw, a practice attended by danger, on account of possible contamination by pathogenic organisms. The boiling of milk, even for a moment, would probably be sufficient to remove the very dangerous quality of tuberculous milk."

In August 1890, on the occasion of the International Medical Congress held in Berlin, and in subsequent publications, Koch announced that by experiment on guinea-pigs he had ascertained that when guinea-pigs, previously made tubercular by subcutaneous inoculation, be inoculated again with extracts (glycerine extract) of sterilised tubercle cultures, the growth itself from the surface of serum or glycerine Agar being rubbed down and extracted with dilute glycerine, or with the filtrate of glycerine broth cultures (previously sterilised), the tubercular glands undergo a rapid necrosis and elimination, brought about by an acute reactive inflammation setting in in the tissues around the tubercle, but the tubercle bacilli themselves are not affected by it. He then applied this method of injecting glycerine extract of tubercle cultures—*tuberculin*—in very small doses, 0·001–0·01 gramme, on the human subject, lupus, bone tuberculosis, early pulmonary tuberculosis. The result was remarkable, since most patients affected with one or another form of tuberculosis reacted very conspicuously to such injection, high temperature, great local congestion and inflammation in lupus and bone tubercle; persons not affected with tubercle, as a rule, not showing any reaction to such small doses. In lupus, bone and joint tubercle, the tubercular tissue becomes necrotic, is either spontaneously eliminated, as in lupus, by the reactive inflammation of the surrounding tissue, or can be removed by surgical aid, as in tuberculosis of bone. Tuberculin is, then, a distinct means of diagnosing tubercle, otherwise not easily diagnosed.

Weyl has analysed the tuberculin, and found that the essential portion of it is a substance related to mucin, not to albumin.

Good therapeutic results have been obtained with the

tuberculin in lupus, bone tuberculosis, and in early pulmonary tuberculosis; in advanced pulmonary tuberculosis the injection of tuberculin has in some cases produced a dissemination of the tubercle bacilli and acute miliary tuberculosis in the lung and other viscera (Virchow). The same or similar results had been obtained in tuberculised guinea-pigs by Baumgarten after injection of tuberculin.

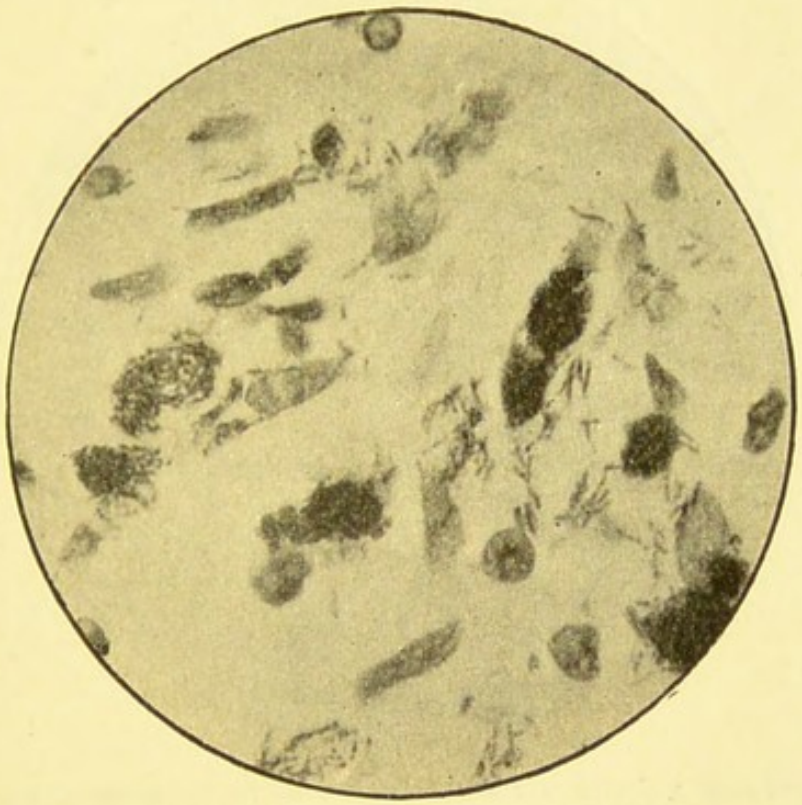


FIG. 141.—FROM A SECTION THROUGH LEPROUS SKIN, SHOWING NUMEROUS LEPROSY BACILLI IN CELLS AND BETWEEN THEM.
× 500.

With Koch's tuberculinum a large number of observations have been made in all countries as to its diagnostic value in bovine tuberculosis, and the result is overwhelmingly in favour of it, since by the positive reaction (raised temperature and constitutional disturbance) produced in a given animal after subcutaneous injection of tuberculinum, it is possible to diagnose tuberculosis even when other (physical

and clinical) signs are wanting. And although the results of the use of tuberculinum for diagnostic purposes are not absolutely uniform, they are nevertheless sufficiently striking to consider such use as of paramount importance.

Bacillus Leprœ.—Armauer Hansen[1] first ascertained the existence of large numbers of minute bacilli in the peculiar

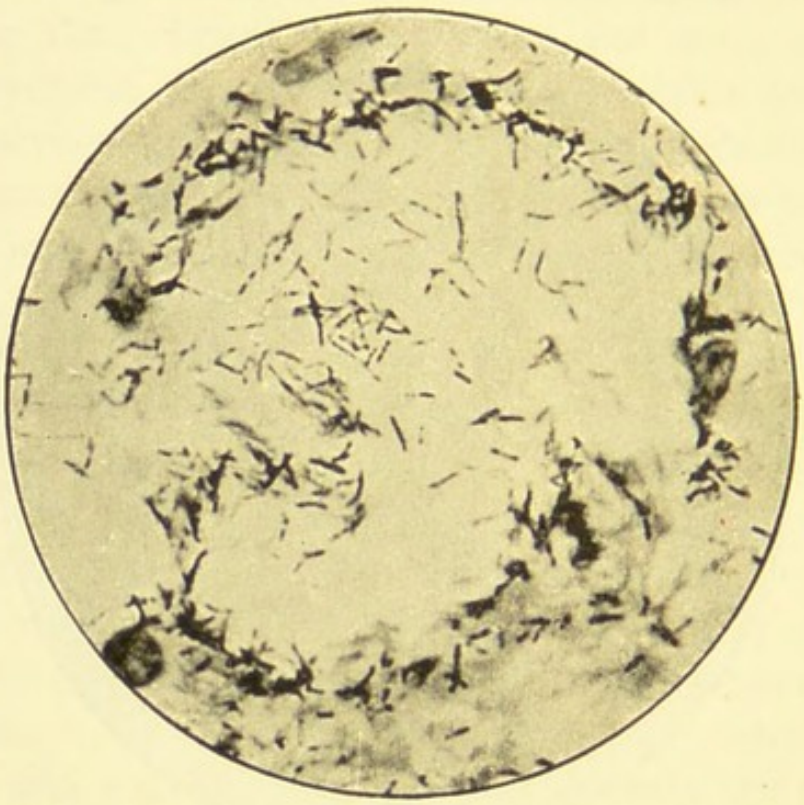


FIG. 142.—FROM A SECTION THROUGH LEPROUS SKIN, SHOWING THE LEPROSY BACILLI. (E. C. BOUSFIELD.)
× 1000.

large leprosy-cells of Virchow, which constitute the nodules of leprous patients. Neisser[2] confirmed this, and considerably extended our knowledge of the bacilli, showing that they can be readily stained with fuchsin or with Ehrlich's acid solution of eosin-hæmatoxylin. The bacilli

[1] *Virchow's Archiv*, vol. lxxix. ; and *Quart. Journ. of Micro. Sci.* 1880.

[2] *Breslauer ärztl. Zeitschr.*, xx. and xxi., 1877, and *Virchow's Archiv*, 84.

are stiff rods about 4 to 8μ long and less than 1μ thick. They are pointed at their ends, and always occur in masses within the large leprosy-cells of the leprous tubercles of the skin and internal organs. But they are also present in the interstitial tissue of the nervous branches in the anæsthetic variety of the disease.[1] Some bacilli are motile, others

FIG. 143.—FROM A SECTION THROUGH THE LARYNX OF A PATIENT DEAD OF LEPROSY.

Huge cells in fibrous connective tissue ; the cells are filled with the leprosy bacilli. Magnifying power 600. (Stained with magenta and vesuvin.)

not ; some are more or less granular and beaded, owing to local collections of the protoplasm within their sheath.

Cover-glass specimens made from a scraping of a leprous nodule or the discharge of a leprous ulcer by spreading out

[1] Compare also Cornil, *Union Médicale*, 1881, Nos. 178, 179, and Babes, *Archives de Physiologie*, July, 1883.

a thin film on a cover-glass, drying and heating then staining after Ehrlich's method of staining for tubercle bacilli (in carbol fuchsin for twenty to thirty minutes at 35° C., then washed in water, then for a few seconds in 33 per cent. nitric acid, washed again in water, dried, and mounted) show the leprosy cells, some small, some very big, all crowded with the stiff, thin, and relatively long bacilli lepræ. Many cells are in a state of disintegration, or broken down into granular débris and in accordance with this numerous bacilli are found free, isolated, or in groups. The large and middle-sized cells are particularly interesting, since their substance is almost entirely occupied with the bacilli arranged in bundles, which bundles often lie towards

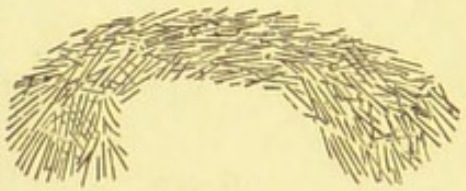


FIG. 144.—FROM AN ARTIFICIAL CULTURE OF BACILLUS OF LEPROSY. (After Neisser.)

one another under sharp angles, and hereby produce a very striking effect. Sections through a leprous tubercle stained in the above manner (in carbol fuchsin passed through 33 per cent. nitric acid, washed in water, then counter-stained in methyl blue anilin water for fifteen to thirty minutes) show the nuclei of the tissue blue, the cells forming the leprous nodule red, owing to the fact that their substance is crowded with the (red) leprosy bacilli; in such sections nothing can be seen of the nuclei or substance of the leprosy cells, the cells being marked merely as groups of densely aggregated leprosy bacilli (fig. 143). While, then, the lepra bacilli have characters in staining by which they resemble the tubercle bacilli, they differ accord-

ing to Baumgarten and others in this, that they stain in alkaline methylene blue with conspicuously greater difficulty than the tubercle bacilli.

Neisser has shown that the characteristic leprosy cells are only wandering cells or leucocytes modified by the growth and multiplication in them of the bacilli. In the blood the bacilli do not occur, and they spread probably only by way of the lymphatics.

FIG 145.—FROM A SECTION THROUGH A NODULE OF THE LIVER OF RHEA.

1. Cells of various sizes filled with minute bacilli; owing to the smallness of the bacilli and to their being crowded in the cells and owing to the comparatively low magnifying power (300) the bacilli appear like dots.

(Stained with fuchsin and methyl-blue.)

Inoculation experiments on domestic animals and monkeys have hitherto failed.[1] Damsch[2] maintains, however, that he was able, by inoculation with leprous tissue into the peritoneal cavity and into the skin, to produce in cats a distinct increase and sprouting of the bacilli.

[1] Köbner, *Virchow's Archiv*, vol. lxxxviii.; Hansen, *ibidem*, vol. xc.
[2] *Virchow's Archiv*, vol. xcii.

Neisser maintained that he had succeeded in cultivating the lepra bacilli, but the evidence he adduced is not deemed sufficient. Bordoni-Uffreduzzi (*Zeitschrift f. Hygiene, iii.*, p. 178) maintains, however, positively that he has produced artificial cultures from the leprosy nodules of bone marrow, on glycerine serum to which peptone and salt had been added, kept at 35–37° C. The line of inoculation became marked as a yellowish irregularly outlined band; the serum was not liquefied. On glycerine Agar, inoculated with considerable quantity of leprous material, the same kind of growth took place. In glycerine Agar plates the colonies that grew on the surface and in the depth, seen

FIG. 146.—TWO CELLS OF THE LEPROSY (?) NODULES IN THE LIVER OF A BIRD (RHEA).

The cell-substance is crowded with minute bacilli, similar to leprosy-bacilli. Magnifying power 700. (Stained with magenta.)

under a magnifying power of 100–200, were rounded reticulated patches, with dark thick centre.

In a section through the liver of a bird (*Rhea*) that died in the Zoological Gardens in London, prepared by Dr. Gibbes after his method of staining for tubercle-bacilli, there were seen innumerable aggregations of larger and smaller pink masses (visible to the unaided eye as dots of the size of a pin's point to that of a pin's head or millet-seed, and larger). Under the microscope these pink masses were seen to be composed of cells of various sizes, each filled with an enormous number of what appeared under a high power very short bacilli, much shorter than tubercle-

bacilli. But they gave the same reaction as tubercle-bacilli. Here and there isolated cells of various sizes could be seen filled with the bacilli. In the large cells the cell-outline was becoming indistinct, and in some the cell-substance was seen to break down, whereby the bacilli became free. In these respects, in the size, distribution, and character of the bacilli, there exists a remarkable similarity between the nodules in leprosy and the nodules just mentioned.

CHAPTER XV

ANAEROBIC BACILLI

THE group of microbes which we now proceed to describe comprises several species of specific pathogenic bacilli which have the following characters in common :—(1) They are obligatory anaerobic, growing therefore best, in fact growing only, when not in contact with air (oxygen) ; (2) they are strong gas-formers—methan or marsh gas ; (3) they are cylindrical bacilli, more or less capable of forming chains and filaments ; (4) they are motile, and possessed of several and sometimes numerous flagella ; (5) they form bright oval spores (endospores) thicker than the bacilli, which spores just like those mentioned of other bacilli (bac. subtilis, bac. mesentericus, bac. anthracis) have a great resisting power to heat, so that while the sporeless bacilli are killed by heat of 65–70° C. in ten or five minutes respectively, the spores do not lose their germinating power by being heated up to 80°–85° for ten or fifteen minutes ; (6) they grow well in the depth of grape-sugar gelatine and liquefy this.

They differ among themselves in (*a*) the nature of the disease they cause in the animal body, (*b*) their distribution in the body of the infected animal, (*c*) the nature and rapidity of their growth in artificial media, (*d*) the rapidity

of the liquefaction of grape-sugar gelatine, (*e*) the size of the bacilli and spores and the position of the latter in the former, and (*f*) the distribution and number of flagella and the greater or lesser tendency of the bacilli to form filaments.

To this group belong : the bacillus of malignant œdema of Koch, the bacillus of tetanus, the bacillus of symptomatic charbon, quarter evil, or Rauschbrand, and the bacillus enteritidis sporogenes.

Material, containing the spores of either of the above microbes, is transferred to sterile grape-sugar gelatine contained in a test-tube plugged with sterile cotton-wool—the gelatine to the height of about four inches—the test-tube is then placed in water of which the temperature is maintained at 78–80° C. for from ten to fifteen minutes, then in cold water so as to allow the gelatine to set, and after sealing the top with gutta-percha paper is finally incubated at 20-21° C. After twenty-four hours or latest after thirty-six or forty-eight hours a number of spherical colonies are noticed in the deeper parts of the sugar gelatine, the rapidity with which these grow, their general aspect, and the rapidity and nature of the liquefaction differ for the different species. As the colonies increase in size they become gradually confluent, and the liquefaction of the gelatine extends till the whole is liquefied, at the same time more or less copious gas evolution takes place, the gas bubbles being on the top of the liquefied gelatine, and when the liquefaction has extended to the top the gas bubbles escape into the space between the gelatine and cotton-wool plug.

In order to obtain uniform and characteristic growths in sugar gelatine tubes from an already established pure gelatine culture, the method of inoculation described in a former chapter as the capillary pipette method is by far the most reliable and best : by means of a freshly drawn-out

capillary glass pipette a droplet of the liquefied culture material is drawn up and allowed to ascend into the end of the capillary glass pipette that had been pushed down into the liquefied culture, or if it does not ascend is drawn up by gentle aspiration at the other end of the capillary pipette; this so charged capillary tube is then withdrawn and pushed

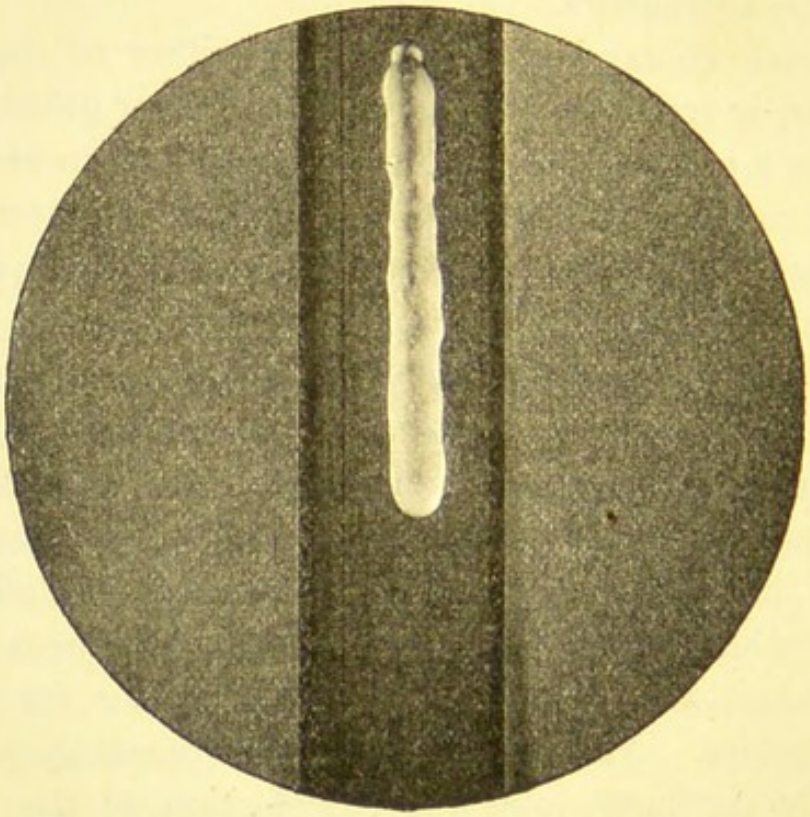


FIG. 147.—STAB CULTURE OF BACILLUS OF MALIGNANT ŒDEMA IN THE DEPTH OF SUGAR GELATINE INCUBATED FOR THREE DAYS AT 20° C.

The growth is indicated by a cylinder of liquefied, slightly turbid gelatine; on the top of the growth a gas bubble.

Natural size.

down into the lowest part of the fresh sugar gelatine tube, and a trace of the material is by blowing forced out, the capillary tube is withdrawn, the plug replaced, and the top part of the new gelatine liquefied by holding this part of the culture tube over the flame till the gelatine at this point bubbles; the tube is then placed in an upright position in

cold water in order to set the top layers of the gelatine quickly; after this the tube is sealed with gutta-percha paper and placed in the incubator. The result will be apparent in twenty-four to forty-eight hours by the appearance of a linear growth in the deep parts, which, as time proceeds, enlarges and shows all the differential characters of aspect, progress, and liquefaction.

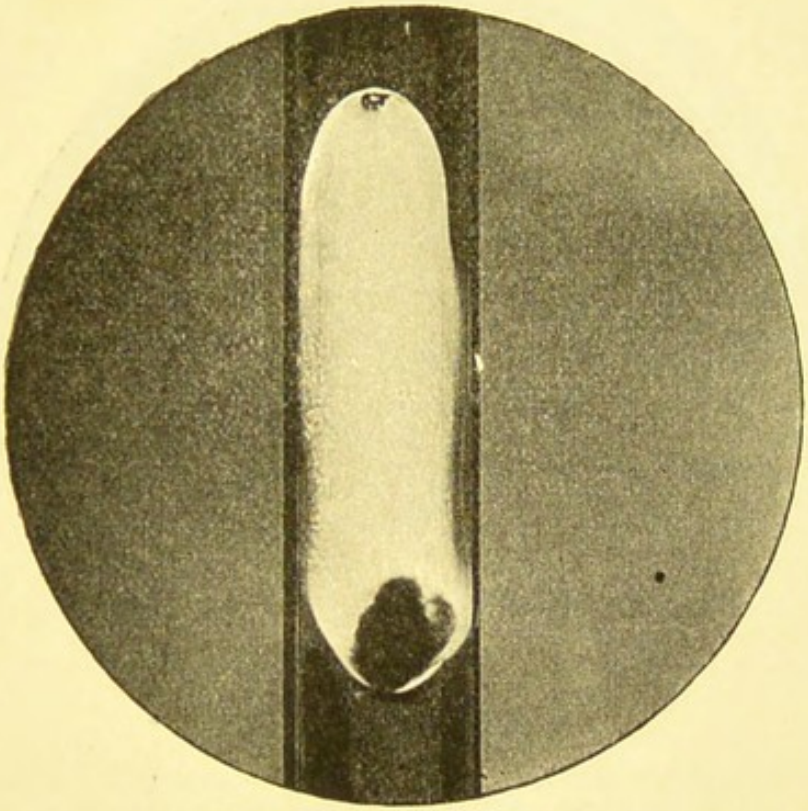


FIG. 148.—STAB CULTURE OF BACILLUS ENTERITIDIS SPOROGENES IN DEEP SUGAR GELATINE, INCUBATED FOR FORTY-EIGHT HOURS AT 20° C.

Liquefaction has proceeded very rapidly; the liquefied gelatine is fairly translucent; at the bottom is a fluffy floccular mass, on the top is a gas bubble.

Natural size.

In Figures 147, 148, 149, and 150 four such stab cultures in the depth of grape sugar gelatine are shown in which the inoculation had been carried out by the capillary glass pipette method, and from these will be seen the uniformity of this method and the striking differences noticeable between the four species here dealt with. In all four tubes

liquefaction is proceeding along the line of growth, but at greatly different rates.

From a liquefied sugar gelatine culture a subculture is easily made by rubbing a liberal amount of the material over the slanting surface of solidified grape sugar Agar and 0·5 per cent. formate of soda (Kitasato and Weyl); this is

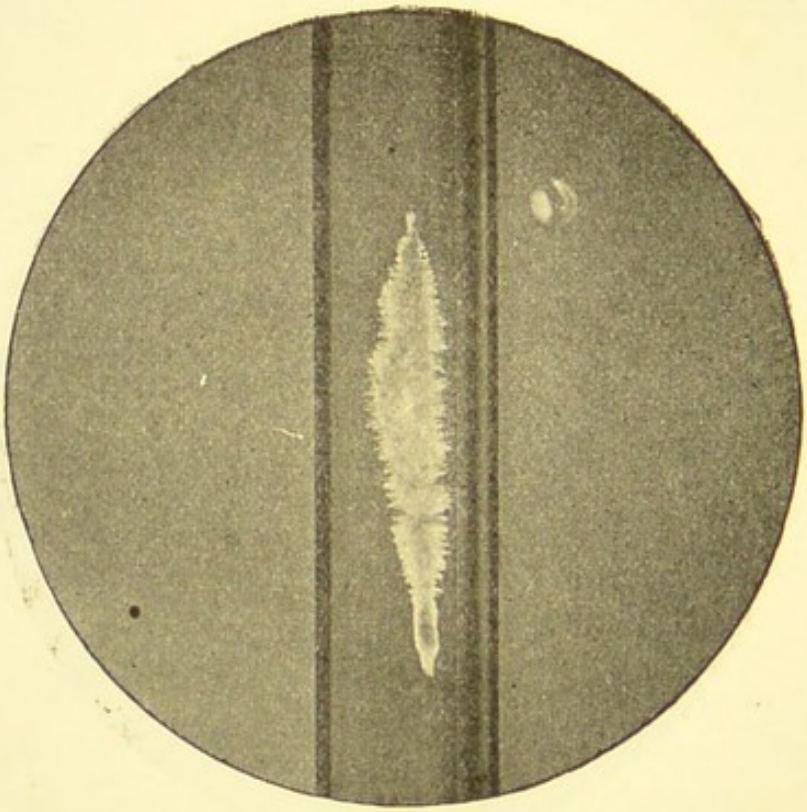


FIG. 149.—STAB CULTURE OF BACILLUS TETANI IN THE DEPTH OF SUGAR GELATINE, INCUBATED FOR THREE DAYS AT 20° C.

The growth is indicated by a spindle-shaped mass of threads extending laterally; the gelatine is liquefied to the extent of the growth.

Natural size.

then placed after Buchner's method in a glass tube containing for each gramme of pyrogallic acid one cc. of liquor potassæ closed by a well-fitting indiarubber plug (*see* a former chapter) and incubated at 37° C. As soon as colonies make their appearance on the Agar surface, a little of it can be withdrawn by the platinum needle or loop, and

used for flagella staining (*see* a former chapter), or the culture can be left to go on for some time till spores have made their appearance; in some of the above microbes spore-formation does not occur in the grape sugar gelatine (malignant œdema, symptomatic charbon) and for this reason cultures on solid media (stab culture in grape sugar Agar, or,

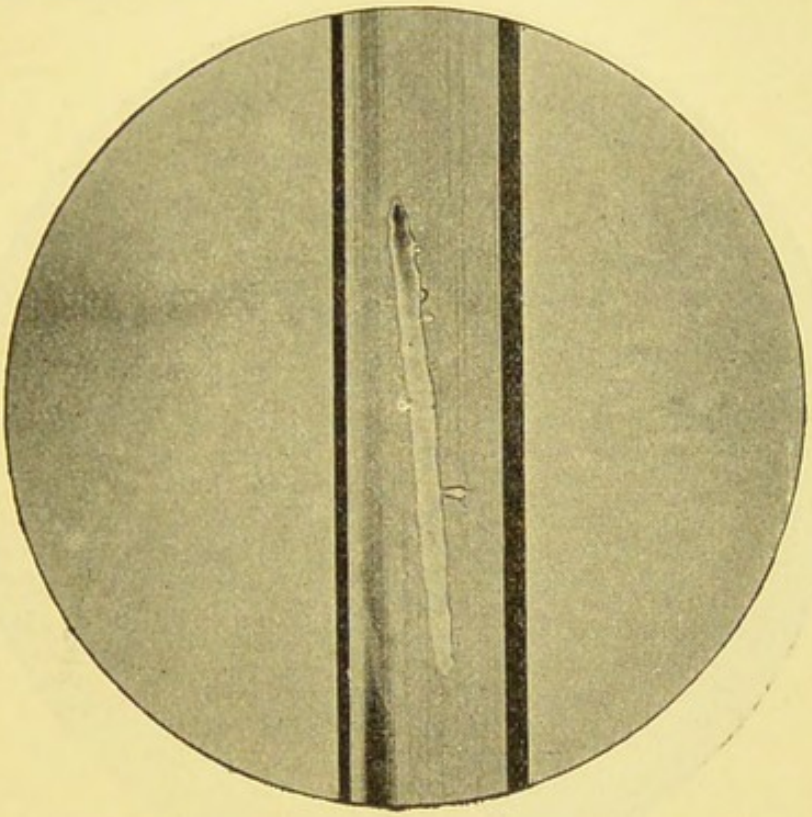


FIG. 150.—STAB CULTURE OF BACILLUS OF SYMPTOMATIC CHARBON IN SUGAR GELATINE, INCUBATED FOR THREE DAYS AT 20° C.

The growth is a cylinder of turbid liquefied gelatine with lateral outgrowths. Natural size.

better, the just mentioned culture on the slanting surface of sugar Agar) must be resorted to.

Besides the above media, glycerine broth peptone and broth peptone (ordinary nutrient broth) are useful for obtaining copious growths. These are employed when it is required to obtain the toxins produced by the microbe during its growth. Thus in the case of bacillus of tetanus

important experiments have been made by Behring and Kitasato, Kitasato, Roux and Vaillard as to the nature and action of the tetanus-toxin (*see* Immunisation and Antitoxin), obtained in the filtrate of broth cultures.

Different kinds of flasks and test tubes have been designed which permit replacing the air above the broth

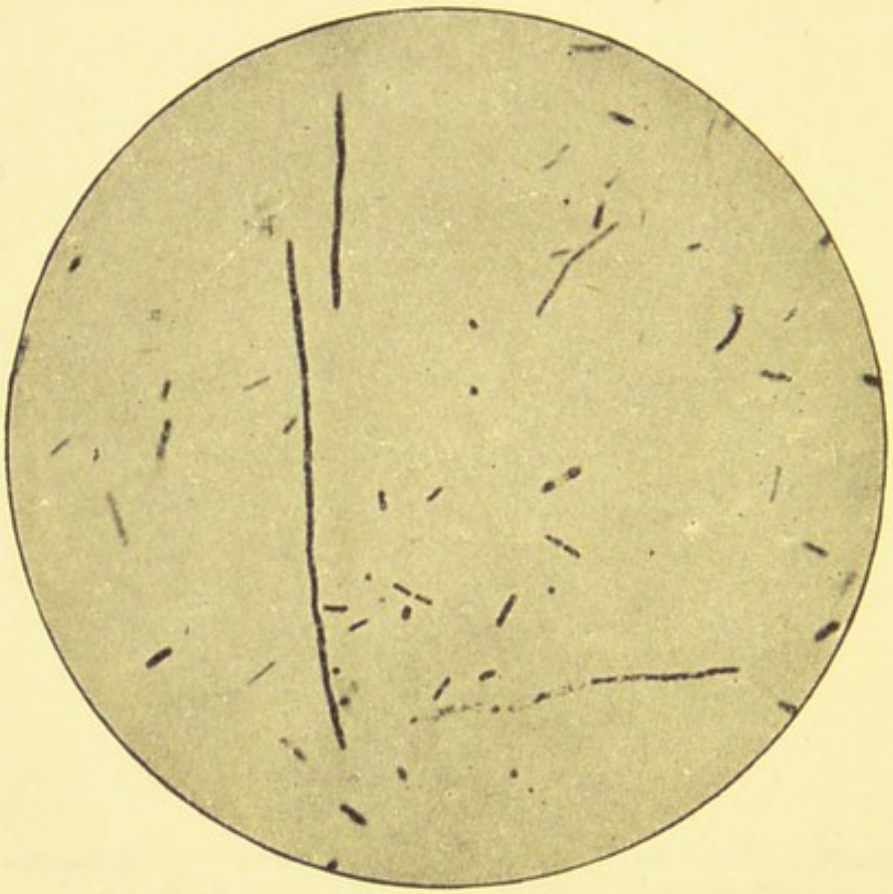


FIG. 151.—FILM SPECIMEN OF SUBCUTANEOUS ŒDEMA FLUID OF A GUINEA-PIG DEAD OF MALIGNANT ŒDEMA; FILAMENTOUS FORMS OF THE BACILLUS OF MALIGNANT ŒDEMA.

× 1000.

(after inoculation) by hydrogen gas—generally the flask or test tube possesses a lateral tube so as to allow of this replacement being easily effected. I find, however, that the anaerobic microbes grow well in ordinary flasks and test tubes, provided there is a large amount of the broth (three-quarters or more of the volume) and the flask or test tubes

are sealed with gutta-percha paper immediately after inoculation.

1. *Bacillus œdematis maligni* (Koch).—This disease has been produced by Koch in guinea-pigs by the subcutaneous injection of recently manured garden earth. An extensive œdema occurs at and about the seat of inoculation; the œdema is accompanied by hæmorrhage into the sub-

FIG. 152.—BACILLI OF MALIGNANT ŒDEMA CONTAINING SPORES, CHIEFLY SITUATED IN THE MIDDLE PART OF THE BACILLI.
× 1000.

cutaneous tissue, and is of an offensive odour; it spreads during the second day, leads to gangrene of the subcutaneous and muscular tissues with the formation of gas bubbles, and the animals die in from twenty-four to forty-eight hours: the spleen is found congested, so also are the liver, kidney, lungs, and intestines. In the œdematous exudation and in the spleen long mobile bacilli are present, either singly or in filaments and long chains; their number

in the blood is comparatively small immediately after death, but they soon multiply therein and if the examination of the blood (heart's blood) is delayed, the bacilli may be found present in considerable numbers. The bacilli do not stain after Gram. The size of the short bacilli is 2-3·5 μ in length, and 1 μ in thickness; their ends are more or less rounded. If the flagella are stained, it is seen that the bacilli possess several (4–5) flagella attached laterally near the ends of the bacillus. Many bacilli are in the form of chains and filaments. The œdematous fluid, and the blood, inoculated into fresh guinea-pigs produce the fatal disease.

Rabbits are also very susceptible to the disease; and at the seat of inoculation œdema is produced. Mice are very susceptible, and die before the end of the first day, but no œdema is present at the seat of inoculation. All the viscera are congested, and the spleen is enlarged; the blood of the spleen, the exudation of the peritoneum, and the pleura, contain the bacilli. A sure diagnosis, and differentiation from anthrax bacilli, to which the œdema bacilli bear a certain likeness, can with certainty be made by cultures.

The cultural characters of this bacillus show that it is altogether different from that of bacillus anthracis; although in size and general aspect in the fresh state, and in stained cover-glass specimens, it is not unlike, in its action on animals, in the condition of the spleen of the inoculated animals, and in its small numbers in the blood of these it is quite different from bacillus anthracis. When cultivated it shows the following characters: The œdema bacillus is anaerobic, since it does not show growth on the surface of nutritive media; it grows only when planted in the depth; in grape sugar gelatine (in the depth) it forms characteristic globular colonies of different sizes, opaque and liquefied, their margin more opaque than the centre and finely striated. The growth and liquefaction

proceed gradually and slowly, till all the gelatine is liquefied; at the bottom of this is a voluminous greyish-white filamentous mass. It grows best in gelatine to which 1–2 per cent. of grape sugar has been added. In solidified sugar Agar it grows well, producing uniform turbidity all through the medium, with floccular condensations and

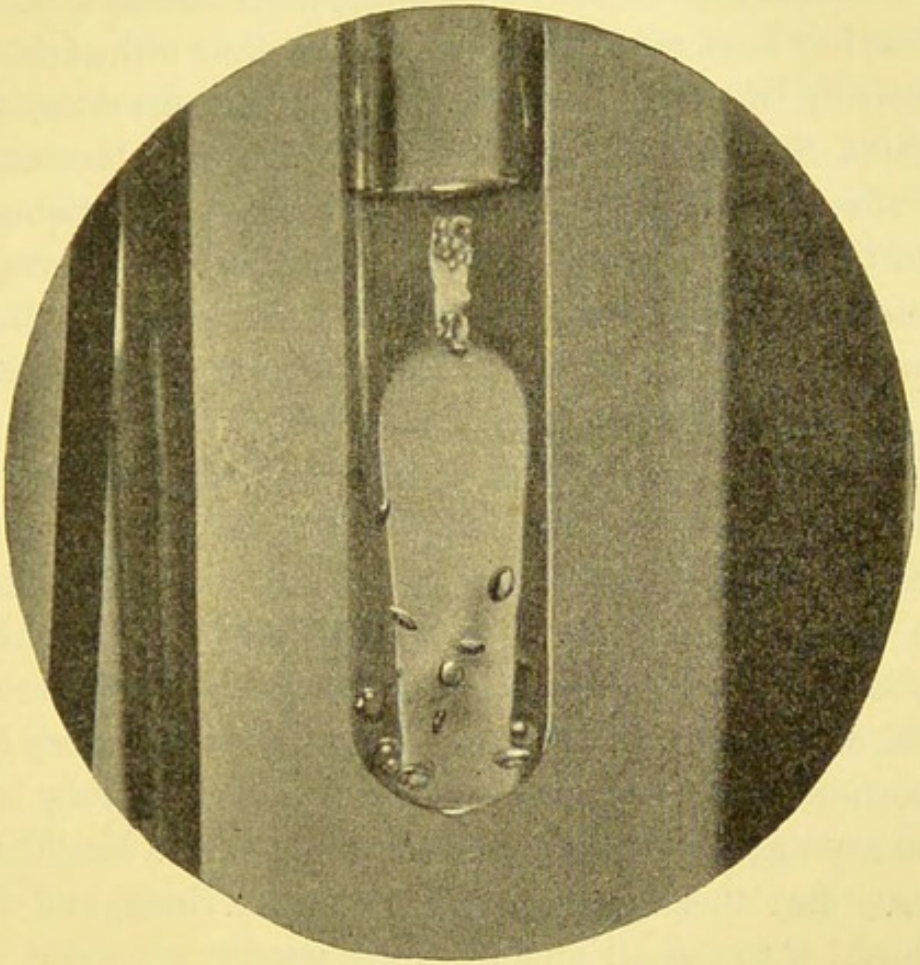


Fig. 153.—Stab Culture of Sugar Gelatine with the Bacillus of Malignant Œdema; numerous Gas Bubbles are shown above and in the Liquefied Growth.

Natural size.

numerous gas bubbles. Solidified blood-serum is liquefied by the bacillus. The cultures act virulently on animals, provided comparatively large quantities are injected.

Oval bright spores are formed in the short bacilli, either in the middle or at one end; the spores are thicker than the bacilli themselves; and some of the bacilli in the

œdematous fluid contain spores, particularly if the examination be delayed after death. The œdema bacillus is of great importance, since by the observations and experiments of Chauveau and Cornevin, Brieger, and others, it has been shown that surgical gangrene (progressive gangrenous emphysema) in the human subject is caused by the same bacillus. It seems that many a soil containing putrid animal substances, such as hay dust, rag dust, offensively smelling filth of dustbins, offensively smelling exudations, gangrenous discharges, &c., contains the œdema bacillus or its spores. Horses, pigs, and sheep are susceptible to this malignant œdema, provided large doses are inoculated; cattle are not susceptible. As mentioned above, guinea-pigs are the best experimental animals, since inoculation produces a typical, emphysematous, spreading œdema, with fatal result.

Pasteur has studied this septicæmia on guinea-pigs, and it is also called Pasteur's septicæmia, and the bacillus is called by him vibrio septique. Roux and Chamberland have demonstrated in the broth cultures of this microbe chemical substances which separated by filtration from the bacilli and injected into animals cause a transitory illness proportionate to the amount injected, and hereby confer immunity against the injection of the virulent bacilli themselves. But this immunity does not last long, and is not produced if too small quantities are used.

Flügge has isolated from recently manured garden earth a pseudo-malignant œdema bacillus which resembles Koch's malignant œdema bacillus, but is non-pathogenic.

2. *Bacillus tetani.*—Carle and Rattone (*Giorn. dell. r. Accad. d. Med. Torina*, 1884) were the first to show that tetanus is a communicable disease. They succeeded in producing typical tetanus, terminating fatally, by inoculating into rabbits pus taken from the ulceration of a human

being in whom tetanus had set in. Purulent exudation was taken in these rabbits from the place of inoculation and transferred to fresh rabbits, and here typical tetanus was again produced. In human tetanus the place of infection (in the skin of the hand, foot, or other part, by a tainted splinter, earth, or other material) becomes marked as a purulent inflammation leading to ulceration; the tissue surrounding the ulceration is much infiltrated, and there is always hæmorrhage in it. After death the membranes of the brain and cord are found much injected, and so also the grey matter of the medulla and cord; occasionally there is a slight accumulation of red and white blood-corpuscles around the vessels.

Nicolaier (*Inaugural Diss.*, Göttingen, 1885) made the important discovery that earth taken from superficial layers of the soil is often capable of producing, when inoculated into the subcutaneous tissue of the mouse, rabbit, or guinea-pig, a local suppuration and hæmorrhagic effusion about the seat of inoculation, rapidly followed by typical tetanus and death. In that earth and in the pus and exudation of the seat of inoculation he demonstrated the constant presence of fine, straight "drumstick" bacilli, which he considered as the *tetanus bacilli*. The purulent matter containing these bacilli, inoculated into fresh mice, rabbits, or guinea-pigs again produces tetanus. Rosenbach (*Archiv f. klin. Chirurgie*, Band xxxiv., 1886) showed that the same bacilli exist in the exudation at the place of infection in human tetanus. Hochsinger, Beumer and Peiper, Bonone, Shakespeare, Raun, and many others have confirmed the existence of these bacilli in tetanus, but no one of these succeeded in cultivating them in pure cultivations. Though numerous cultivations have been established, and tetanus been produced in animals with them by the aid of foreign bodies—

cotton-wool, splinters soaked with the cultures—yet these cultivations were always in an impure state, until Kitasato (*Zeitschrift f. Hygiene*, Band vii., p. 225) has succeeded in cultivating the tetanus bacillus of Nicolaier in pure cultivations and in producing tetanus with such pure cultures. Minimal doses inoculated into mice produced tetanus in twenty-four hours, death in two to three days.

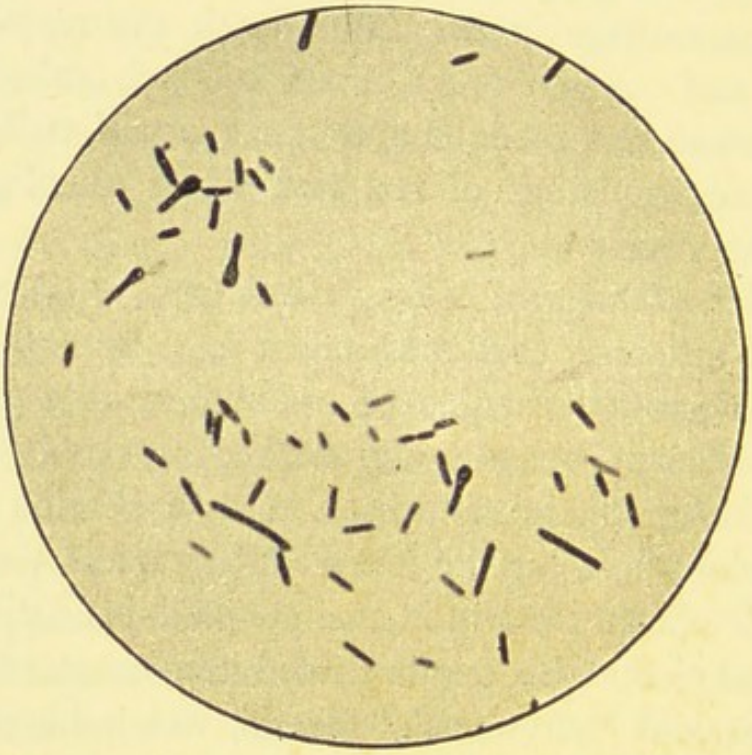


FIG. 154.—FILM SPECIMEN OF BACILLUS TETANI FROM A CULTURE IN SUGAR GELATINE; SOME OF THE BACILLI SHOW A TERMINAL SPORE, "DRUMSTICKS." × 1000.

In the case of rats, rabbits and guinea-pigs the dose had to be somewhat larger, 0·3–0·5 cc. of broth culture. Rats and guinea-pigs are ill with tetanus already after twenty-four to thirty hours, rabbits not before two to three days. On *post-mortem* examination of such animals there is no suppuration at the seat of the inoculation, but only hyperæmia; hence the suppuration observed in other cases is not an essential feature, and in former experiments and in the case

of human beings is probably only due to the presence of the foreign bodies themselves (earth, splinters, &c.) which were the vehicles of the tetanus bacilli; in the internal organs there is no definite change. In the organs there are no bacilli present, nor was it possible to produce tetanus in other animals by inoculating them with the cord, nerves, blood or spleen of the animals dead of tetanus. In rabbits

Fig. 155.—Similar Specimen as in preceding figure.
× 1000.

Kitasato produced typical tetanus by injection of 0·5 cubic centimetre of broth culture of the tetanus bacillus into the vein of the ear. Also by injection of the culture after trephining into the dura mater Kitasato produced typical tetanus; but neither in the brain nor in the cord, nor in the blood or other viscera of these animals, could the tetanus bacilli be found.

The tetanus bacilli are straight cylindrical bacilli, in culture varying between 1μ and 4μ, occasionally forming longer chains and filaments; in the fresh state they show sluggish motility, but in suitably stained specimens show numerous flagella, often arranged in bundles (*see* Figs. 21, 22, 23). They form rapidly (already in 30 hours at 37° C.) a terminal spore which gives them the shape of a "drumstick"; the spores are at first spherical, later oblong, bright and glistening. Pus containing the tetanus virus preserves its virulence in the dried state for many months, owing to the presence of the spores (Kitt).

The bacilli stain well after Gram.

The success of obtaining pure cultures of the tetanus bacilli was achieved by Kitasato by cultivating tetanus pus anaerobically; preliminarily to this he watched for the time when in ordinary cultures of the tetanus pus on serum or Agar amongst the different species of bacteria present he found such which by their peculiar shape and containing a terminal thick spore—"drumstick forms"—he recognised as the tetanus bacilli. By exposing such impure cultures three-quarters to an hour in water at 80° C. all bacteria were killed except those spores. With material thus treated he made gelatine plate cultures, and gelatine tube cultures, but in such a way that the air was excluded by filling them with hydrogen gas or by planting the bacilli into the depth of the gelatine. Under these anaerobic conditions he obtained pure cultures.

It appears, then, from these exact researches that the introduction of the tetanus bacilli under the skin is followed by the production by them of a chemical virus, which, as it is being produced at the seat of inoculation, is absorbed into the system and sets up the disease; but the bacilli themselves appear to remain limited to the seat of inoculation, and do not live in the blood or any other tissue, and

therefore only the seat of the inoculation contains the infective principle, *i.e.*, the bacilli; for this reason brain, cord, nerves, blood and viscera have no power to produce infection.

The disease tetanus is then, like that of diphtheria, not a true infection but intoxication.

Brieger has, as a matter of fact, isolated from the exudation at the seat of infection in human tetanus a toxic principle, *tetanin*, the injection of which produces tetanus symptoms in animals; and Kitasato showed this to hold good also for the tetanin obtained from the cultures of the tetanus bacilli.

In his "Experimental Researches on the Poison of Tetanus" (*Zeitschr. f. Hygiene*, x. 2) Kitasato gives a full account of the influence of light, heat, drying and of various chemical substances on the tetanus poison.

Behring and Kitasato [1] have shown that by repeated injection of non-fatal doses—using at first small doses of active culture or tetanus toxin, or by using attenuated virus (by the addition of trichloride of iodine, carbolic acid)—it is possible gradually to increase the amount of virulence of the dose without causing a fatal issue in the experimental animals (rabbits).

Hereby the animals were rendered insusceptible to fatal doses of tetanus bacilli or tetanus toxin, and further it was shown that the blood-serum of such (artificially) highly immunised animals when injected into an otherwise susceptible animal (mouse) possesses the remarkable power of neutralising the effect of a fatal dose of tetanus bacilli or tetanus toxin injected before or after into that animal (mouse). It is from these researches that the scientific

[1] *Deutsche Med. Woch.*, 1890, No. 49, and Behring, *Zeitschrift f. Hygiene und Infekt.* xii.

basis for the use of blood-serum of animals, artificially immunised against tetanus, for the cure of human tetanus —the antitoxic power of that blood-serum—is derived; researches carried on by Behring, by Roux and Vaillard (*Annales de l'Institut Pasteur*, 1893), and others have led to the obtaining of tetanus antitoxin blood-serum both for protective and curative purposes (*see* Chapter on Immunity).

Tizzoni and Cattani, in a series of memoirs, had already demonstrated the means by which animals possessed naturally of slight or great susceptibility respectively can be made altogether insusceptible to tetanus. Further, the blood-serum of animals, made previously insusceptible, injected into animals possesses a decided antitoxic action. They have isolated from such blood-serum this substance—the tetanus antitoxin—by precipitating with alcohol, drying in vacuo, and dissolving in water. In four cases of human tetanus, by the injection of the antitoxin of Tizzoni the disease was arrested and the patients recovered (*Centralbl. f. Bact. und Parasit.*, Band x., No. 24, p. 785.)

3. *Bacillus of symptomatic charbon* (*quarter evil, Rauschbrand*).—This disease affecting young cattle and sheep occasionally produces great mortality amongst them, particularly amongst the former. Owing to its involving chiefly one of the hind extremities in the form of a large subcutaneous tumour, in which, on incision, a quantity of sanguineous, discoloured, almost black fluid is shown, the disease is called *quarter evil* or *black leg*. Owing to its slight resemblance to anthrax (large tumour containing serous sanguineous fluid) it is called in France *charbon symptomatique;* in Germany it is called *Rauschbrand* on account of the emphysematous nature of the tumour, and on account of the gangrenous nature of the infiltrated

tissues. The disease, when it appears, rapidly spreads amongst young cattle and sheep; rare amongst horses, it is unknown amongst swine or poultry. The animals affected are quiet, do not feed, and show high temperature; on one or the other quarters—generally one of the hind—there appears a large diffuse swelling, on account of which

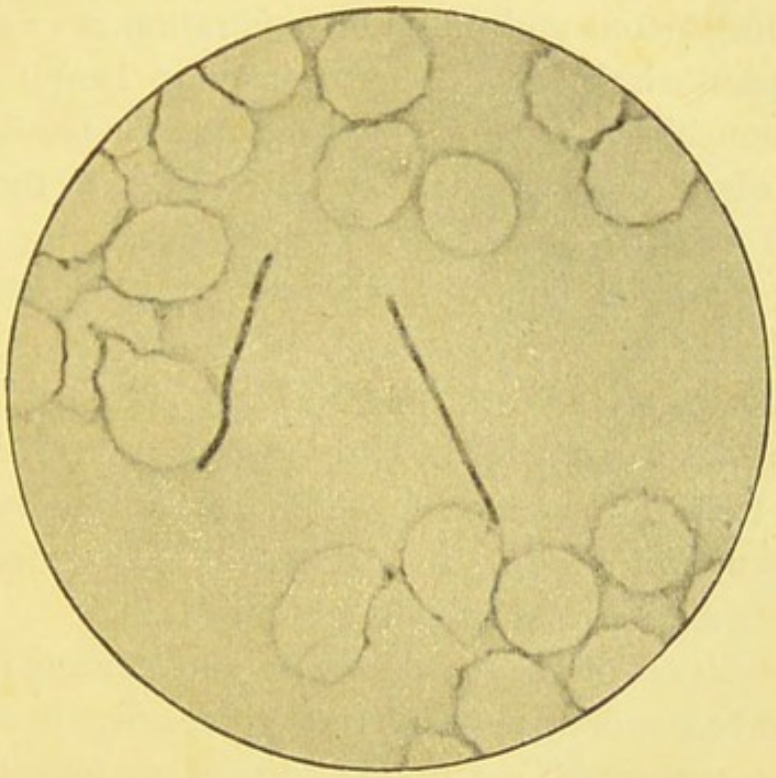


Fig. 156.—Film Specimen of Blood of a Guinea-pig dead after subcutaneous Inoculation of the Bacillus of Symptomatic Charbon.

Blood discs and long chains of bacilli.

× 1000.

the animal is lame and cannot move that extremity. In the course of thirty-six to forty-eight hours death takes place. On post-mortem examination the tumour is seen to be located subcutaneously; here the connective and muscular tissues are dark, almost black, gangrenous, and contain a large quantity of sanguineous serum and a large quantity of gas bubbles (said to be CO_2 and methane).

The infiltration with sanguineous serous fluid extends for some distance into the adjacent parts in the muscular tissue; in the viscera: congestion of the liver, spleen, kidney, and particularly the subcutaneous lymph glands; these, beginning from near the tumour, are found swollen, dark, and on incision a sanguineous fluid oozes out from them. The spleen is only very slightly enlarged. Cover-glass specimens of the subcutaneous and muscular infiltration at or near the tumour, particularly of the subcutaneous lymph glands, show in considerable numbers small motile bacilli 3–5 μ long, and about 0·5 μ thick: they are rounded at their ends, and some contain terminally a bright oval spore; others possess a terminal enlargement without spore (Bollinger, Arloing, Cornevin, and Thomas[1]).

Immediately after death the bacilli are not easily demonstrable in the heart's blood because present only in small numbers, though they can be shown to be present in the liver, spleen, and kidney, but always more numerously if some hours are allowed to elapse after death.

The exudation of the tumour or the surrounding muscular tissue injected subcutaneously into guinea-pigs in comparatively large quantities ($\frac{1}{2}$–1 Pravaz syringe) produces the same kind of emphysematous gangrenous change with sanguineo-serous exudation at or near the place of inoculation; the animals die between twenty-four to sixty hours, the internal viscera show great congestion; in the subcutaneous tumour, in the blood of the heart, and in the juice of the viscera the bacilli can be easily demonstrated; in the blood and viscera they are fairly numerous if some hours have elapsed after death. Rabbits are far less susceptible than guinea-pigs.

If only a drop or two are injected the guinea-pigs, though they become affected with the local disease, do not succumb,

[1] *Bull. de l'Acad. Française*, 1881.

but show themselves refractory against infection with large quantities, such as in control animals would invariably produce death.

Arloing, Cornevin, and Thomas have brought to light various important facts connected with the action of the bacilli. These authors cultivated the bacilli in broth, but they found that the bacilli grow best in chicken broth,

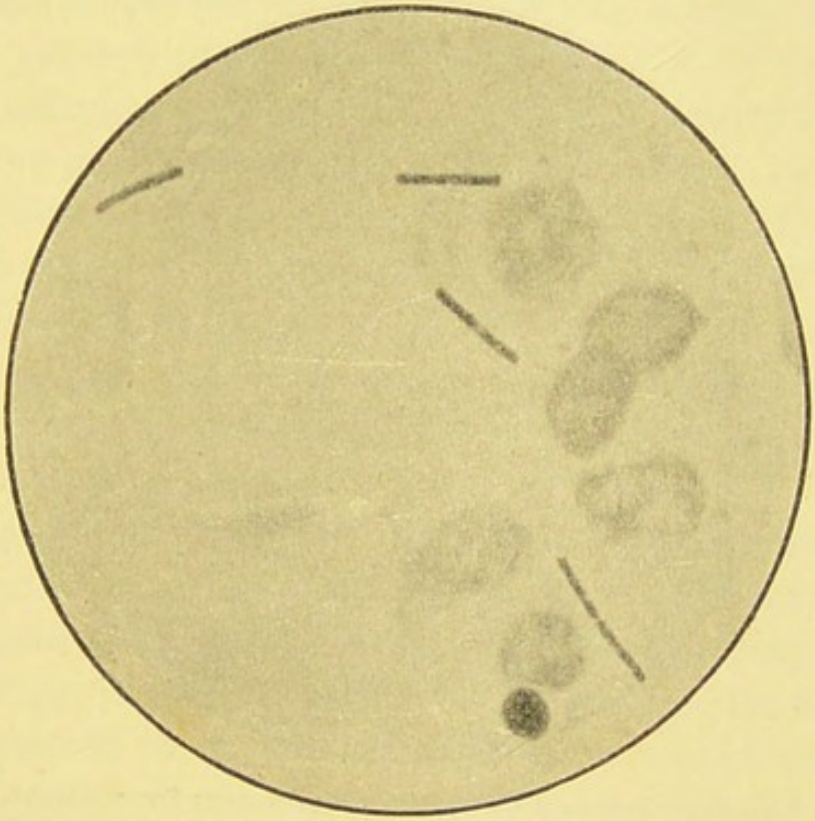


Fig. 157.—Film Specimen of Spleen Juice of a Sheep dead of Symptomatic Charbon, showing a few Nuclei and the Specific Bacilli.
× 1000.

glycerine and sulphate of iron, provided oxygen (air) is excluded; they are, therefore, true or obligatory anaerobic bacteria. They grow well in grape sugar gelatine, but must be inoculated into the depth of it. The character of the growth in a stab culture in sugar gelatine has been described already, and is shown in Fig. 150. Though similar to that of the anaerobic bacillus of malignant œdema

it differs from it in growing slower than this latter, the liquefaction proceeds slower, and there are not present the voluminous fluffy masses at the bottom of the liquefied gelatine. The spores in the bacillus of symptomatic charbon are generally situated terminally in the bacilli.

Arloing, Cornevin, and Thomas have shown that if small quantities of the fluid of the natural tumour (muscle fluid)

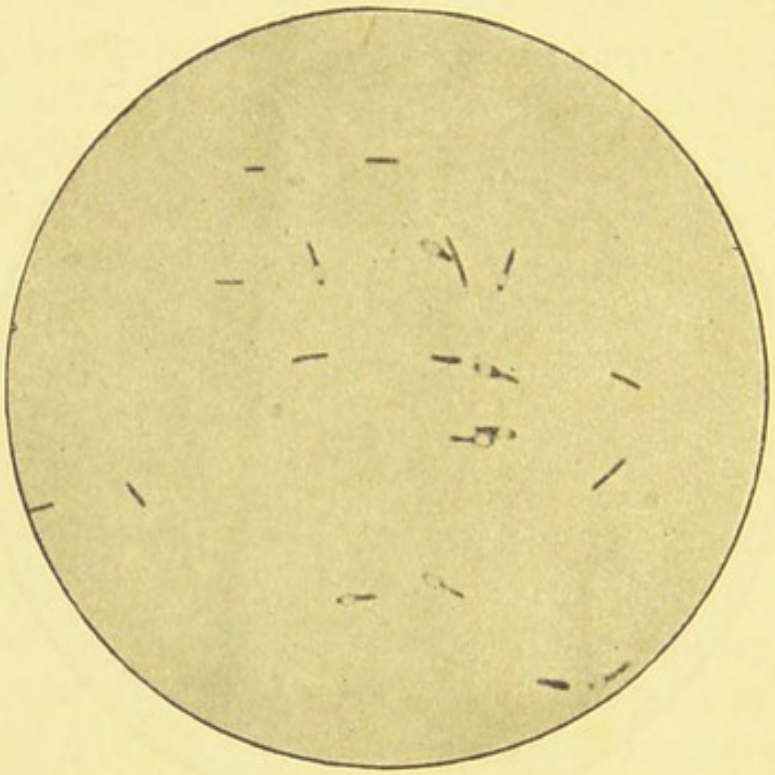


FIG. 158.—FILM SPECIMEN OF A CULTURE OF BACILLUS OF SYMPTOMATIC CHARBON SHOWING THE OVAL SPORES, ONE IN EACH BACILLUS SITUATED TERMINALLY. × 700.

be injected subcutaneously into cattle only a local though typical tumour is the result; the animals recover, and then are possessed of immunity against further inoculation with otherwise fatal doses.

Further they found that three to five drops of the tumour fluid injected into the vein of cattle—but without inoculating the subcutaneous tissue around the vein—produce only a transitory febrile disturbance; the animals quickly

recover, and show themselves refractory against subcutaneous fatal doses. A safe mode of protective inoculation used by these observers successfully on a large scale is this: The tumour fluid (the juice of the gangrenous muscular tissue) is rapidly dried at 32–35° C., then it is rubbed up with water and heated to 100° C. Another lot is treated in the same way, but heated only to 85° C.; the first lot represents a first vaccine (*premier vaccin*), the second lot a second vaccine (*deuxième vaccin*); both can be dried and sent to distances; when required for use the dried matter is rubbed up in 100 parts of water, and of this 1 cc. per animal is subcutaneously injected. The *premier* (weaker) vaccine must be used first; after the lapse of about ten or twelve days the *deuxième* (stronger) vaccine is injected. Animals thus twice vaccinated proved themselves completely protected against a fatal and virulent dose taken from the natural tumour.

Though there exists, both as regards the pathology and the microbes, a certain resemblance between the malignant œdema and the charbon symptomatique, this resemblance is only superficial, and there can be little doubt that the two diseases in their pathology, in their microbes, and their transmissibility or non-transmissibility to certain animals are *totally different* diseases. The differences between the non-motile aerobic bacillus anthracis and the motile anaerobic bacillus of symptomatic charbon morphologically, culturally, and in their effect on the guinea-pig are very conspicuous.

4. *Bacillus Enteritidis sporogenes.*[1]—During the night of 27th–28th October, 1895, there occurred suddenly an epidemic of severe diarrhœa among the patients in the wards of St. Bartholomew's Hospital; the number of cases

[1] Illustrations of the morphology of this bacillus could not be got ready in time for this edition.

amounted to fifty-nine, of the twenty-eight wards of the hospital fifteen were attacked.

The first cases occurred about midnight, the majority about 2 A.M., and a few between 5 and 6 A.M. of October 28th. By noon of the 28th, the epidemic was practically over, no further cases occurring. Dr. Andrewes, the sanitary officer of the hospital, has investigated the clinical and etiological facts of this epidemic, and from his notes I gather that the cases were not of the choleraic type, vomiting and cramps being conspicuously absent. In all cases the onset was sudden and consisted in abdominal pains followed by copious watery evacuations with numerous mucus flakes; in the severe cases, the discharges were considerable in amount and frequency and contained much blood; in such cases there was also prostration and even collapse. All cases recovered.

Examining specimens of the evacuations under the microscope, they were found to contain numerous red and white blood corpuscles and crowds of bacteria. Amongst these a very large number of oval glistening spores attracted attention; these were either free, isolated and in continuous masses, or they were contained within cylindrical bacilli, each of these bacilli containing one spore nearer to one end. These spores and spore-bearing bacilli were found abundantly in every one of the evacuations that had been examined. As the occurrence of such an abundance of spores and spore-bearing bacilli in the human intestine is an unusual feature, special attention was directed to them and cultivations were made to isolate them. The only two known species of spores and spore-forming bacilli in the intestine that had to be here considered were (1) the aerobic Bacillus mesentericus and (2) the anaerobic Bacillus amylobacter. The last-named could be at once excluded, from

the fact that in the above microscopic specimens no clostridia could be discovered, Bacillus amylobacter being noted for the clostridia forms of its sporing bacilli. The first species, viz., the Bacillus mesentericus, could with probability be excluded from the microscopic examination of fresh specimens alone, since its bacilli show conspicuous motility, whereas in our cases the motility of the bacilli was extremely feeble and could be recognised only on very few examples. But the culture test soon proved that our spores were not those of Bacillus mesentericus. Aerobic gelatine and Agar plates, surface cultures on gelatine and Agar, brought forth the colonies of Bacillus coli only ; the Bacillus mesentericus being aerobic, if it had been present in the evacuations, would undoubtedly have made its appearance in these cultures. More direct proof, however, was obtained by placing a mucus flake of the evacuation in gelatine or Agar, heating these to 78–80° C. for ten to fifteen minutes, then preparing ordinary aerobic plates and incubating them at 20° and 37° C. respectively : no colonies of any description made their appearance. The spores of Bacillus mesentericus, like other well-known spores (of Bacillus subtilis, of Bacillus anthracis, of tetanus, of quarter evil, of malignant œdema, &c.) when heated to 80° C. for ten or fifteen minutes do not hereby lose their power of subsequent germination, although all non-sporing bacilli—*e.g.*, Bacillus coli—are thereby killed. Since then in the aerobic plates of the heated gelatine and Agar no growth took place, there could not have been any Bacillus mesentericus present. Besides the above aerobic, also anaerobic cultures were made of the evacuations ; a flake was placed into grape-sugar gelatine and grape-sugar Agar, heated to 78–80° C. for ten to fifteen minutes, then allowed to set and incubate. In both the sugar gelatine and sugar Agar cultures already

after twenty-four hours numerous colonies were noticeable in the depth, those in the sugar gelatine were spherical translucent masses of liquefied gelatine, those in the sugar Agar whitish small dots not liquefying the Agar; at the same time gas bubbles were present in connection with the colonies, particularly in the sugar Agar. After forty-eight hours the growth had so advanced, and the liquefaction of the gelatine had become so extensive, that the lower half in the test tube was completely liquefied, very slightly turbid by the growth; on the surface of the liquefied growth gas bubbles may be present or they may be altogether absent, in the depth whitish cloudy flakes (*see* Fig. 148).

Examining under the microscope such a liquefied culture after two or three days' growth it was found to be made up of rod-shaped or cylindrical bacilli, generally singly or in chains of two, three or more rods; they were mostly apparently stationary, but here and there feeble locomotion could be noticed, consisting in a wobbling or rolling slightly progressive movement; but only few such motile bacilli could be seen. In some of the bacilli there was present a bright oval spore, occasionally in the middle, but oftener near one end. In the floccular masses at the bottom of the culture tube spore-bearing bacilli were numerous, and even occasionally a free spore. After three or latest after four days the whole of the sugar gelatine in the tube had become liquefied by the growth, on the top there were gas bubbles; in the depth a white powdery precipitate which on microscopic examination shows numerous free spores. When such a culture is opened it has a distinct smell of butyric acid; when the liquefied gelatine is disturbed by moving in it a platinum or glass rod, numerous gas bubbles rise up; the liquid when sucked up in a capillary pipette emits a considerable amount of gas bubbles. When the culture tubes

are exposed to the light, numerous gas bubbles rise up and collect on the surface. A large number of subcultures in various media were made from the primary anaerobic sugar gelatine cultures and of the results the following deserve special mention.

(*a*) The rapidity of liquefaction of the sugar gelatine by the growth stands in an inverse ratio to the amount of gas bubbles escaping through the gelatine as the growth proceeds. If, after inoculation of the sugar gelatine by stab, there are found, after one or two days' incubation, numerous gas bubbles distributed in the upper part of the gelatine and escaping to the free surface, it may be predicted with certainty that the growth in, and the liquefaction of, the gelatine in such a culture will proceed very slowly; and conversely, if after one or two days' incubation the progress of liquefaction in the depth (after inoculation of the depth) is conspicuous, there is very little or nothing to be seen of gas bubbles on the surface.

(*b*) The formation of spores stands in direct relation to the rapidity of liquefaction; in tubes in which the growth and the liquefaction proceed very slowly, there are at no time spores formed in the bacilli; in old cultures of this kind the bacilli are found as longer or shorter threads, some undergoing involution and death by granular disintegration. Whereas, in tubes in which liquefaction proceeds rapidly—the whole of the gelatine liquefied in two to three days—there is always copious spore formation.

(*c*) Milk inoculated with the bacillus and incubated at 37° C. shows, as a rule, already after twenty-four hours, sometimes a little later, distinct changes consisting in the separation of flocculi of coagulated caseïn from the slightly turbid whey, numerous gas bubbles being present in the creamy layer on the surface; after forty-eight hours the

separation is complete, most of the caseïn flocculi are on the surface mixed with numerous gas bubbles, the cream being so altered that only a thin layer of fluid yellow oil is present on the surface of the culture. Examined under the microscope, the clear whey is full of short cylindrical bacilli. Spore formation in milk cultures is observed only when the culture is made strictly anaerobically and there is no marked spontaneous evolution of gas bubbles; under these conditions, whitish cloudy flocculi are found in the whey, which are full of spores.

(*d*) The spores do not lose their power to germinate if exposed to 80° C. for fifteen minutes; they are, however, killed if immersed in boiling water for two minutes.

(*e*) The cultures in gelatine, as also in milk, have a distinct smell of butyric acid, this is more pronounced the older the culture.

Cultures in sugar gelatine, as also cultures in milk, while young, not more than a week old, when injected into the subcutaneous tissue of guinea-pigs or mice prove virulent. Half to three-quarters of a cubic centimetre of the liquefied gelatine culture, or of the whey of a milk-culture, per 200 grammes body-weight of guinea-pig, injected under the skin of the groin, causes distinct illness already in six to eight hours: the animals are quiet, do not feed, they have œdematous swelling about the seat of injection, and the body temperature is lower than normal; their muscular movements become gradually greatly impaired, and they are found dead between twenty and twenty-four hours. Smaller quantities produce the fatal result in two, or even three days, and very small quantities cause only temporary illness and transitory local swelling.

On post-mortem examination, the subcutaneous and muscular tissue of the groin, of the whole of the abdomen

and chest, and even of the neck, are found deeply congested, separated from the skin by accumulations of gas, and the tissues infiltrated with copious sanguineous malodorous exudation. This, under the microscope, is densely filled with rod-shaped or cylindrical bacilli, few of these motile, most of them without motility. While the local appearances produced in the animal by our cultures bear a considerable general resemblance to those produced by injection of Koch's bacillus of malignant œdema, which, as is well known, is also an anaerobic microbe, there exist marked differences between the two ; in malignant œdema the sanguineous exudation contains, besides cylindrical bacilli, numerous characteristic, thread-like bacilli, in our cases these threads are quite absent, besides, the bacilli of malignant œdema are generally longer than those in our case ; in malignant œdema most of the bacilli are actively motile, in our cases very few are motile, and these only feebly so. A further difference is brought out by the examination of microscopic specimens, both of the cultures and of the subcutaneous exudation, in which the bacilli have been submitted to the process of staining after Gram. While the bacillus of malignant œdema after staining with the dye is decolourised by Gram, our bacillus retains the dye well. The bacillus of malignant œdema does not cause the rapid curdling of milk, as our bacillus does.

Another noteworthy difference between the two microbes is the rapidity of liquefaction of sugar gelatine in anaerobic cultures : although the colonies in sugar gelatine look alike for both these microbes, our bacillus liquefies the gelatine conspicuously faster than the bacillus of malignant œdema, and the gelatine liquefied by the former is less turbid than that by the latter. Also, in respect of flagella, a marked difference is noticed between the two microbes. The

bacillus of malignant œdema possesses numerous flagella fastened along its cylindrical body, our bacillus possesses flagella only near the rounded ends ; the short rods possess, as a rule, flagella at both ends, one, two, or three at one, a bundle of three to eight at the other end, and the flagella are always attached at one point laterally to the rounded end ; the cylindrical bacilli have one to three flagella at one end. Some of the flagella are very long—six to ten times the length of the bacillus—and spiral, others are shorter and wavy. In a preparation in which the flagella are successfully stained (by Van Ermengem's modification of Löffler's method), besides those that are still attached to the bacillus there are numerous flagella—single or in bundles, wavy or spiral—which are free, that is, had become detached during the process of preparation. It is certainly very surprising to find in such specimens what a large number of bacilli do possess numbers of long spiral flagella, and to compare with this the extremely feeble motility shown by the few in the fresh state ; from a flagella-stained specimen one would conclude that the majority of the bacilli are possessed of brisk motility, such a conclusion is, however, very conspicuously contradicted by actual observation.

A further difference between our bacillus and that of malignant œdema is the distribution and morphology of the bacilli in the infected animal : while in animals that succumb after infection with the malignant œdema bacillus numerous bacilli are present in the spleen, many of them as the characteristic threads, in our case the spleen contains the bacilli very sparingly, and then only as short rods, and considerable masses of spleen tissue have to be used for obtaining successful cultures ; the same applies to the blood of the circulation. But in the size and the position of the spores in the bacilli our bacillus closely resembles the

bacillus of malignant œdema. As in the case of the bacillus of malignant œdema so also with our bacillus, larger doses of culture are required for infection of guinea-pigs than of the subcutaneous exudation, this latter on subcutaneous injection proving more virulent than the artificial culture. In our case, subcutaneous injection of five minims of the subcutaneous exudation suffices to produce fatal infection within twenty to twenty-four hours in a guinea-pig of 200 grammes weight.

Spores alone, or cultures five to seven days old in which spore-formation is nearly completed, do not act as virulently as young cultures when injected subcutaneously into the guinea-pig, larger doses of the former being required to produce the same result as smaller doses of the latter. Doses, which taken from recent cultures produce fatal results in twenty to twenty-four hours, when taken from old cultures full of spores produce only a transitory local swelling and transitory constitutional disturbance. Neither mice nor guinea-pigs are susceptible to infection by feeding with spores.

Injected into the peritoneal cavity of the guinea-pig the bacilli of young cultures produce fatal results in six to eight hours; the peritoneal cavity containing after death copious sanguineous exudation full of the bacilli.

	μ.
The size of the bacilli is, length.....	1·6 to 4·8
,, ,, thickness.....	0·8
,, free spores is, length.... ..	1·6
,, ,, thickness....	0·8 to 1

In size, shape, feeble motility, in the rapid liquefaction of sugar gelatine, in the characteristic changes produced in milk, our bacillus resembles the anaerobic *Bacillus butyricus* described by Botkin,[1] but Botkin's bacillus differs from our

[1] Botkin, "Ueber einen Bacillus butyricus," *Zeitschrift f. Hygiene u. Infectionskrank.*, bd. xi. p. 421.

microbe by the character and aspect of its young colonies in gelatine[1] and Agar, and by not being pathogenic. Our organism is as strongly pathogenic as that of malignant œdema, from which, however, as pointed out above, it differs, both morphologically and culturally, in several important points.

Bacillus variolæ—vacciniæ.—In the Report of the Medical Officer of the Local Government Board for 1892–1893 I described a peculiar extremely minute bacillus as occurring in the calf-lymph and in human variola lymph during the early phases; in the calf-lymph 72 to 96 hours after vaccination, in the human variola during the third or fourth day; in both instances the lymph was collected aseptically and only clear lymph and as much as possible without any epidermal adnexa was used for film specimens; after heating and treatment with 30 p.c. acetic acid for some minutes, were subjected to prolonged staining in alcoholic gentian violet. Some of the films of calf-lymph (collected after removal of the epidermis as a whole) showed an abundance of these minute bacilli, generally in small and large masses; some of the specimens look like film specimens of an artificial culture (Figs. 159 and 160). Lymph of early human variola vesicles showed the same bacilli, but not so abundantly. Calf-lymph of later stages (five or six days old) showed no bacilli or only here and there a trace. In the bacilli, when abundant, forms may be recognised in which some globules of the nature of spores were present, in Fig. 161 this is shown in the bacilli magnified 2000. The presence of these spore-like bodies and the absence of the bacilli in the lymph of later stages led me to the conclusion that we have here to deal with a spore-forming bacillus, and

[1] The colonies of Botkin's bacillus butyricus grow slower, and are in their early phases more opaque and distinctly filamentous.

that after the multiplication of the bacilli in the early phases has reached its climax spores begin to be formed, and it is these which prevail in the lymph of the later phases. This would well accord with the known facts concerning the preservation of the active principles, for it is established that the active principle of vaccine is preserved in glycerine, although as is also known pure glycerine acting for long times is a germicide for cocci and sporeless bacilli; likewise lymph dried in thin layers on points preserves its efficacy for long periods, although such prolonged drying would kill all but spores.

Large numbers of species of microbes, have been described as occurring in vaccine lymph: cocci, bacilli, torula, and there is no difficulty in demonstrating by film specimens and particularly by culture their occurrence in lymph collected in the usual fashion—*i.e.* without special precautions in avoiding surface or epidemic admixtures. My experience, extending over a very considerable number of experiments, is this, that from carefully and properly collected vaccine lymph (humanised) such as is sent out by the Vaccine Department of the Local Government Board, and such as it is possible to collect aseptically from a calf-vesicle (after scraping off the crust) from a percentage of tubes containing proved active vaccinia, no cultures are obtainable in the ordinary media (nutrient gelatine, nutrient Agar, sugar gelatine, sugar Agar, solidified serum) although the cocci and bacilli which are present and have been described in many samples of vaccinia are easily cultivable in these media: staphylococcus albus and cereus, staphylococcus aureus, bacillus mesentericus, torula, &c. From my own observations, which are in complete accord with those of Dr. Copeman, I maintain that none of those ordinarily cultivable microbes are an essential inhabitant in vaccinia, and can have

anything to do with its active principle. Now, the above minute bacilli which I have described above as occurring abundantly in early phases in calf-lymph—in some instances so abundant that the lymph looks like a culture of them—are not cultivable in the ordinary culture media. The very lymph from which the specimens of Figs. 159 and 160 were derived was tested by culture and by transference to the calf,

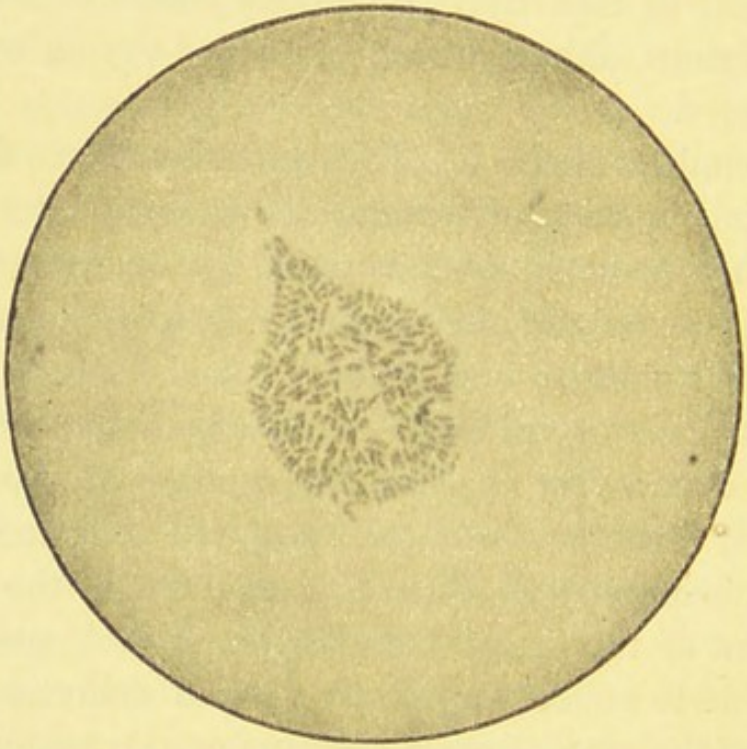


FIG. 159.—FILM SPECIMEN OF CALF-LYMPH 72 HOURS, CLUMPS OF MINUTE BACILLI.

How minute these bacilli are, is shown by the fact that the photogram is taken at a magnification of 1000.

and while in this latter it produced typical vaccinia it failed to produce any growth whatever in the culture media (solidified blood-serum, glycerine Agar, ordinary Agar, sugar gelatine, and ordinary gelatine). From these observations I concluded that the above minute bacilli are most probably the microbes of vaccinia. Dr. Copeman, who has worked at the same subject, has completely confirmed the presence of these

bacilli in active lymph and their inability to grow in the ordinary culture media.

L. Pfeiffer (*Die Protozoen als Krankheitserreger*, Jena, 1890) describes the presence of coccidia in the epithelium in variola, vaccinia, varicella, herpes zoster, and other vesicular eruptions. From his description and the illustrations given by him (Figs. 28–34, pp. 88–99) he has no doubt that they occur in the substance of the epithelial

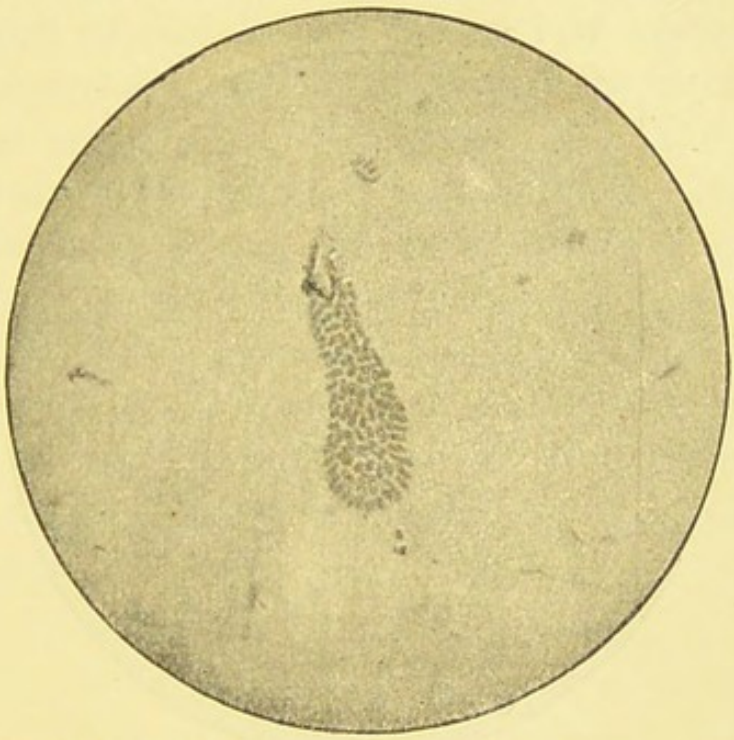


FIG. 160.—FROM A SIMILAR SPECIMEN AS THE PRECEDING FIGURE. × 1000.

cells; that here they (the coccidia) multiply by division, and form in their interior the spores. It can be easily shown that certain peculiar bodies do occur in the epithelial cells in these affections, which bodies are not the typical ordinary nuclei, and which can be brought out by various dyes, and thereby can be differentiated both from the cell-protoplasm and from the ordinary cell nucleus. In sections through the vesicles of sheep-pox, as also of human small-pox, stained

first with rubin and then with methyl blue, many of the epithelial cells in the region of the vesicle contain each an oval or spherical homogeneous body, which by its pink colour is well marked off both from the cell protoplasm and the swollen and hydropic cell nucleus, both these being stained blue; but it is extremely difficult—and it seems premature and improbable—to identify them as of the nature of extraneous parasites, viz., coccidia: on the contrary, these bodies look extremely like derivatives of the cell nucleus.

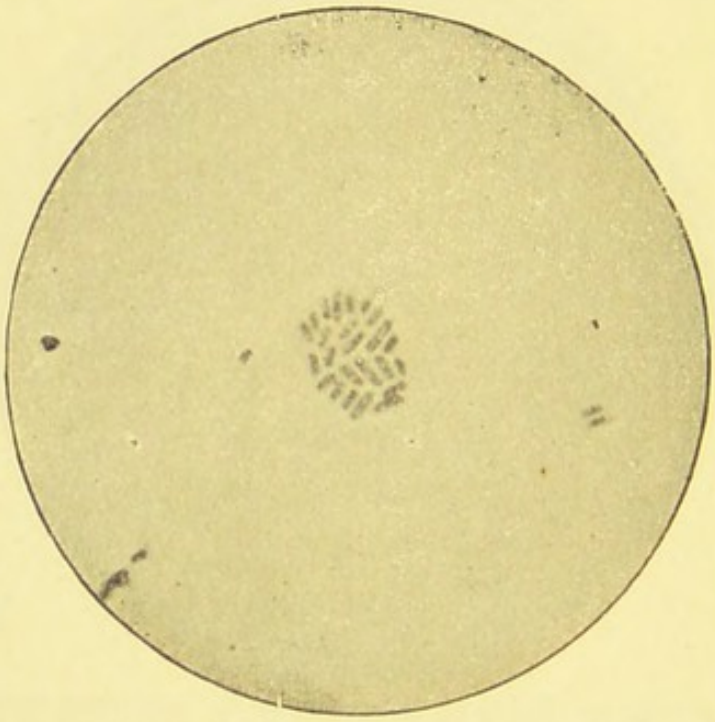


FIG. 161.—FROM A SIMILAR SPECIMEN.
× 2000.

In 1892 Guarneri describes after inoculation of the cornea of rabbits with variola and vaccinia in the epithelial cells of the cornea a peculiar parasite, called by him Citoryctes. L. Pfeiffer, J. Clarke, and Sicherer confirmed their occurrence in the corneal epithelium under the same conditions.

Quite recently Ernst Pfeiffer (*Centralblatt f. Bakt. und Parasitenk.* xviii., No. 25) describes the same bodies:

spherical, oval, crescentic, granular, spindle-shaped or threadlike bodies of about the size of red blood discs, or larger and smaller fractions of them, after a few hours to a few days after insertion of the lymph, in the tissue of the corneal substance or in the corneal epithelium. These bodies stain in dyes somewhat like red blood discs or hæmoglobin masses, and to my mind, after reading the description and illustrations given by E. Pfeiffer, the probabilities are very great that these bodies are in reality blood discs or part of such, as well as nuclei of leucocytes, that had been introduced into the cornea with the vaccine lymph. What must appear extremely curious is—(1) the indefinite shape and size of these bodies, and (2) the fact that according to these observers they should be capable of growth and multiplication in the rabbit's cornea, which animal is well known to be insusceptible to vaccinia.

It cannot be said from the facts adduced that we have really to do with a living parasite; it seems to me for the above reasons more probable that these bodies are not parasites at all.

CHAPTER XVI

VIBRIO AND SPIRILLUM

VIBRIONES are called those bacteria which have the shape of a more or less curved cylindrical rod—comma bacilli, and when after division the two new individuals remain joined end to end they form a characteristic S-shaped microbe. Vibriones elongate and by repeated divisions and the new elements remaining joined end to end produce wavy, spiral, or corkscrewlike filaments or spirilla. Spirilla may be uniform without being composed of jointed commas or they may be composed of separate vibrios. Some species of vibrios form uniform unsegmented spirilla, others may have less tendency to do so or may produce short segmented spiral chains. When growing in fluid some species form readily long spirilla apparently showing no segmentation. There exist considerable differences both with regard to the length of the spirals and the amount of curvature, for in some media or in some species the comma bacilli or vibrios form readily well twisted spirals, while in another medium or of another species the spirilla are short, or if long are only slightly wavy. Many of the species of vibrios and spirilla are distinctly motile, and where flagella staining had been applied have been seen to be possessed of one or

two fine spiral or wavy flagella. Owing to the curved shape their movement is always characteristically corkscrewlike, and therefore already by observing their movement in the fresh specimen (hanging drop) they can be recognised as comma bacilli or vibrios. This is particularly striking in the S-shaped forms.

The individual comma bacilli in stained and well-washed specimens show the same distinction into sheath and protoplasm as was mentioned of the bacilli, and also the presence of a vacuole in the middle of the individual comma bacilli and the terminal easily stained collections of protoplasm. Though in some species of bacilli, *e.g.*, bacillus of glanders, bacillus of diphtheria, there exist rods which are more or less curved, they do not form spirals, and their curved character is not permanent ; but in the true vibrios and spirilla, however slight the curvature of some elements—and in some species and under some media the curvature of some of the elements is very slight indeed—they nevertheless are capable of forming spirals. Above all only vibrios and spirilla form S-shaped forms, and the presence of these is as typical a character as the formation of spirals themselves. Anthrax bacilli growing on alkaline gelatine assume occasionally a curved shape, while Finkler's spirilla, or those found in noma and in cholera Asiatica, appear in some media only to show the very slightest curve ; but from subcultures of the above anthrax bacilli in broth or gelatine the typical straight anthrax bacilli result, while of the above spirilla subcultures made in broth, in gelatine, &c., the typical spirilla will be the result. This shows that the first, though they may occasionally become curved rods, are not spirilla but bacilli, and the latter, though the individuals may occasionally appear almost straight, are not bacilli but spirilla.

Similarly, some of the bacilli of proteus vulgaris, of diphtheria, and of glanders are of a curved shape, but they do not form S-shaped forms or spirilla. Cohn[1] has described a number of vibrios and spirilla occurring in various decomposing fluids.

(*a*) *Vibrio rugula* consists of rods of about 8 to 16 μ in length, and curved either like a C or like an S. They are single, or form chains of two. Their protoplasm is always slightly granular. They are found in putrefying organic

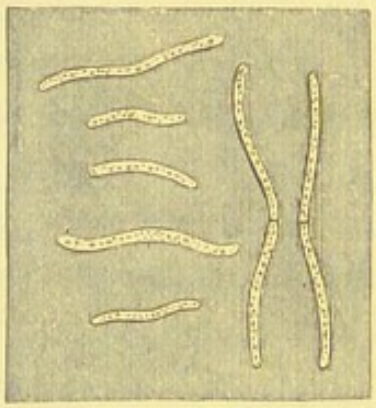


FIG. 162.—VIBRIO RUGULA (AFTER COHN).

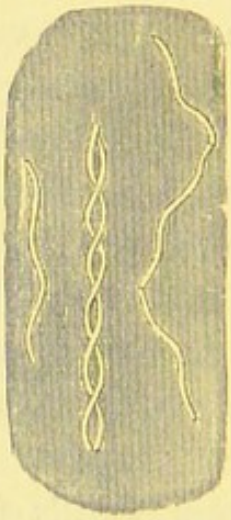


FIG. 163.—VIBRIO SERPENS, ISOLATED (AFTER COHN).

substances, and often form continuous masses, the individuals interlacing in all directions.

(*b*) *Vibrio serpens.*—This is also a septic organism, much thinner and longer than the previous one, more wavy, as a rule, curved into a single or double wave. The length varies between 11 and 25 μ. It is motile; and also forms continuous masses, the individuals interlacing in all directions.

(*c*) *Spirillum tenue.*—This is much finer and more wavy than vibro serpens, the turns being closer together and

[1] *Beiträge z. Biol. d. Pflanzen*, vol. ii.

spiral. Its length varies between 2 and 5 μ; it often forms continuous felted masses; it is motile.

Occasionally the spirilla grow to a great length—two, three, and more of them forming a chain; the individual

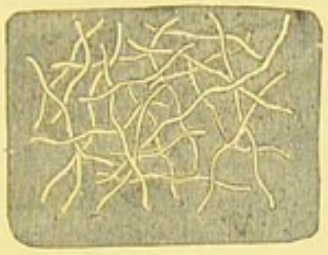


FIG. 164.—VIBRIO SERPENS IN SWARMS (AFTER COHN).

spirilla are not arranged in a linear series, but folded into a zigzag. This form, which in reality is not a special kind of spirillum, is called by Cohn[1] *spirochæta plicatilis*. The

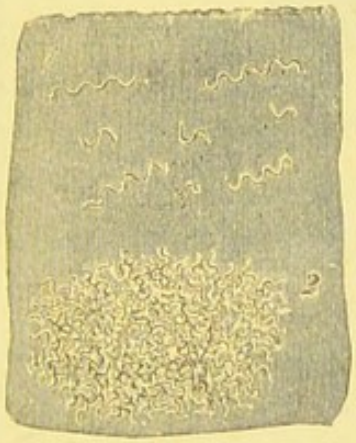


FIG. 165.—SPIRILLUM TENUE, (1) SINGLY AND (2) IN SWARMS (AFTER COHN).

spirillum found in the tartar of the teeth is of this form, *spirochæta denticola*. But there exist all intermediate forms between a single spirillum tenue and a spirochæta. In stained specimens the construction of the spirochæta from

[1] *Beiträge zur Biologie d. Pflanzen*, vol. ii

several spirilla tenua is very distinct in some, though not in others.

(*d*) *Spirillum undula* is much thicker and shorter than the former; there are all forms between such as are only half a turn to such as are of a whole turn of a spiral. It is motile and forms chains of two or more elements, occurring

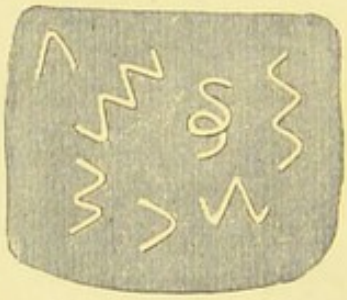


FIG. 166—SPIRILLUM UNDULA. (AFTER COHN).

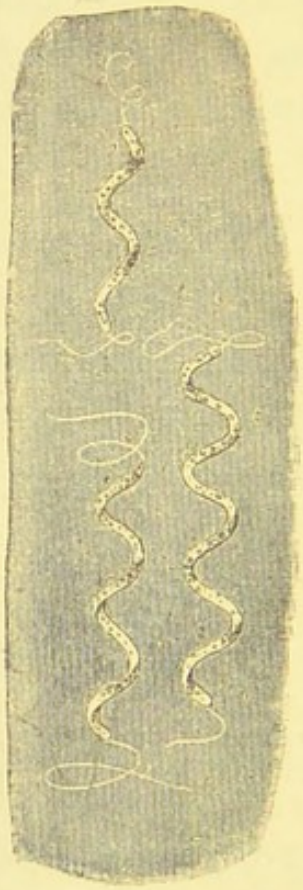


FIG. 167.—SPIRILLUM VOLUTANS (AFTER COHN).

also in continuous masses, occasionally held together by a hyaline interstitial substance.

(*e*) *Spirillum volutans.*—These organisms are giant spirilla; long and thick, with granular protoplasm; 25 to 30 μ long; motile, and with a flagellum at each end.

(*f*) *Spirillum rosaceum.*—I have seen on paste a spirillum, morphologically identical with spirillum undula; it is of a

pale pink or rosy colour.[1] It is motile, and forms a kind of zoogloœa, the individuals being closely placed and therefore producing a rosy colour of a more decided tint. Where they form continuous masses, the naked eye can detect the rosy tint.

(*g*) *Spirillum sanguineum* (*Ophidomonas sanguinea*, Ehrenberg).—This was observed by Cohn and Warming[2] in pond-water. Morphologically it is identical with spirillum volutans. It is motile, with a flagellum either at one or both ends. Warming occasionally saw two and three flagella at one end. It is about 3 μ thick; all forms occur between such as have half and such as have two and a half turns of a spiral. Lankester also saw the same kind of organism among his peach-coloured bacteria.[3]

(*h*) *Spirillum rubrum* (von Esmarch[4]) forms long, very motile spirilla, possessed of numerous flagella attached to the sides of the spirilla; it does not liquefy gelatine. Its colonies are of a deep red colour.

(*i*) A variety of species of vibrios have been described by Weibel[5] as occurring in sewage and on cultivation formed coloured growths: vibrio aureus, flavescens and flavus; none of them liquefy the gelatine and are apparently not possessed of motility.

(*j*) Elwers and also Dunbar have isolated a vibrio or spirillum phosphorescens which in cultivation has the power to form phosphorescence; it liquefies gelatine, and is motile.

(*k*) Dr. Lingard has found in, and I have isolated from,

[1] "On a Rose-coloured Spirillum," *Quar. Journ. of Micr. Sci.*, vol. xv. New Series.
[2] *Beitr. z. Biol. d. Pflanzen*, vol. i.
[3] *Quarterly Jour. of Micr. Science*, vol. xiii. New Series.
[4] *Centralbl. f. Bakt. und Parasit.*, vol. i., p. 225.
[5] *Ibid.*, vol. iv., p. 258.

the necrotic tissue of the tumour in noma of a child a motile vibrio, which does not liquefy gelatine; it forms on it in streak a moist brownish growth; in film specimens the vibrios are found as commas, as S-shaped forms, and as wavy or corkscrewlike longer or shorter spirilla. It grows well at 37° C. on Agar and forms also here in streak a brownish moist filmy growth. In gelatine plate it forms

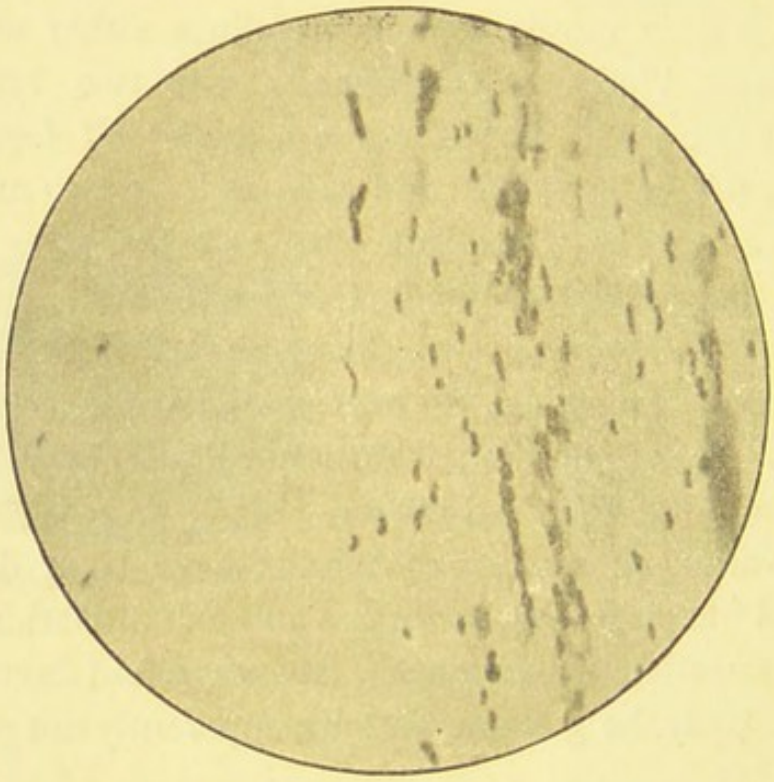


FIG. 168.—FILM SPECIMEN OF A FLAKE OF A RICE-WATER STOOL, SHOWING THE VIBRIOS IN LINEAR ROWS; ONE SHOWS A FLAGELLUM.
× 1000.

greyish round colonies which slowly enlarge, and after a week or ten days are not more than a few millimetres in diameter. (*Bacteria in Asiatic Cholera.* Macmillan, 1889, p. 103.)

(*l*) *Vibrio or spirillum choleræ Asiaticæ* (Koch), comma bacillus of Koch.—Examining microscopically the intestinal discharges of acute cases of cholera one notices, besides

detached epithelial cells and lymph-corpuscles, numerous bacteria belonging to different species of micrococci and bacilli. Some there are amongst them which are comma-shaped, *i.e.*, curved, cylindrical rods, single or double, or S-shaped; they are motile, spinning round or moving in a spiral; they are of different lengths and of different amount of curvature, but, as cultivation experiments show, belong all to

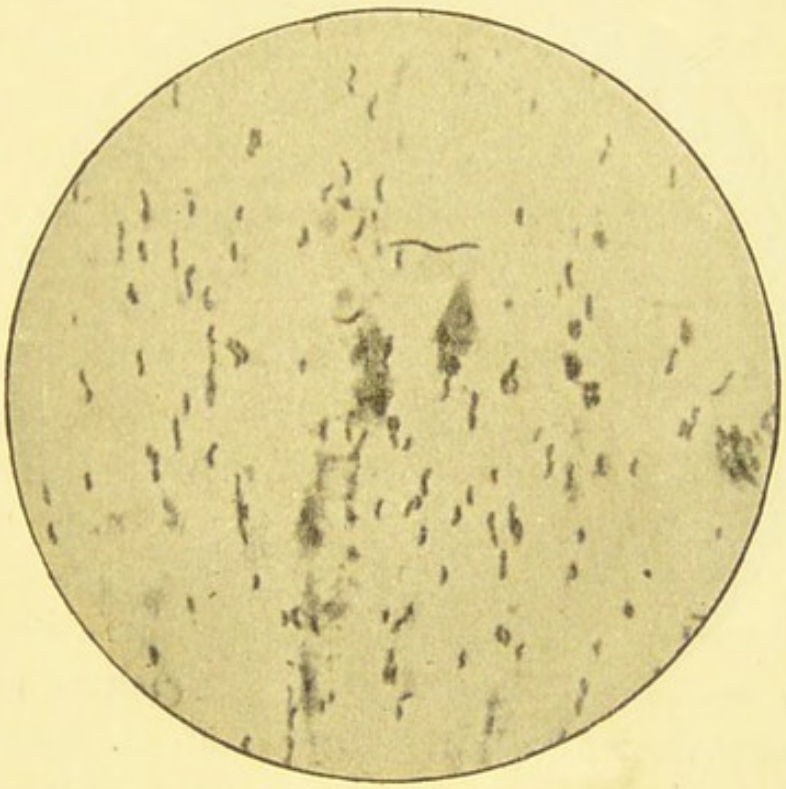


FIG. 169.—FILM SPECIMEN OF A FLAKE OF THE RICE-WATER FLUID OF A DIFFERENT CASE.

× 1000.

the same species: namely, the comma bacilli, or vibrios or spirilla of Koch, discovered by him as constantly present in the acute stages of cholera Asiatica, and as showing definite cultural characters.[1] There exist, however, considerable differences with regard to the number of these comma bacilli

[1] Conferenz zur Erörterung der Cholerafrage, *Berliner kl. Woch.* 31, 1884.

present. In some acute cases the mucus flakes of the typical rice-water stools or of the intestinal fluid contain these comma bacilli in enormous numbers, almost to the exclusion of other bacteria; such is the case in some typical cases in the mucus flakes taken directly from the watery contents of the ileum, though the mucus flakes taken in the same

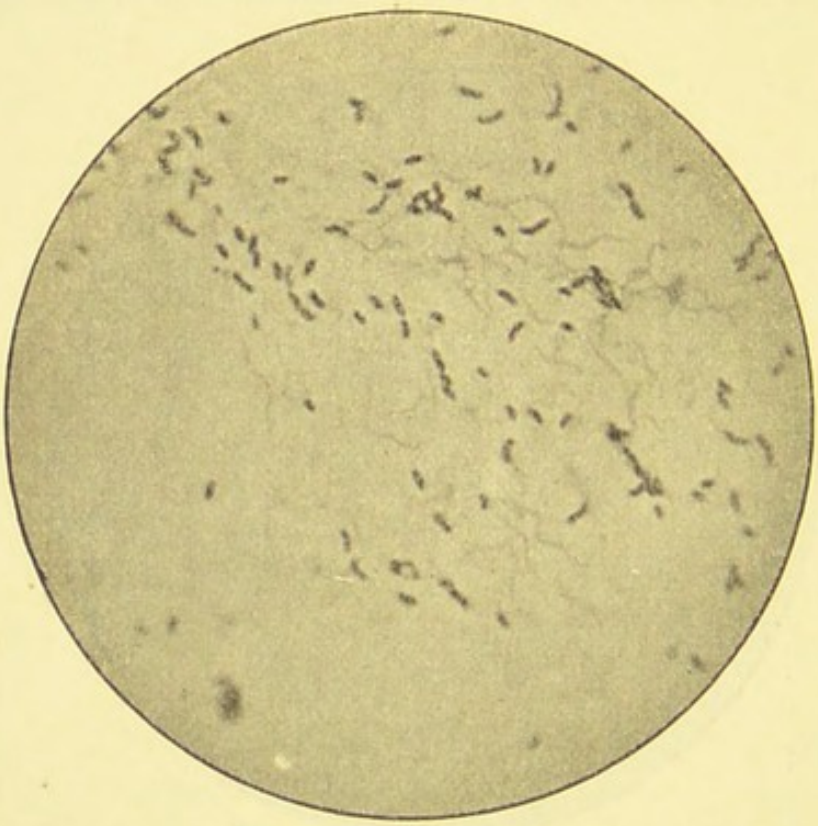


FIG. 170.—FILM SPECIMEN OF A FLAKE OF THE RICE-WATER FLUID OF A FURTHER CASE

Numerous flagella are seen.

×1000.

body from the jejunum, in all other respects identical, contain but few of these comma bacilli. In other equally typical acute cases they are mixed up with other bacteria.

There is no definite relation between the number of Koch's vibrios in the intestinal fluid and the severity, acuteness or purity of the case. Some cases there no doubt are in which the mucus flakes of the rice-water

stools or of the contents of the lower ileum are crowded with the comma bacilli, but in a considerable percentage of typical cases this condition does not obtain; there are comma bacilli present, but they are mixed up sometimes to a considerable amount with other bacteria. The epithelial flakes detached and suspended in the contents of the ileum, as well as the epithelial flakes loosened but not quite detached from the mucous membrane, both of the villi as also of the mouth of the Lieberkühn's follicles, contain comma bacilli as well as other bacteria. In sections through the hardened mucous membrane of the ileum one can find sometimes comma bacilli as well as other bacteria within the tissue of the superficial mucosa denuded of epithelium, in the cavity of the Lieberkühn's follicles and in spaces artificially produced by the loosening and detachment of the epithelium of the Lieberkühn's follicles, but their presence in these localities is due to immigration from the free surface into a disorganised mucous membrane, and neither bears any relation to the onset nor to the severity of the illness. Where the comma bacilli are scarce in the intestinal contents, they, or other bacteria, are altogether missed from the mucosa, where they are abundant in the contents and on the surface they may penetrate from the surface into the mucous membrane.

In Figs. 168 and 169 film specimens of mucus flakes of typical acute cases of Asiatic cholera are represented, in which the cholera vibrios are present in fairly pure state, and it will be noticed that as Koch has pointed out they are arranged more or less in linear rows, "fish-in-stream arrangement"; this condition and distribution of comma bacilli in mucus flakes of watery stools is so characteristic of cholera asiatica that it alone is sufficient to make a correct diagnosis, although as a matter of routine further experiments of culti-

No.	A. Derivation of Material.	B. Microscopical Characters of Stool or of Intestinal Contents.	C. General Characters of Cultures.	D. Cholera Red Reaction.
I.	Hull, No. 1	Typical	Positive	Distinct
II.	Grimsby, No. 1	,,	,,	,,
III.	Grimsby, No. 2	,,	,,	,,
IV.	Hull, No. 2	,,	,,	,,
V.	Rotherham	,,	,,	,,
VI.	Westminster	,,	,,	,,
IX.	Boston	Not altogether typical	,,	,,
X.	Morton (Gainsboro' R.)	,, ,, ,,	,,	,,
XII.	Leicester	Typical	,,	,,
XIII.	Handsworth	Doubtful	,,	,,
XIV.	Retford	Typical	,,	,,
XV.	Fulham	Not typical	,,	,,
XVII.	Kennington (Lambeth)	,,	,,	,,
XVIII.	Ashbourne	,,	,,	,,
XXII.	Croydon Borough	,,	,,	,,
XXIV.	Derby	Typical	,,	,,
XXVII.	Accrington	,,	,,	,,
XXX.	Ilkeston	Not typical	,,	,,
XXXIII.	Appleton-le-Street, No. 1	Fairly typical	,,	,,
XXXVI.	Great Yarmouth, No. 1	Typical	,,	,,
XXXVIII.	Tividale (Rowley Regis)	Fairly typical	,,	,,
XXXIX.	Southwark (St. George the Martyr)	Not typical	Not liquefying gelatine in stab, slowly liquefying in plate culture	,,
XL.	Great Yarmouth, No. 2	Typical	Positive	,,
XLI.	Liverpool	Not typical	,,	,,
XLIII.	Coton Hill (Stafford R.)	Fairly typical	,,	,,
XLV(a).	North Bierley, No. 2	Typical	,,	,,
LI.	Balby (Doncaster R.)	,,	,,	,,
LII.	Rawmarsh	,,	,,	,,
LIV.	Bingley (Township)	Not typical	,,	,,
LV.	Keighley	Doubtful	,,	,,

E. Growth in Gelatine Stab Culture.	F. Growth on Potato Culture at 37° C.	G. Milk Culture at 37° C.	H. Amount of Agar Culture required for production, by Intra-peritoneal Injection, of Fatal Result in Guinea-pigs.
Liquefied fairly quick ; good pellicle	No growth after 10-14 days.	Coagulated after 11 days.	$\frac{1}{6}$ of a tube.
,, ,, ,,	Light yellow after 14 days.	Fluid after 14 days	$\frac{1}{8}$,,
Liquefies quickly; no pellicle	No growth after 14 days	Coagulated after 6 ,,	$\frac{1}{6}$, $\frac{1}{4}$,,
,, ,, ,,	,, 14 ,,	,, 11 ,,	$\frac{1}{8}$,,
,, ,, slight pellicle	,, 14 ,,	Fluid after 14 ,,	Not tested.
,, ,, ,,	,, 14 ,,	,, 14 ,,	$\frac{1}{6}$ of a tube.
Fairly quick ; good pellicle -	,, 14 ,,	,, 14 ,,	Not tested.
,, no pellicle -	Light yellow after 14 days.	Coagulated after 5 ,,	,,
Moderate ; good pellicle -	Light brown after 5 days.	,, 5 ,,	$\frac{1}{8}$ of a tube.
Fairly quick ; slight pellicle	No growth after 14 days	Fluid after 14 ,,	Not tested.
Moderate ; slight pellicle -	,, 14 ,,	Coagulated after 5 ,,	,,
Fairly quick ; good pellicle -	,, 14 ,,	Fluid after 14 ,,	$\frac{1}{6}$, $\frac{1}{4}$ of a tube.
Quick ; slight pellicle - -	,, 14 ,,	Coagulated after 6 ,,	$\frac{1}{6}$, $\frac{1}{4}$,,
Slow ; no pellicle - -	,, 14 ,,	,, 10 ,,	$\frac{1}{6}$, $\frac{1}{4}$,,
Quick ; good pellicle - -	,, 14 ,,	,, 6 ,,	Not tested.
,, no pellicle - -	,, 14 ,,	Fluid after 14 ,,	$\frac{1}{8}$ of a tube.
Slow ; good pellicle - -	Light yellow after 5 ,,	,, 14 ,,	$\frac{1}{6}$,,
,, ,, - -	,, 5 ,,	,, 14 ,,	Not tested.
Fairly quick ; good pellicle	No growth after 14 ,,	Coagulated after 10 ,,	,,
,, ,, ,, -	,, 14 ,,	,, 11 ,,	$\frac{1}{6}$ of a tube.
Slow ; no pellicle - -	,, 14 ,,	,, 5 ,,	Not tested.
Not liquefying - - -	,, 14 ,,	Fluid after 14 ,,	$\frac{1}{8}$ of a tube.
Quick ; slight pellicle -	,, 14 ,,	Coagulated after 11 ,,	$\frac{1}{8}$, $\frac{1}{6}$,,
Fairly quick ; good pellicle	,, 14 ,,	,, 5 ,,	Not tested.
Moderate ; good pellicle -	Light yellow after 5 ,,	,, 10 ,,	$\frac{1}{6}$ of a tube.
Quick ; no pellicle - -	No growth after 14 ,,	,, 14 ,,	Not tested.
,, slight pellicle - -	,, 14 ,,	,, 5 ,,	,,
,, good pellicle - -	,, 14 ,,	,, 5 ,,	,,
Very quick ; no pellicle -	,, 14 ,,	,, 6 ,,	,,
,, ,, - -	Light yellow after 14 days.	,, 6 ,,	$\frac{1}{6}$, $\frac{1}{4}$ of a tube.

vation are resorted to for confirmation. Unfortunately such a condition is present only in a percentage of cases; amongst the fifty odd cases of Asiatic cholera occurring in England in September and October of 1893 (*see* Tabular Statement) such a condition was found in fifteen cases, that is to say, when from the number and distribution of the vibrios in the flakes of the intestinal contents alone the diagnosis could be made.

Koch's cholera vibrios or Koch's comma bacilli can be demonstrated in almost all cases of cholera Asiatica, beginning with those that show as yet only diarrhœa, more or less profuse, up to those that have shown all the typical characters, with vomiting and purging of copious rice-water evacuations. After the acute stage has passed, and the typhoid stage has set in, the comma bacilli become less numerous, and gradually disappear, so that when after three, four, or five days the evacuations assume again the character of fæces the comma bacilli are either only found with difficulty or are altogether missed; in fact, in cases in which they are scarce at the earlier stage they are not to be seen later than the third day.

If cholera stools, particularly rice-water stools, are kept for a day or so, one meets with comma bacilli which have formed spirilla; some wavy threads, others distinctly corkscrew-shaped, some short, others long; in dried and stained preparations many of these spirilla are seen to be chains of comma bacilli; spirilla are found occasionally already in the fresh stools or fresh mucus flakes, but as a rule the comma bacilli are present as single vibrios or as dumb-bell vibrios, *i.e.*, S-shaped forms. As regards the amount of curvature and length of the individuals there exist variations. Moreover as cultures prove and as has been already mentioned (*see also* Tabular Statement of Cholera Cases in

England in 1893) the commas derived from different undoubted cases of cholera represent different varieties, that is to say they are in their general characters and reactions cholera vibrios, but in the details of the appearances of their growth in the different media they differ in a definite manner, which are not merely of an accidental or transitory character but are differences maintained by them in sub-culture through a number of successive transferences. These facts fully confirm the statements first made by D. D. Cunningham (Scientific Memoirs) derived from observation of cholera in Calcutta, and although at first doubted (as for instance by Hueppe and Gruber at the International Congress of Hygiene held in London in 1891) they are now admitted, by no one more so than by Hueppe and Gruber. In this I am not referring to changes which are well known to occur in individual varieties in course of many transferences, *e.g.* the gradual decrease or increase in rapidity with which the gelatine is liquefied, or the differences that can be observed in subcultures through many transferences as regards the more or less distinct alteration in the formation of a pellicle on gelatine or on broth, &c., but I am referring to pronounced differences present from the outset on the different commas of different stock, and persisting for many generations unaltered.

For the object of demonstrating in a rapid manner the presence of the cholera vibrios in the evacuations, even when present in very small numbers, the method of Dunham is the best : a flake or a loopful of the dejecta or contents of the ileum is placed in a watery solution of pure peptone 1 per cent., common salt 0·5 per cent. After incubation at 37° C. already after 10–12 hours, better after 16–24 hours, greater or lesser turbidity (according to the number of comma bacilli present in the original intestinal material) is

noticed in the culture-tube; with a platinum loop a droplet is taken from the superficial layers of the culture fluid and examined in the living state (hanging drop) or in stained film specimens. In the former the individual commas and the characteristic S-shaped forms can be easily recognised under the microscope both by their shape and by the peculiar corkscrewlike movement; in the stained film specimen the presence of commas and particularly of S-shaped forms is of importance.

From these peptone cultures subcultures in Agar plates (at 37° C.) or in nutrient gelatine plates are then made for further isolation, and if the peptone culture on microscopic examination (stained film specimen) be found fairly pure the addition of a few drops of pure sulphuric acid to the peptone culture produces the nitroso-indol reaction of Bujwid[1] and Dunham[2], *i.e.* a pink colouration of the culture—*cholera-red reaction.* If the cholera vibrios are, however, mixed with other bacteria (bac. coli or proteus) then they must be first purified by plate cultures, and from the colonies of cholera vibrios of these plates pure peptone cultures can be made for the Bujwid-Dunham test.

Löffler, as has already been stated in a former chapter, was the first to stain the flagella of the cholera vibrios, and he found that each comma bacillus possesses one spiral flagellum at one end; but it can be shown by van Ermengem's modification that, though this is the rule, occasionally more than one such flagellum is present. I have shown[3] that by staining the flakes of a typical rice-water (cholera) stool with gentian violet the flagella of the cholera vibrios can be demonstrated as stained wavy or spiral appendages, and in some cases I

[1] *Zeitschrift f. Hygiene*, vol. ii. 1, p. 52.
[2] *Ibid.*, vol. ii. 2, p. 337.
[3] *Centralbl. f. Bakteriol. und Parasit.*, vol. xiv. No. 19.

have seen these flagella attached more than as a single flagellum for each vibrio, sometimes they were present as bundles (Fig. 170), still attached or free (detached in the course of preparation). Abel, Aufrecht, and others have described "fine faintly stained spirilla" in addition to the typical vibrio in cholera stools, and Abel thinks that what I considered to be detached free flagella were really only these

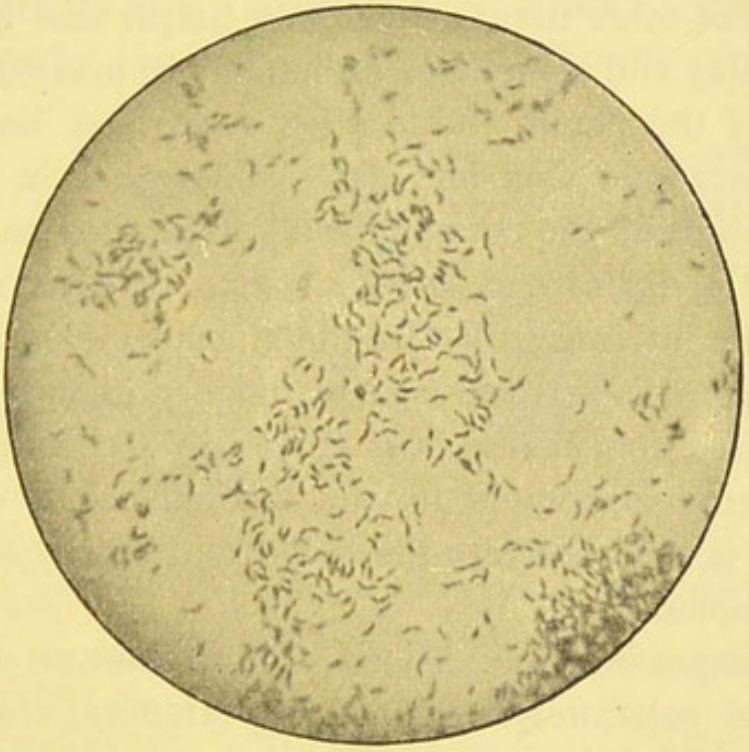


FIG. 171.—FILM SPECIMEN OF A RECENT AGAR CULTURE OF CHOLERA VIBRIOS × 1000.

"fine spirilla." Such "fine faintly stained spirilla" can be seen in every flagella-stained film specimen of bacillus coli, particularly of the typhoid bacillus taken from a pure Agar culture of these microbes, and I have seen free flagella and flagella attached to bacillus coli from flakes in the watery evacuations of severe acute diarrhœa, they resembled the above "fine faintly stained spirilla." Neither Abel nor anybody else has succeeded in cultivating the above "fine

faintly stained spirilla," and until this is done I maintain that they are detached flagella (probably of bacillus coli) which have become stained, and that there exists something in the watery stools which acted like a mordant and which makes the flagella susceptible of becoming stained.

The comma bacilli occur in cholera as a rule only in the cavity of the small and large intestines, chiefly the lower part of the ileum and large intestine; no bacteria occur in the blood or other tissues. Comma bacilli and also other bacteria may and sometimes do immigrate into the tissue of the wall of the ileum, and in a few cases have been traced even as far as the liver and gall-bladder; but in the large majority of cases the comma bacilli are limited to the contents of the ileum and large intestine and the superficial parts of the internal surface of the mucous membrane of the ileum. For this reason Koch maintained that the disease is an intoxication, that is, it is caused by a chemical poison which, being elaborated by the comma bacilli within the intestine, is absorbed into the blood, and hereby sets up the disease cholera.

The comma bacilli of Asiatic cholera show on cultivation in nutrient gelatine well-defined appearances, which enable us to recognise them, so much so that in suspicious cases of cholera their demonstration by cultivation in the evacuations is of diagnostic value. But in connection with this it must be borne in mind that in some cases or in non-typical cases their demonstration by the gelatine culture test, owing to the vast predominance of other bacteria, is a matter of some difficulty. Where they are present in large numbers their demonstration by the gelatine culture test is a matter of comparative ease. All that is necessary is to place a small flake of the evacuation into a few (8–10) cubic centimetres of sterile (well-boiled) salt solution, shake it well up,

and then with a droplet of this inoculate nutrient gelatine, contained in a test-tube, liquefy this in warm water, shake up and then pour it into sterile glass dishes for the object of plate cultivation. A particle of a mucus flake of a rice-water stool rich in the comma bacilli first diluted in several cubic centimetres of sterile salt solution and a trace of this mixture being used for plate cultivation yields large numbers of colonies of the comma bacilli. These show themselves (at 20° C.) already after thirty to forty hours as greyish-white minute specks just visible to the unaided eye; after two to

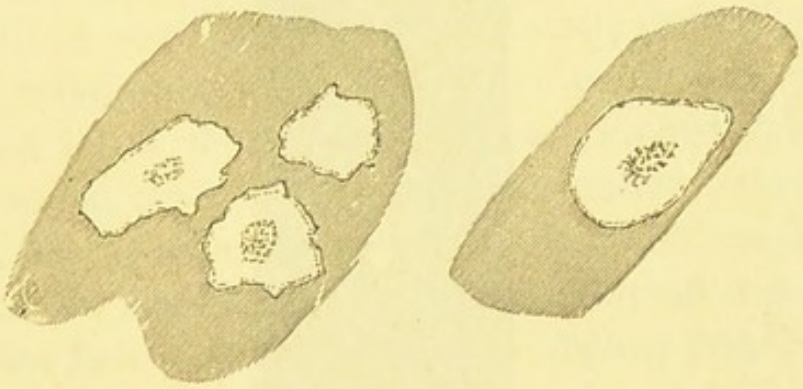


FIG. 172.—PLATE CULTIVATIONS IN NUTRITIVE GELATINE, AFTER THREE DAYS GROWTH AT 20°C., SEEN WITH THE UNAIDED EYE.

Colonies of cholera comma-bacilli.
The clear part is due to liquefaction of the gelatine.

three days they are distinctly visible as clear, circular depressions, due to liquefaction of the gelatine within this depression. In the centre of the depression is a round, greyish mass surrounded by clear, liquefied gelatine; looked at under a magnifying glass this mass appears like a mass of minute glass splinters, with a more or less uneven margin; in the centre of the mass is a more opaque larger granule. Each of the colonies gradually enlarges; the zone of clear, liquefied gelatine becomes broader, and the whitish central granular patch enlarges; where the colonies lie closely together at the outset, the progressing liquefaction produces

soon a coalescence of the adjoining colonies, and then we get a number of circular zones of clear, liquefied gelatine, each with a central gray granular mass, the zones being fused at the points of contact. When during the further growth the gelatine becomes liquefied over extensive areas,

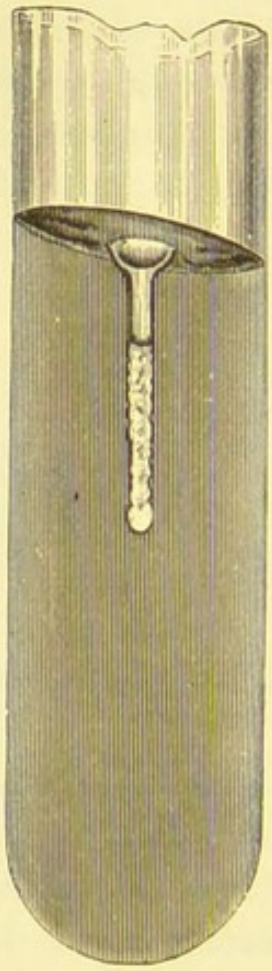


FIG. 173.

Part of a test-tube containing gelatine-peptone; in it pure cultivation of choleraic comma-bacilli. The funnel-shaped opening of the channel in which the growth of the comma-bacilli is going on contains a long air bubble.

the outlines of the original colonies are lost, and on the surface of the clear, liquefied gelatine are thin, filmy flakes, and at the bottom are minute whitish granules. In all stages before and after the liquefaction of the gelatine has become well pronounced, there are found under the microscope

rapidly motile vibrios, single commas, S-shaped dumb-bells and numerous longer or shorter spirilla, some wavy chains of commas, others uniformly spiral; the above-named "granules" and "flakes" are masses of commas and spirals intimately matted together, and when examined in the hanging drop look like so many clumps rapidly revolving.

In stab culture in gelatine the characters of these comma bacilli are also well marked; they are accurately represented in Fig. 176, and need not further be described, except that there are considerable differences as regards the rapidity with which the growth causes liquefaction in the gelatine; in all cases however it starts from the surface.

After several days to a fortnight there is noticed a distinct pellicle on the surface of the liquefied gelatine in some cases, in others such a pellicle is absent: the gelatine is clear, but contains a few whitish granules marking the outline of the funnel-shaped channel of liquefied gelatine.

Alkaline broth (at 36–38° C.) is slightly turbid already after twenty-four hours' growth: this increases during the succeeding days. After a week or so the superficial layers become gradually clearer, and this extends gradually and insensibly towards the deeper layers; hand in hand with this goes the deposit of a grayish-white powdery precipitate; a more or less distinct pellicle is noticed already after a few days, and this gradually increases in thickness. In some cases the pellicle is distinct and complete, in others it is absent. Under the microscope the comma bacilli in the fluid and in the pellicle are seen to be connected into beautiful spirilla, some of these measuring great lengths, some as many as twenty to thirty turns, the long spirilla more or less plicated and bent.

The growth on Agar mixture is not characteristic, being in the form of thin, translucent patches and films with

rounded or knobbed outline, assuming as growth goes on, *i.e.*, after some days, a slight brownish tint.

On boiled potato the comma bacilli grow only at temperatures above 25° C.; at 36° they form after a few days a thick, smeary, brown film. In some cases the growth is a transparent film, in others no growth takes place on potato. Comma bacilli grow well and rapidly, if mucus flakes of a cholera intestine containing numerous comma bacilli are placed on linen kept damp. After twenty-four hours the comma bacilli have increased to an enormous extent, almost to the exclusion of other bacteria originally present, provided these were at the outset less numerous than the comma bacilli.

Cholera vibrios show rapid growth at 37° C. on solidified blood-serum, which becomes liquefied by the growth.

In cultivations of the comma bacilli one meets with forms which in so far differ from the typical curved, cylindrical vibrios, as they are much thicker, plano-convex, or bi-convex, or even approaching the spherical shape with a clear vacuole in the middle. In well-stained and well-washed specimens also the most typical comma bacilli show within a sheath the protoplasm collected at the ends—as a granule at each end—whereas the middle part remains clear. The above atypical forms are merely a further development of their normal constitution, being derived from them by enlargement of the central clear space or vacuole. Such atypical forms are to be met with in all cultures; they are as actively motile as the typical commas; their number, however, varies greatly with the character of the culture. If comma bacilli, originally derived from the cholera intestine, are carried through many successive sub-cultures in gelatine, say one or two dozen, the number of such atypical bi-convex or spherical forms is found larger.

Comma bacilli when in culture rapidly undergo degeneration into granular débris; in fact, a good deal of the white deposit in gelatine and broth cultures is due to débris of comma bacilli. Degeneration goes on comparatively more rapidly in Agar culture than in gelatine cultures. It is a notorious fact that on the surface of Agar cultures the whole of the growth is found dead after from a few to several months, so that no new culture can be started from such an old culture. This degeneration and death occur sooner or later in all cultures after the lapse of some time; this alone proves sufficiently that the comma bacilli do not form permanent seeds or spores. Koch has proved by many experiments of drying that the comma bacilli are invariably killed by drying, unlike spore-bearing bacilli, and at no time do the comma bacilli form spores. Heating cultures (old or recent) of comma bacilli to 60° to 65° C. for five minutes invariably kills the cultures—proof that no spores are formed. The assertion of Hueppe that the terminal granules observed in comma bacilli are spores, viz., arthrospores, is definitely negatived by the above direct experiments.

Comma bacilli of cholera mucus flakes or of cultures, recent or old, are killed by acids, *e.g.*, a fluid containing 0·2 per cent. hydrochloric acid,[1] so that the normal acid fluid of the stomach kills the comma bacilli; also this is opposed to there being present spores in the comma bacilli.

Comma bacilli grow well and luxuriantly between 17° and 40° C., on almost anything—paste, boiled egg, turnip, cucumber, cabbage, bread, meat, various fruits, &c. They grow best at 35–37° C., if the medium is faintly alkaline, they nevertheless grow also on neutral medium, and even on some media like potato and fruit, which are slightly acid. I have seen comma bacilli which, having started on

[1] Koch, *l.c.*; Watson Cheyne, *Brit. Med. Journal*, 1885.

nutrient gelatine kept for a few days at 20° C., continued to grow slowly but steadily after the gelatine was then kept at 15–16° C. Comma bacilli gradually die off if nutriment is insufficient, *e.g.*, in water; they are gradually killed in fæcal matter (Kitasato); and they do not grow well when oxygen is absent from the culture (Koch).

Comma bacilli obtained from typical cases of Asiatic cholera grow well in milk at 37° C., they herein rapidly multiply and in some cases cause no visible change, while in others they cause coagulation of the milk; but also in regard to this latter phenomenon there exist considerable differences, for while some varieties cause coagulation after five or six days others take several weeks. Most varieties of cholera vibrios (derived from cases of Asiatic cholera) produce alkali in culture media (*e.g.*, in Petruschki's neutral whey), but some varieties undoubtedly produce slight acid. Another difference noticed between the vibrios derived from different cases of Asiatic cholera refers to their action when injected subcutaneously into guinea-pigs. Koch[1] had already succeeded in producing acute septicæmic infection of mice by intraperitoneal injection of large doses (*see* later), with rapid multiplication of the vibrios in the blood; Ferran and D. D. Cunningham[2] have succeeded in producing septicæmic infection by subcutaneous injection into guinea-pigs; after death the blood, the smeary exudation on the serous covering of the intestine and the intestinal contents containing an abundance of the cholera vibrios. I have produced this effect both with gelatine cultures of cholera vibrios as also of Finkler's vibrios, using 0·5–2 cc. of the liquefied culture per guinea-

[1] Conferenz zur Erörterung d. Cholerafrage, *Berl. klin. Woch.* 31, 1884.

[2] *Scientific Memoirs*, Calcutta, 1891.

pig ; the animals died in thirty to forty hours, the blood and the intestine, liver, and spleen containing numerous vibrios. Now, when testing the cultures of cholera vibrios derived from different cases of undoubted cholera asiatica and grown on the slanting surface of solidified nutrient Agar for a day or two it will be found that they possess different degrees of virulence. Of some varieties $\frac{1}{6}$ or $\frac{1}{8}$ of a culture produces distinct tumour at the seat of inoculation and

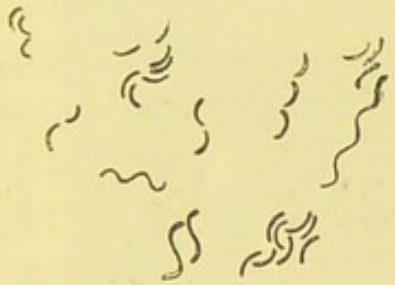


FIG. 174.

From an Artificial Cultivation of choleraic Comma-bacilli in Gelatine Peptone. Magnifying power 700. Most of these are single curved bacteria, a few are joined end to end in twos, thus forming S-shaped organisms; and a few are in chains of several placed end to end.

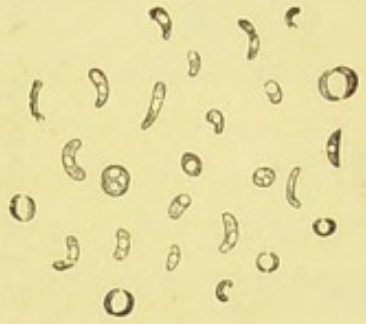


FIG. 175.

From an Artificial Cultivation of choleraic Comma-bacilli in Agar-Agar Peptone at the ordinary temperature of the room after several weeks. The Comma-bacilli change by vacuolation into plano-convex, then biconvex organisms. Magnifying power about 700.

death in thirty to forty-eight hours with all the appearances of general septicæmic infection, while with other varieties double and treble this dose produces only a transitory tumour with transitory constitutional disturbance; after several days the animals completely recover, or at most ulceration of the skin about the seat of the tumour and ultimate recovery takes place. R. Pfeiffer and Metschnikoff have had cultures of cholera vibrios which in small doses produced general septicæmic infection of the guinea-pig after subcutaneous injection. The greater or lesser virulence of the cholera vibrios (tested by subcutaneous injection of

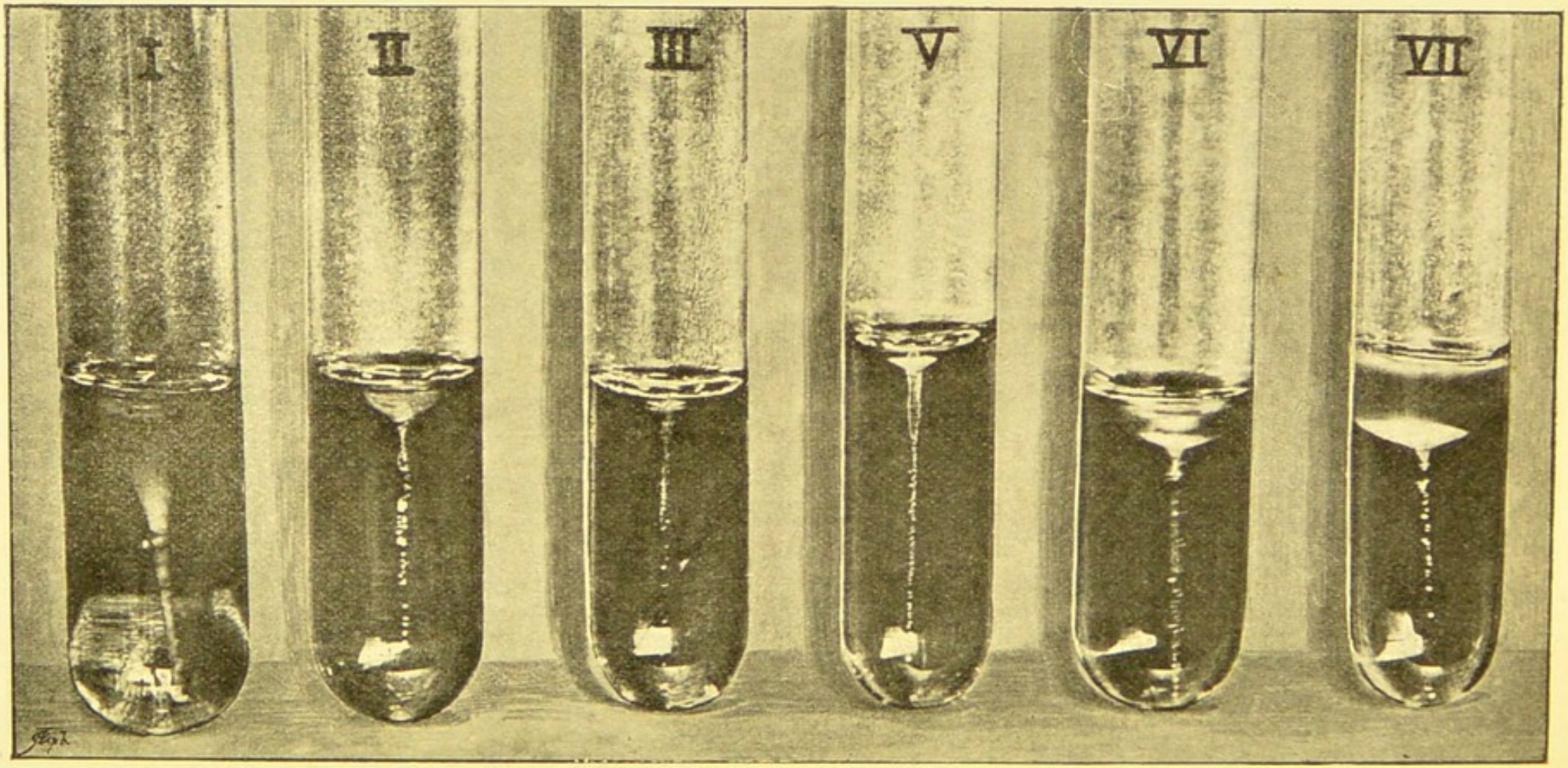


FIG. 176.—GELATINE STAB CULTURES OF CHOLERA VIBRIOS DERIVED FROM DIFFERENT CASES OF CHOLERA OCCURRING IN ENGLAND IN 1893. The tubes had been kept under precisely the same conditions; the amount of liquefaction varies considerably in the different varieties. Natural size.

the guinea-pig) stands in no definite relation to the severity of the cholera case from which they are derived.

Haffkine has on the other hand shown that by successive transference from guinea-pig to guinea-pig of the peritoneal exudation produced in the first of the series by intraperitoneal injection of a fatal dose of cholera (Agar) culture, after as many as twenty and more transferences the cultures of cholera vibrios obtained from the peritoneal fluid of the last guinea-pig reach a high degree of virulence, so much so that minute quantities of such a culture injected intraperitoneally are capable of causing fatal general septicæmic infection of the guinea-pig.

It has been shown by Sabolotny (*Central. f. Bakt. u. Paras.* vol. xv. p. 150) that the marmot is particularly susceptible to subcutaneous injection with the vibrio, acute septicæmic infection and death being the result.

Similarly also for the guinea-pig the virulence of a given stock of vibrios can be materially increased by adding to the culture medium potassium nitrate, or even a larger proportion of sodium chloride.

The same holds good for the degrees of virulence shown by the cholera (Agar) cultures when injected intraperitoneally into guinea-pigs. Of some varieties $\frac{1}{12}$ of an Agar culture is sufficient to produce a fatal result in a guinea-pig of 300 grammes weight in twenty to twenty-four hours, while of others as much as $\frac{1}{6}$ or even $\frac{1}{4}$ of a culture tube is required. The slanting surface of nutrient Agar is inoculated over its whole extent, then incubated at 37° C. for forty-eight hours. By this time the whole surface (six centimetres by two) is covered with a translucent gray film of growth; to the culture tube are then added four, five, or six cc. of sterile bouillon, and by means of a sterile platinum loop the growth is rubbed completely down into the

bouillon ; this distribution is then poured into a sterile watch-glass or capsule, and $\frac{1}{4}$, $\frac{1}{6}$, $\frac{1}{8}$, $\frac{1}{10}$, or $\frac{1}{12}$ or less of the culture, as the case requires, is drawn up into a hypodermic sterile syringe and injected intraperitoneally into a guinea-pig of known weight. The result is always that according to the virulence and the relative proportion of the dose and body-weight the

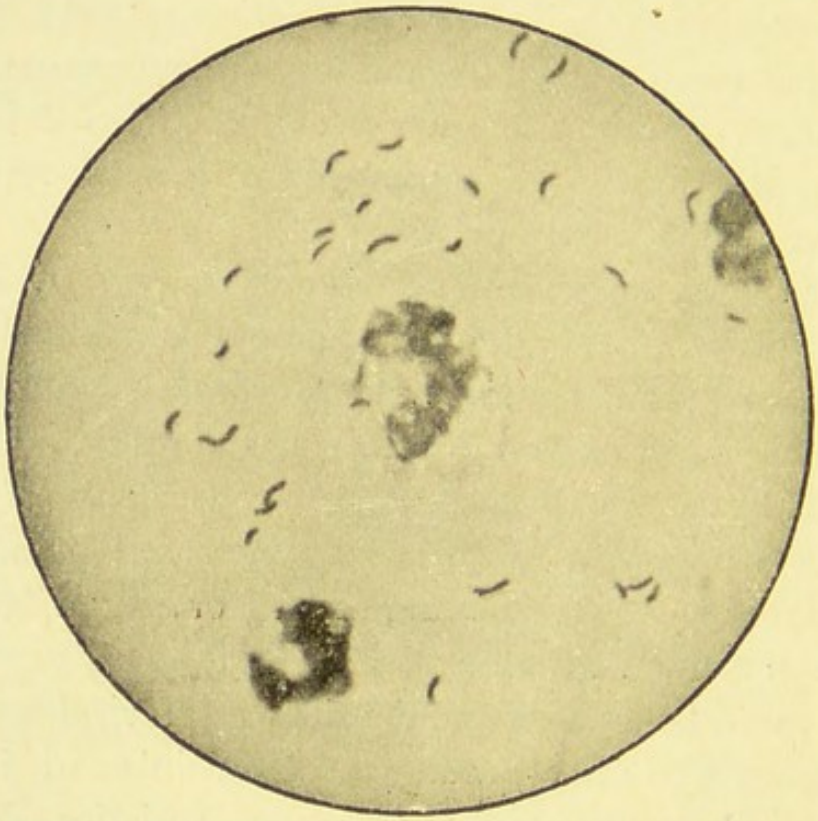


FIG. 177.—FILM SPECIMEN OF THE PERITONEAL FLUID OF A GUINEA-PIG DEAD FROM ACUTE PERITONITIS AFTER INTRAPERITONEAL INJECTION OF CHOLERA VIBRIOS.

× 1000.

guinea-pig is distinctly ill after from a few to several hours, the animal is quiet, does not feed, its coat becomes rough, the temperature gradually falls, movement becomes more and more impaired, and the animal is found dead after sixteen, eighteen, twenty, twenty-four hours, or as late as thirty-six hours. If it does not die after thirty-six hours it as a rule again recovers.

The fatal dose—producing death in or within twenty-four hours—differs according to the initial virulence and the size of the animal. The fatal dose of living vibrios from an Agar culture is always a little smaller than if the dose to be injected is first sterilised, either by boiling or, as I generally do, by heating it to 70° C. for five or ten minutes, or, as was done by R. Pfeiffer, by killing the vibrios by chloroform.

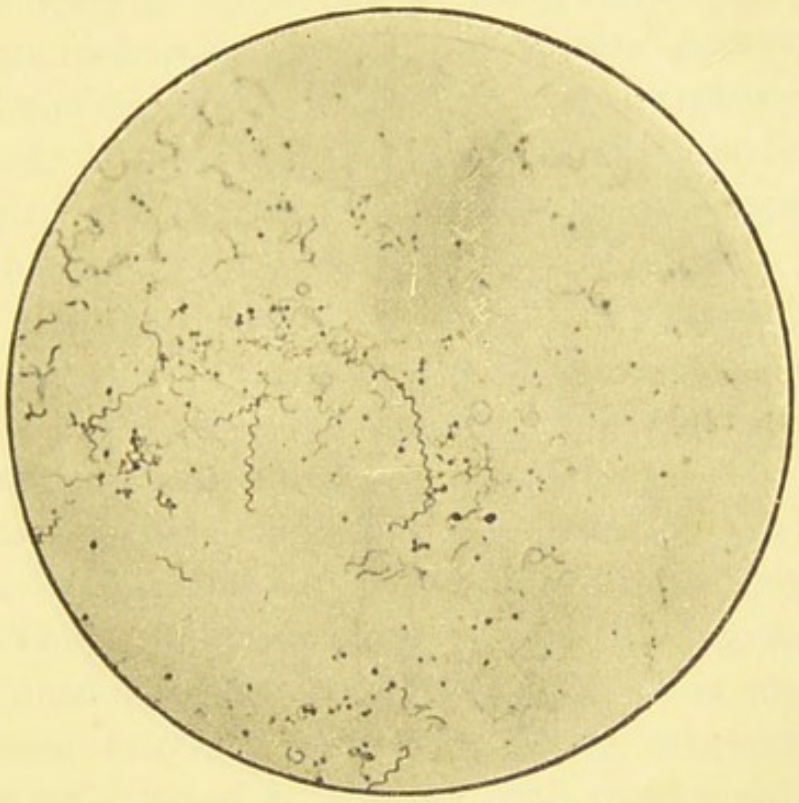


FIG. 178.—FILM SPECIMEN OF AN AGAR CULTURE OF CHOLERA VIBRIOS, A FEW WEEKS OLD, SHOWING NUMEROUS LONG SPIRILLA.

About 400.

On post-mortem examination the peritoneum is found intensely inflamed : hyperæmia of the serous covering and of the wall of the intestine, sanguineous copious fluid or slightly viscid peritoneal exudation, turbid by being densely crowded with the living motile vibrios (if the culture injected was not previously sterilised), flocculi of lymph on the omentum, on the intestine, and particularly the surfaces of

the liver. The intestine is relaxed, and filled occasionally but not always with sanguineous mucus. The same symptoms and the same post-mortem appearances are observed if the culture injected was first sterilised, only, as stated above, the dose has to be a little larger to produce fatal issue. Examining by cultivation, the peritoneal fluid (after living culture had been injected) is found crowded with

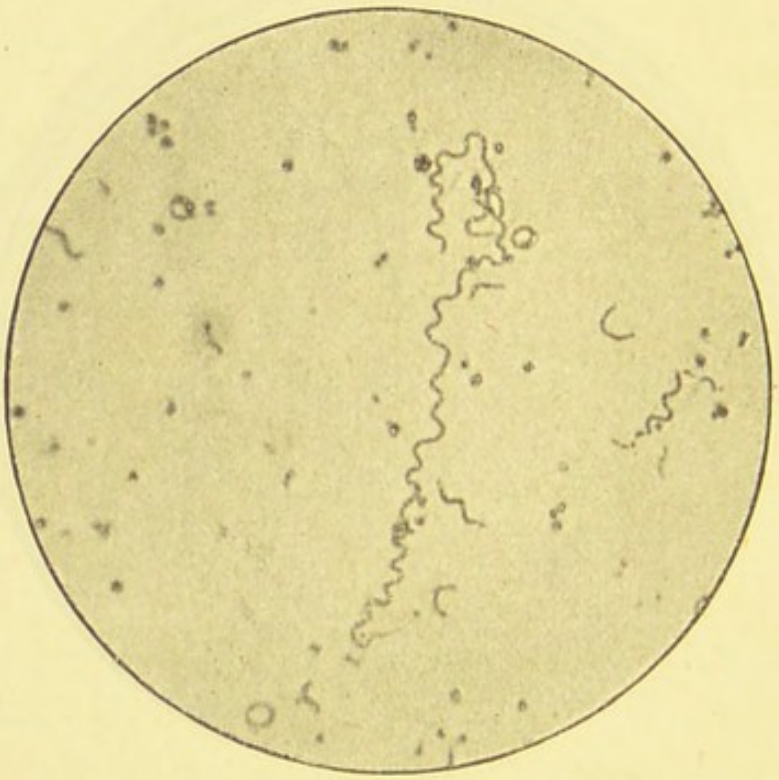


FIG. 179.—FILM SPECIMEN OF THE SAME CULTURE AS IN PREVIOUS FIGURE, SHOWING A LONG SPIRILLUM.

× 1000

living cholera vibrios; from the heart's blood as a rule colonies of cholera vibrios can be recovered by culture, in some cases fairly abundant, in others relatively sparingly. In some cases the intestinal cavity contains fluid mucus filled with the cholera vibrios. All these results, as I have shown, are obtained, of the same kind and the same degree,

by vibrio of Finkler, by proteus vulgaris, by bacillus prodigiosus, by bacillus coli, and bacillus of typhoid, and I found that bacillus prodigiosus, coli, and typhoid are in this respect more virulent than vibrio of cholera or of Finkler.

The result of the intraperitoneal injection of living or dead vibrios taken from Agar cultures does not therefore in any way throw any light on the specific action of the cholera vibrio, it being an action due to the presence of poisonous substances, *intracellular poisons* or *protein poisons*, within the bodies of the vibrios or in those of many other microbes mentioned. Under all these injections of the bodies of the most varied microbes the same disease and the same pathological changes are produced.

That there are different degrees of virulence amongst different cultures of the same species, amongst the different varieties of a species and amongst the different species themselves, has been already mentioned.

A dose of living microbes need be smaller than of dead microbes of the same culture in order to produce a fatal result. This is easily explained by remembering that in the case of dead microbes no further addition is made after introduction into the peritoneal cavity, whereas if the microbes are injected in a living state their multiplication within the peritoneal cavity—as mentioned above, the peritoneal exudation is found crowded with them—adds considerably to the original intracellular poisons as also the metabolic products, specific toxins, produced in consequence of this multiplication act towards bringing about a fatal result. That the cholera vibrios create poisonous metabolic products, toxins, in a culture is well established; in some fluids not much of it—*e.g.* in broth or in ordinary gelatine—but in aqueous humour or in serum the cholera vibrio produces this toxin rapidly and in considerable quan-

tity, as has been first shown by Van Ermengem;[1] and McLeod and Mills[2] found this toxin in a very effective and concentrated form produced in the intestinal fluid of the guinea-pig infected *per os* after Koch's method (*see* below).

The effect of non-fatal doses of cholera vibrios (dead or living) or of other microbes injected intraperitoneally into the guinea-pig will be considered later on in connection with artificial immunisation, at present suffice it to say that the *fatal* effect of cholera vibrios injected intraperitoneally into the guinea-pig proves nothing whatever as to any specific action any more than is the case with bacillus prodigiosus, and that the greater or lesser virulence of one microbe as compared with another (as judged by the relative amount injected intraperitoneally) proves nothing whatever for or against intrinsic specific action. I have shown[3] that by repeated intraperitoneal injection of sterilised vibrios (of cholera, of Finkler) or of sterilised bacilli (coli, prodigiosus, typhoid, proteus) taken from the surface of recent Agar cultures and used in non-fatal doses, the guinea-pigs become furnished with a high degree of resistance against a subsequent intraperitoneal injection of fatal doses of living vibrios or bacilli respectively. In the case of the cholera vibrios, starting for the first injection with a $\frac{1}{6}$ sterilised culture, and increasing the dose up to $\frac{1}{5}$, then $\frac{1}{4}$, $\frac{1}{3}$, and finally $\frac{1}{2}$ sterilised culture, allowing eight to ten days to intervene between each two injections, it will ultimately be found that such a prepared animal does not react any further to the intraperitoneal injection of a double or even treble otherwise fatal dose of living Agar

[1] *Recherches sur le Microbe du Choléra asiatique*, Bruxelles, 1885.

[2] *Reports from the Laboratory of the Royal College of Physicians, Edinburgh*, vol. i.

[3] *Centralbl. f. Bakt. und Parasit.*, 1893, and *Report of the Medical Officer of the Local Government Board for* 1893.

culture. It follows from this that in this animal from the purely intercellular substances—only dead bacillary bodies having been used—substances have been produced which can immunise—*i.e.*, can act germicidally against the intraperitoneal growth and multiplication of the cholera vibrio. Testing the blood-serum, "cholera serum," of such an animal immunised by dead bacillary bodies only as to its immunising or germicidal action against living cholera vibrios after the method of Pfeiffer—*i.e.* mixing a definite amount of "cholera serum" with an otherwise fatal dose of living cholera vibrios, and injecting the mixture into the peritoneal cavity of a fresh guinea-pig, at the same time injecting into a control guinea-pig of the same weight the same dose of living vibrios without the cholera serum—it will be found that that serum exhibits in the peritoneal cavity marked and definite germicidal power. R. Pfeiffer[1] has described numerous experiments, by which it was clearly established that by repeated intraperitoneal injections of doses of living cholera vibrios, starting with non-fatal doses and gradually increasing the dose till no reaction follows any longer, and the animal after the last injection again gains in body-weight, the blood-serum of such an artificially or "actively" immunised guinea-pig has potential, powerful, germicidal power, inasmuch as injected into the peritoneal cavity of a fresh guinea-pig, together with an otherwise fatal dose of living cholera vibrios, it produces a rapid alteration and crumbling away of the vibrios,[2] no multiplication of them and no disease follows—that is to say, the addition of the serum of an

[1] *Zeitschr. f. Hygiene u. Infekt.* vol. xvi.

[2] The peculiar alteration produced in a suspension of cholera vibrios by the addition of such "cholera serum" (Bordet and Durham) will be described and discussed later on.

"actively immunised" animal is capable of giving immunity ("passive immunity") to another guinea-pig against the cholera vibrio injected intraperitoneally. There is no difficulty in confirming this discovery of Pfeiffer as to the presence of potential germicidal substances in the blood-serum of an actively immunised guinea-pig. What I have, however, to add, is that according to the above experiments of immunising against living vibrios by means of the intracellular substances only, I conclude that for the production of germicidal serum it is not necessary that there should be produced in the animal body toxins—by the multiplication of the living vibrios injected—for the immunising substances in the above experiments could have been derived solely from the dead bodies of the vibrios used for immunisation. I may state here also that the same evidence I have obtained in showing that germicidal serum of typhoid immunised guinea-pigs against living typhoid bacilli, as also of germicidal serum of diphtheria immunised guinea-pigs against living diphtheria bacilli, is obtainable by using for immunisation the bacillary bodies only, without allowing these microbes to undergo multiplication and production of toxins within the peritoneal cavity. We shall return to this subject more in detail when treating of immunity.

Guinea-pigs that by repeated intraperitoneal injections of dead cultures of cholera vibrios have acquired resistance by which they can withstand an otherwise fatal dose of living cholera vibrios in their peritoneum, the vibrios not being now able to live and multiply in such a peritoneal cavity, are, however, not proof against cholera toxin. I have made experiments[1] to show that guinea-pigs

[1] *Reports of the Medical Officer of the Local Government Board for* 1894.

well immunised against living vibrios by previous repeated injection of dead vibrios succumb to a dose of toxin produced by cholera vibrios in serum cultures. (The same also holds good for vibrio Finkler and the toxin produced in serum cultures of this vibrio.) So that the distinction on which I have always insisted,[1] between the action of intracellular poisons of a microbe and that of the toxins produced by the microbe as a result of its metabolism is well founded.

Koch[2] in his first pamphlet on cholera told us[3] that he had made every imaginable effort to produce cholera in animals experimentally. The experiments of feeding white mice with cholera dejecta, first made by Tiersch and then by Burdon Sanderson, were repeated by Koch over and over again on fifty white mice fed with this material (dejecta of cholera patients, and the contents of the intestine of cholera corpses) and with choleraic material after it had begun to decompose, but no result whatever followed; the mice remained healthy. "We then made experiments on monkeys, cats, poultry, dogs and various other animals that we were able to get hold of, but we were never able to arrive at anything in animals similar to the cholera process. In precisely the same manner we made experiments with the cultivations of comma bacilli; these were given as food in all stages of development. When experiments were made by feeding animals with large quantities of comma bacilli, on killing them and examining the contents of their stomachs and intestines with a view to find comma bacilli it was seen that the comma bacilli had already perished in the stomach, and had usually not

[1] *Ibidem*, 1892, 1893, and 1894.
[2] The following is copied from my *Bacteria in Asiatic Cholera*.
[3] *Conferenz zur Erörterung der Cholerafrage*, Berlin, 1884, p. 27.

reached the intestinal canal. . . . The comma bacilli had been destroyed in the stomachs of these animals. . . . The experiment was therefore modified by introducing the substances direct into the intestines of the animals. The belly was opened, and the liquid was injected immediately into the small intestine with a Pravaz syringe. The animals bore this very well, but it did not make them ill. We also tried to bring the cholera dejecta as high as possible into the intestines of monkeys by means of a long catheter. This succeeded very well, but the animals did not suffer from it." "I must also mention," says Koch, "that purgatives were previously administered to the animals in order to put the intestine into a state of irritation, and then the infecting substance was given, without producing any different result. The only experiment in which the comma bacilli exhibited a pathogenic effect, which therefore gave me hope at first that we should arrive at some result, was that in which pure cultivations were injected directly into the blood-vessels of rabbits or into the abdominal cavity of mice. Rabbits seemed very ill after the injection, but recovered after a few days. Mice, on the contrary, died from twenty-four to forty-eight hours after the injection, and comma bacilli were found in their blood. Of course they must be administered to the animals in large quantities; and it is not the same as in other experiments connected with infection, where the smallest quantities of infectious matter are used, and yet an effect is produced. In order to arrive at certainty as to whether animals can be affected with cholera, I made inquiries everywhere in India as to whether similar diseases had ever been remarked amongst animals. In Bengal I was assured such a phenomenon had never occurred. This province is extremely thickly populated, and there are many kinds of

animals there which live together with human beings. One would suppose, then, that in that country, where cholera exists in all parts continually, animals must often receive into their digestive canal the infectious matter of cholera, and in just as effective a form as human beings, but no case of an animal having an attack of cholera has ever been observed there. Hence I think that all the animals on which we can make experiments, and all those, too, which come into contact with human beings, are not liable to cholera, and that a real cholera process cannot be artificially produced in them."

Koch,[1] starting from the idea that the comma bacilli are killed by the gastric juice, and that in order to develop their pathogenic powers they have to get unscathed and living into the small intestine—their natural breeding-ground—it occurred to him that this difficulty might be obviated by first neutralising or making alkaline the contents of the stomach, and introducing *per os* the comma bacilli. He therefore kept guinea-pigs for twenty-four hours without food, and then injected into their stomach *per os* 5 cubic centimetres of a 5 per cent. watery solution of carbonate of soda. This does not noticeably injure the stomach, and, as direct observation proved, kept the contents of the stomach in an alkaline condition for three hours. Some minutes (twenty) afterwards he introduced by catheter 10 cubic centimetres of a cultivation of the comma bacilli in meat infusion.

The result is noteworthy. Seven guinea-pigs thus experimented upon remained perfectly well: "They were killed after twenty hours," says Koch, "and the contents of their stomach, intestine, and cæcum were examined by gelatine plate cultivations. In six of the seven animals the

[1] *Second Conference on Cholera*, Berlin, May, 1885.

cholera bacteria could be demonstrated in the small intestine. The experiment had thus in so far succeeded that the cholera bacilli had passed uninjured through the stomach, but they had not set up any disease in the animals." Similar experiments were then made on eight other guinea-pigs. These animals also remained quite healthy. Finally four guinea-pigs were similarly experimented upon (5 cc. of solution of sodium carbonate, the 10 cc. of cultivation of the comma bacilli in meat infusion); three remained well, the fourth appeared ill next day, looked shaggy and did not eat; on the following day it was very ill; paralytic weakness of the posterior extremities came on, the respiration was weak and slow, the head and extremities were cold, and the animal died in this condition. On *post-mortem* examination the small intestine was markedly reddened and full of a flaky, watery, colourless fluid. The stomach and cæcum contained a large quantity of fluid. "The examination with the microscope and with gelatine plates," says Koch, "showed that the contents of the small intestine contained a pure cultivation of the choleraic comma bacilli." "That this one animal only should have died, out of a series of nineteen, uniformly experimented upon, suggested some peculiar condition that had obtained in this one animal, and as a matter of fact on examination it was ascertained that this animal had aborted immediately before the injection, and on *post-mortem* examination it was found that the abdominal walls were very flaccid and the uterus still greatly enlarged. This led me to the idea that either the abortion *per se*, or perhaps its unknown cause, had acted on the other abdominal organs, more especially on the small intestine, in such a way as to produce a temporary relaxation with arrest of peristaltic movement; and thus had rendered it possible for the comma bacilli to

remain longer and gain a footing in the intestine." This conclusion appeared quite justifiable, inasmuch as by direct experiment it had been proved that the contents of the stomach pass too rapidly through the small intestine, and since the comma bacilli could only unfold their poisonous action, *i.e.*, could multiply and produce the chemical poison, if they had time to remain there and to multiply. Consequently if they were not delayed on their passage through the small intestine they would not multiply there, and once in the cæcum, where the reaction is acid, they would become harmless.

In order to produce a condition similar to the one in the above single successful experiment on the guinea-pig, Koch injected tincture of opium into the peritoneal cavity after the introduction of the sodium carbonate and the cultivation of the comma bacilli : this answered well for achieving positive results. Immediately after the administration of the 10 cc. of the culture of the comma bacilli, 1 cc. of German tincture of opium for every 200 grms. of the animal's body-weight were injected into the peritoneal cavity : the animal became thereby narcotised for half an hour, and died after one and a half to three days, with the same symptoms as the above guinea-pig. "Eighty-five guinea-pigs have been infected in this way with cholera."

Now the following criticisms can, I think, be justly applied to these experiments : (1) According to Koch's own showing, it cannot be the narcosis which is essential, even allowing for the present that relaxation of the intestine may have been produced by the intraperitoneal injection of opium tincture, since alcohol alone was injected by Koch into the peritoneal cavity, and he says that thereby "we were most successful in making the animals susceptible to the cholera infection." (2) Can narcosis

of the animal be produced by opium without furthering in the least the process of the experiment?[1] This has been tried over and over again; watery extract of opium is injected into the peritoneal cavity, and narcosis lasting for one hour is produced, but the animals remain well; tincture of opium is subcutaneously injected, the animals fall into narcosis, lasting for from forty to eighty minutes, but no result is obtained from the previous introduction of the comma bacilli; in fact, the experiment as designed by Koch was repeated by me on a large number of guinea-pigs, thirty in all, but instead of producing narcosis by injection of tincture of opium into the peritoneum I produced it by intraperitoneal injection of watery extract of opium, or subcutaneous injection of tincture of opium and watery extract of opium, but all in vain. The comma bacilli used were of recent broth culture, or of gelatine culture, and were beyond question or doubt the choleraic comma bacilli.

From all these considerations it appears to me unwarranted to conclude that the multiplication of the comma bacilli in the small intestine, and their fatal action by the chemical products they elaborate, takes place on account of a relaxation and arrest of the peristaltic movement by the opium. Another explanation appears to me much more probably correct. It is this—provided the intestine is first made diseased, either in consequence of slight peritonitis, as was probably the case in the guinea-pig that had aborted, or in the experiments when tincture of opium is injected into the peritoneal cavity, or from other reasons, the comma bacilli that are present in the intestinal cavity undergo rapid multiplication, and by their chemical products not only increase the disorder of

[1] *The Practitioner*, 1886 and 1887.

the mucous membrane, but eventually poison the animal. And from this I conclude, further, that a multiplication of the comma bacilli can and does take place only when the intestine is previously brought into a diseased state. Under this view all Koch's and Van Ermengem's results become at once intelligible.

I maintain, then, that the living choleraic comma bacilli *per se*, however large their number, when introduced into the normal small intestine of the guinea-pig are quite innocuous, but they are rendered capable of great multiplication if the intestine is previously, from some cause or another, diseased. The chemical products, the toxins, of such multiplication act as poisons analogous to the ptomaines obtained from putrefactive bacteria.

That this is the true explanation I find proof in some of Koch's experiments with other bacteria, notably with Finkler's and Deneke's comma bacilli. With both these organisms on experimenting in the above manner he obtained positive results; not so constantly, it is true, but still he did obtain positive results, not identical, but similar. Of course it is not to be expected that, seeing these are three different species, they would act in the same manner. Finkler published a large series of experiments, in which, with his comma bacilli, and after the method of experimentation employed by Koch, he produced results identical with those gained by Koch with the choleraic comma bacillus. There can be no doubt, as will be mentioned later, that Finkler's comma bacillus has nothing to do with cholera nostras, or with any other infectious disease, but that it is simply a putrefactive organism. And on the same grounds Koch's comma bacillus cannot be said by these experiments on the guinea-pig to have been proved to have a causal relation to cholera asiatica or that

the disease so produced in the guinea pig is cholera, any more than has Finkler's comma bacillus, or any of the other species of bacteria that are capable of producing chemical poisons analogous to ptomaines. All that can be said is, provided that conditions are established by which the choleraic comma bacilli are enabled to grow and multiply in the intestinal canal, these chemical poisons are produced.

This method of experimentation introduced by Koch cannot therefore be held to prove a specific action of the cholera vibrio on the guinea-pig, since after this method the same result is produced with other bacteria, in no way connected with cholera asiatica. Metchnikoff (Annales de l'Institut Pasteur, 1895) has shown that by choosing very young rabbits, almost immediately after birth, it is possible in a large percentage to produce by ingestion of culture of the cholera vibrio rapid multiplication of the vibrios within the alimentary canal and death of the animal in 24—48 hours; I have repeated these experiments and can confirm them, but I have to add that the same result is obtained with the vibrio of Finkler.

While then the position of affairs, viz., whether the vibrio of cholera (Koch) is or is not the real *causa causans* of Asiatic cholera, is not altered by all these experiments on the guinea-pig (subcutaneous, intraperitoneal, and intro-intestinal injection), there have been made numerous observations within the last three or four years (since the Hamburg epidemic in 1892) which materially alter the circumstances from what they were previously. Since 1886, and up to that date the fundamental fact discovered by Koch that the particular vibrio found by him in Asiatic cholera is peculiar to cases of Asiatic cholera and to no other disease of the intestine, its demonstration being therefore of the greatest

importance for diagnostic purposes, had been conceded and confirmed on almost all sides (*see* this 3rd edition and my *Bacteria in Asiatic Cholera*, 1886 and 1887), the proof, however, as to the experimental production of cholera in the guinea-pig, as we have shown above, was far from a satisfactory kind.

Experiments by ingestion of cultures of cholera vibrios in the *human subject* have been made in Munich (Pettenkofer and von Emmerich), in Vienna (Stricker), and in Paris (Metchnikoff), and the results of these, though not unequivocal, were sufficiently instructive to strengthen the position of Koch's view as to the causal relation of the cholera vibrio to Asiatic cholera.

In a considerable percentage of these experiments it was shown that the ingestion of cultivation of cholera vibrio, that had been kept up through many subcultures in the laboratory, produced more or less severe diarrhœa with the presence of the cholera vibrios in the evacuations as shown by the culture test. In a few the effect was tolerably severe (Pettenkofer and Emmerich), and in one case (a boy) observed by Metchnikoff it was a very good imitation of genuine Asiatic cholera, including the rice-water stools with crowds of the cholera vibrios. It is well established by the older researches of v. Pettenkofer and fully confirmed by the observations made in reference to cholera in India and in Europe down to the most recent times, viz., that in the production of cholera the predisposition of the individual, season, and locality are important factors besides the real *causa causans* or the cholera microbe—the x, y, and z of sanitarians. If then in the above precise and deliberate experiments with pure cultures of Koch's cholera vibrio by ingestion fair results—even few in number—at a time and locality when and where no cholera exists, are

brought about, the inference, it must be admitted, that the cholera vibrio is the microbe of cholera is extremely near. It cannot be expected that in a number of healthy persons the ingestion of laboratory cultures, which, as experiments on the guinea-pig show, are liable to become less and less virulent, should be productive of severe and typical attacks of cholera; even at the beginning and towards the end of an epidemic of cholera we see occasionally a considerable percentage of mild attacks—practically only more or less severe diarrhœa; therefore, that in the above experiments there should have been a percentage of positive results—cases with fairly severe diarrhœa—and in the one case of Metchnikoff's series a severe result should have been actually brought about, and that in all these positive cases the comma bacilli introduced should have multiplied in the intestine and their presence been demonstrated by microscopic and culture test, is in itself a very strong link in the evidence as to the causative relation between the vibrio and the disease cholera.

Another important link of evidence was brought forward by showing that the blood-serum of a person who had recovered from an attack of Asiatic cholera possesses the power to confer passive immunity to guinea-pigs against the action of the cholera vibrio (Klemperer, Botkin, Wassermann), that is to say that serum possesses immunising action against the cholera vibrio. If the cholera vibrio is really the cause of cholera, one can understand that, just as in other communicable diseases a first attack alters or adds something to the blood, so as to furnish this with immunising power, a like effect should be produced by the cholera vibrio after it has been growing and multiplying within the affected individual, that is to say that the blood-serum of such an individual should possess a specific immunising action against the cholera vibrio. And this is actually the

case from the observations recorded. This harmonises well with R. Pfeiffer's discovery of the germicidal action of the serum of actively immunised guinea-pigs against fatal doses of the cholera vibrio. By itself this link of the evidence is not very strong, but it proves this that the blood-serum of an individual after an attack of cholera possesses a specific immunising action against the cholera vibrio, and it is permissible to conclude that this power of the serum was brought about by the action of the cholera vibrio just as is the case in Pfeiffer's active immunisation of guinea-pigs. Not that it proves that the disease produced in the guinea-pig by intraperitoneal injection is a process comparable to cholera in man, but it shows that within the blood of the living body the cholera vibrio is capable of creating specific immunising substances, and taken together with the analogous observations with the bacillus of diphtheria, the bacillus of tetanus, the pneumococcus, the bacillus of septicæmia, and other specific microbes it becomes extremely probable that also in cholera of man the production of immunising serum, is due to a like cause, *i.e.* to the cholera vibrio.

Haffkine in a long continued series of observations has established that by transmission of the peritoneal exudation of a guinea-pig, dead after intraperitoneal injection of living cholera culture, through a large number of successive guinea-pigs ultimately the vibrios present in such exudation (*see* a former page) assume increasingly greater virulence, so much so that cultures made from the peritoneal exudation of the last animal of the series (twenty to thirty transmissions) yield extremely virulent vibrios, a tenth or a twentieth or less of the dose of that with which the series was started, being now sufficient to produce fatal results in sixteen to twenty hours when injected intraperitoneally. Such cultures of "exalted virulence" injected subcutaneously into

guinea-pigs cause even as small doses intensive effects: tumour and, hæmorrhage, constitutional illness, and, if the dose is not too small, death; if the dose is small enough the effect passes off, the tumour leads in most instances to sloughing, but ultimately the skin heals. A second injection has less effect, and a third still less. After a time, *i.e.* when the animal has again quite recovered, it is found to be immunised against the intraperitoneal injection of multiple fatal doses of even the powerful virus.

In order to mitigate the effect of the first injection Haffkine attenuates the virulent living vibrios by the addition of phenol—first vaccine—and only on second or even third injection uses the full virulent living vibrio—second vaccine. Having preliminarily tested the effects by subcutaneous injection of these vaccines into (willing) human subjects (himself, Hankin, and others at the Pasteur Institute), and producing well-marked tumour and constitutional symptoms more or less rapidly passing off, he proceeded to India to test these "vaccines" in reference to protective subcutaneous inoculations of the human subject—two, and in some instances three, separate injections being made—against cholera.

Now, it ought to be here distinctly understood that before Haffkine started on this work in India he was convinced that guinea-pigs successfully protected, "actively immunised," by subcutaneous injection with his "vaccines" against a subsequent intraperitoneal injection of fatal doses of virulent vibrios, were also protected against ingestion of the vibrios administered after Koch's method described on a former page, and therefore concluded that a similar effect might be produced also in human beings by previous subcutaneous injection of his vaccines—that is to say, such persons might be protected, actively immunised, against

natural infection with cholera *per os*. It ought to be further stated, however, that according to the observations of Wassermann and Pfeiffer (*Zeitschr. f. Hygiene und Infect.* vol. XIV., and according to my own observations (Reports of the Medical Officer of the Local Government Board for 1893 and 1894), such intestinal protection of guinea-pigs is not by any means uniformly observed even after intraperitoneal active immunisation. R. Pfeiffer, as a result of his recent experiments, finds that guinea-pigs passively immunised by the intraperitoneal injection of "cholera serum" are still susceptible to intestinal infection after Koch's method.

But, be this as it may as regards the guinea-pig, Haffkine has in India, during 1894 and 1895, made a large number of double and treble vaccinations, and has collected a large body of statistics as to cholera-vaccinated persons that have been exposed to cholera in India living in the same locality and conditions side by side with non-vaccinated persons. The latest statistics published in India, and in the *British Medical Journal*, during the last months of 1895 by medical men who had assisted in these vaccinations and had observed the results are of a most encouraging nature; when seeing it stated that in a large body of unvaccinated persons of a given locality (tea plantations) the incidence of attacks is enormous, and in an equally large body of vaccinated persons living side by side and under the same conditions with the former the incidence of attacks is incomparably smaller, in fact in some of the latest statistics is very small indeed as compared with that in unvaccinated persons—that is to say, while of unvaccinated persons the disease kills off many, of the vaccinated persons only few—seeing all these statements one cannot help arriving at the conclusion that the protective inoculations practised by

Haffkine in India with cultures of cholera vibrio have had positive results, and further that these observations form a strong link in the chain of evidence that the cholera vibrio is the cause of cholera. The evidence that Koch's vibrio is the microbe of Asiatic cholera, and as such forms an essential—though not the only—factor in the production

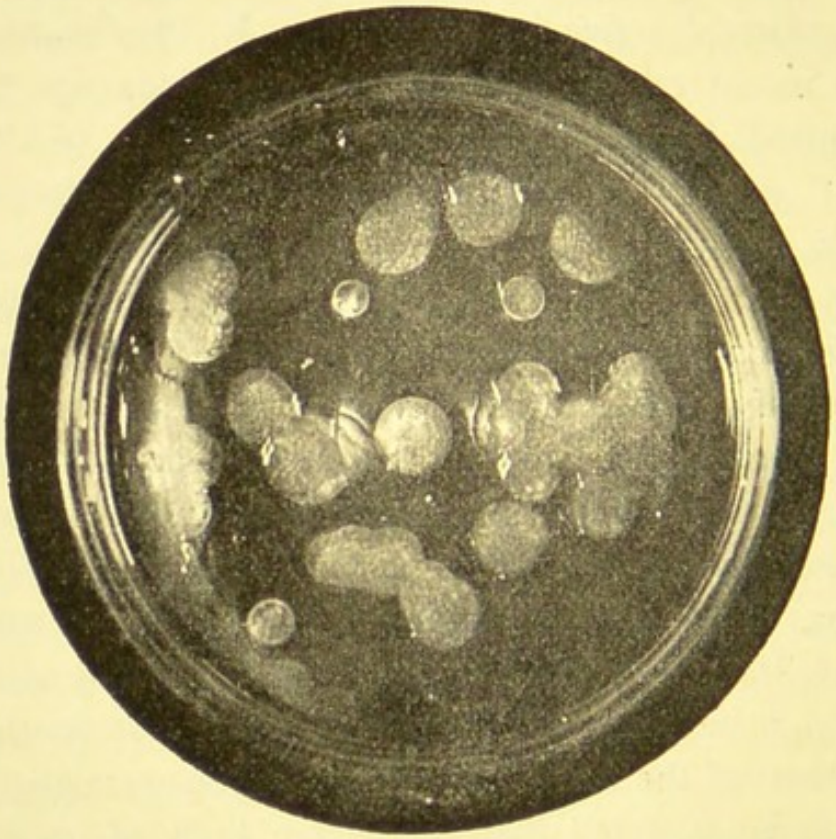


FIG. 180.—PLATE CULTIVATION IN GELATINE OF VIBRIO FINKLER—PRIOR, INCUBATED AT 20°C. FORTY-EIGHT HOURS; THE COLONIES ARE ROUND, LIQUEFIED, TURBID, SOME ISOLATED, OTHERS CONFLUENT.

Natural size.

of cholera asiatica—disposition, locality, season, being other factors—is then a chain in which the individual links taken separately are open to criticism, but when all are taken together—notably: the diagnostic value of the cholera vibrio for cases of Asiatic cholera, the capability of the vibrio to produce powerful toxin, the result of experiments on ingestion of cultures of cholera vibrio on human beings, the immunising

action of cholera serum of human beings against the cholera vibrio, taken together with Pfeiffer's germicidal action of cholera serum of immunised guinea-pigs, and the results of Haffkine's protective inoculations on human beings—form as strong a body of evidence as can be expected—seeing that animals are not subject to cholera—to confirm Koch's original view as correct, viz., that the vibrio is the microbe

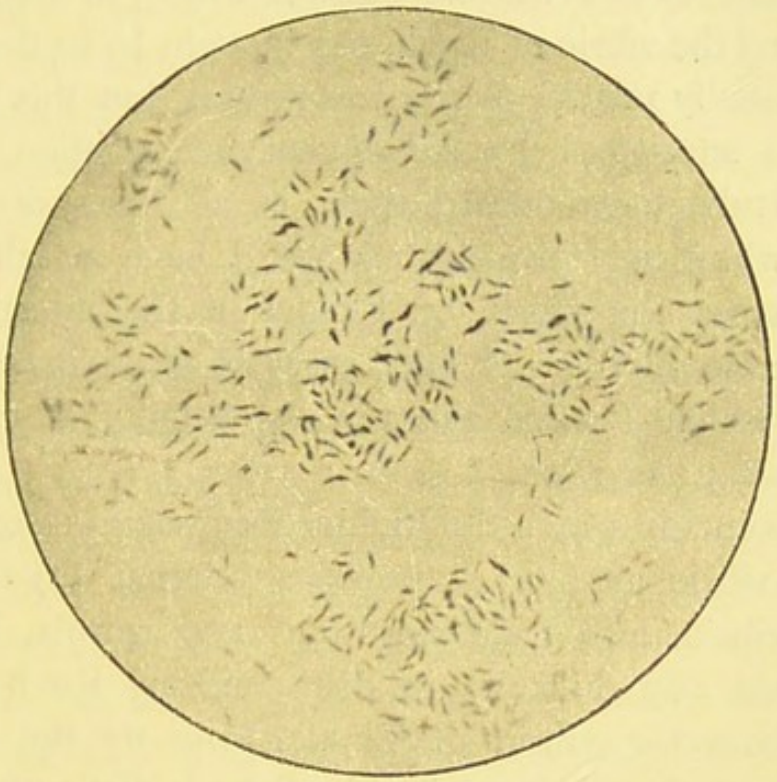


FIG. 181.—FILM SPECIMEN OF PERITONEAL EXUDATION OF A GUINEA-PIG DEAD AFTER INTRAPERITONEAL INJECTION OF CULTURE OF VIBRIO FINKLER-PRIOR.

× 1000.

of cholera, that the growth and multiplication of this within the cavity of the intestines produces toxins which absorbed into the system cause the disease cholera. Consequently consumption of articles of food, water and solids, contaminated with cholera vibrios, derived directly or indirectly from the discharges of a cholera case, as also direct contamination and introduction of cholera vibrios into the alimentary canal, are capable of causing cholera.

A vibrio was isolated by Finkler and Prior[1] from decomposing stools of a case of sporadic cholera, cholera nostras (English cholera); this was done at a time when Koch's discovery of the comma bacillus in Asiatic cholera was but recent, and when for the first time a vibrio was isolated from the human intestinal discharges. The *vibrio of Finkler-Prior* (or Finkler comma bacillus, or Finkler vibrio, or Finkler vibrio proteus) is a comma bacillus which, in many points resembling the vibrio of Koch, was thought by its discoverers to be causally related to cholera nostras, but this view has not been supported by subsequent investigation. Frank and Kartulis have missed them in all cases of sporadic cholera which they investigated, and I have myself not yet come across the vibrio of Finkler in the intestine of a considerable number of fatal cases of sporadic or English cholera which I have had the opportunity of examining during 1894 and 1895.

The points in which the Finkler-Prior vibrio resembles the cholera vibrio are: (1) it liquefies gelatine, (2) and it is a motile vibrio, also S-shaped forms and spirilla, but there never was any difficulty in distinguishing Koch's cholera vibrio from the vibrio of Finkler-Prior by the following characters: the vibrio of Finkler-Prior is distinctly larger—longer and thicker—than the cholera vibrio, and it grows incomparably faster at 20° C. in gelatine, and liquefies this incomparably quicker than the cholera vibrio. Besides, the colonies in the gelatine plate are always round and the liquefied gelatine is uniformly turbid; in these respects the Finkler vibrio compares well with the proteus vulgaris; in the stab gelatine the vibrio of Finkler forms already after forty-eight hours considerable growth and liquefaction, and the liquefied gelatine is uniformly turbid; also herein it

[1] *Centralblatt für allg. Gesundheitspflege*, vol. i., Nos. 5 and 6.

resembles the proteus vulgaris. Gelatine liquefied by the cholera vibrio has no smell, whereas in the case of vibrio Finkler it has a more or less putrid smell, just like that of a growth of proteus vulgaris.

The cultures of vibrio Finkler act on the guinea-pig in the same way as the cholera vibrio, subcutaneously, intraperitoneally, and by ingestion; there is no difference generally in this respect between the two vibrios. There are, as stated on a former page, particularly virulent cultures of the cholera vibrio which in intensity of action on the guinea-pig surpass the vibrio of Finkler, but they surpass also other less virulent cultures of undoubted cholera vibrios.

Vibrio Finkler grows best at 20–21° C.; it does not at all grow well at 37° C., that is at a temperature when the cholera vibrio grows best. If the peptone salt solution is inoculated in one set of tubes with the cholera vibrio, in a second set with the vibrio of Finkler, and of each set one tube is kept in the incubator at 20° C., and likewise of each set one tube is kept at 37° C., a very marked difference will be observed between the two species of vibrios after twenty-four hours, viz. the peptone culture of cholera vibrio incubated at 20° C. is only very slightly turbid, there is just an indication of growth having taken place, while the peptone culture of vibrio Finkler shows marked turbidity, good growth having taken place; whereas the peptone culture of cholera vibrio incubated at 37° C. shows uniform good turbidity, the peptone culture of Finkler vibrio shows no turbidity. The same holds good for cultures in broth peptone. By incubating the peptone cultures at 37° C. for even from twelve to eighteen hours the difference between the two species is marked. Also on growing on the slanting surface of Agar at 37° C. vibrio Finkler shows faint growth after twenty-four or even after forty-eight hours, while the vibrio of

cholera has produced already in twenty-four hours a conspicuous film.

If to a peptone salt culture of pure cholera vibrio, as soon as it shows turbidity (no matter whether incubated at 20° C. or at 37°), a few drops of *pure* sulphuric acid are added, as was mentioned on a former page, a distinct rose-red tint, cholera red, is produced; a peptone salt culture of vibrio Finkler which in order to produce turbidity had been incubated at 20° C.—it does not become turbid at 37° C.—treated with a few drops of pure sulphuric acid gives no cholera red reaction. The assertions to the contrary are based on the sulphuric acid used not being pure but containing nitrites; with such impure sulphuric acid also in a peptone culture of proteus vulgaris a red reaction is obtainable.

Another point in this connection worth mentioning is that for the demonstration of the pure cholera red reaction the peptone used for peptone salt culture ought to be pure and free from nitrites. Pestana of Lisbon, who isolated from the intestinal discharges of cases of cholerine that occurred in epidemic form in Lisbon in 1894 a vibrio (*see* below), has shown that a culture of it in peptone salt, when the peptone used was free from nitrites, gives no cholera red reaction, but a culture of it in peptone salt made with nitrite-containing peptone gives a faint but distinct cholera red reaction.

Soon after Koch's discovery Deneke[1] isolated from stale cheese a spirillum—*spirillum tyrogenum*, which in morphological and cultural respects bore a very great resemblance to Koch's cholera vibrio, in fact, looked at in the light of the present knowledge of different varieties of cholera vibrio, cannot be distinguished from this latter. In size, shape,

[1] *Deutsche Medicin. Wochenschrift*, 1885, No. 3.

motility, growth in peptone salt, and cholera red reaction, in gelatine, on Agar, on blood-serum, in its action on the guinea-pig (administered *per os* after Koch), it is difficult to distinguish it from the cholera vibrio; perhaps it grows a little faster on gelatine in the plate and in the stab, but, as has been stated on a former page, such differences are also observed between the individual varieties of the cholera vibrios.

The same has to be said of a number of vibrios and spirilla that have been isolated in the course of the last three or four years by various observers in different waters: vibrio berilonensis, vibrio danubicus, vibrio of Warsaw, vibrio Nordhafen, vibrios of the Elbe, various species of vibrios isolated from water (Seine and other rivers near and around Paris) by Sanarelli (*Annales de l'Institut Pasteur*, November 1893). With the exception of the vibrio phosphorescens of Elwers and Dunbar, most of the others differ from the typical vibrio of Koch so little and in so few details—in fact, less so than do the individual varieties of vibrios isolated from noted cases of cholera—that from their morphological and cultural characters, including the cholera red reaction which they all show to a greater or lesser degree, and from their intraperitoneal pathogenic action on the guinea-pig, they cannot be distinguished from the different varieties of the true cholera vibrios. And for this reason I think Sanarelli's contention that, inasmuch as the water vibrios which he found in the Seine and other rivers in France, that had been subject to notorious pollution with the dejecta of cholera cases which had occurred in Paris, its suburbs, and elsewhere in France, during the preceding years, resemble in many respects the cholera vibrio, those water vibrios are genetically related to the cholera vibrio, this contention, I say, does not deserve to be set aside in the off-

hand manner that R. Pfeiffer does when criticising Sanarelli's results. Nor do I think that the discoverers of the various water vibrios (berilonensis, danubicus, Dunbar's vibrio found in the Elbe in 1894, and other similar finds) are justified by the small differences observable between these vibrios and the typical cholera vibrio in denying a genetic relation. I do not for a moment intend to imply that any or all were so related, but because the waters, in which these vibrios were found, did not produce cholera in the consumers, is not sufficient arugment, as for the production of cholera it would require a virulent cholera vibrio and various other factors (mentioned on a former page), and all these may have been absent in these cases.

I have isolated a vibrio from drain water (Hull, Sutton drain) which was described on page 193 in the Cholera Report of the Medical Officer of the Local Government Board, 1894; the cultural characters of this vibrio were in some respects distinctly different from the typical cholera vibrio, in others they were identical, but in etiological respects there was strong evidence that the water of that Sutton drain had an important relation to causing cholera asiatica (*see* Dr. Theodore Thomson's report, *ibidem*, pp. 101, 102).

On the other hand, in certain filth-polluted well-water, to the consumption of which an epidemic of Asiatic cholera at Ashbourne in September, 1893, had been clearly traced (*see* Dr. Bruce Low's report, *ibidem*, p. 127), I have found in the floccular suspended matter crowds of comma bacilli (Fig. 182) which in morphological and cultural characters completely resembled the typical cholera vibrio (*ibidem*, p. 194).

The vibrio isolated by Pestana (*Centralbl. f. Bakt. und Parasitenkunde*, 1894) from the flakes of the dejecta of cases of epidemic cholerine in Lisbon grows much slower

in gelatine than the typical cholera vibrio; on Agar the growth is also slower and much more transparent, it does not give the cholera red reaction with pure sulphuric acid when grown in pure peptone salt, and when injected into the peritoneal cavity of the guinea-pig is far less virulent than the typical cholera vibrio. The epidemic of cholerine in Lisbon had a very low mortality—few cases of death out

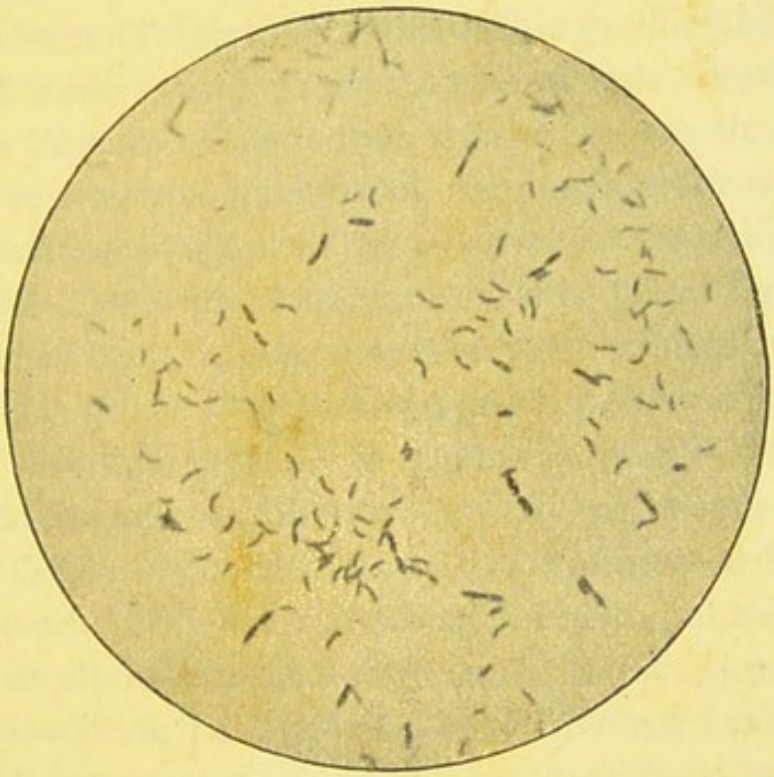


FIG. 182.—FILM SPECIMEN OF A FLOCCULUS FROM THE WATER OF THE POLLUTED WELL OF ASHBOURNE THAT HAD CAUSED AN EPIDEMIC OF ASIATIC CHOLERA.

× 1000.

of over 140 attacks, the normal mortality in an epidemic of cholera being 50 per cent. and sometimes more—all these facts justified Pestana in declaring that the vibrio is not the cholera vibrio and the disease is not Asiatic cholera. Recent observations, which I have carried out for the Medical Department of the Local Government Board, and which will be published in their Reports in 1896, seem to show

however, that Pestana's and similar conclusions, notably those arrived at with regard to the non-choleraic origin of the water vibrios previously mentioned, are not to be accepted unconditionally. The observations to which I refer were made on oysters kept in sea-water tanks to which previously culture of the typical cholera vibrio had been added. These vibrios had been originally derived from the flakes of the rice-water contents of a typical fatal case of cholera that had occurred on board of a steamer arriving in the Thames in August 1894, from a cholera-infected port; these vibrios had in cultivation all the characters of the typical Koch's vibrio, and tested on the guinea-pig's peritoneum showed a considerably high degree of virulence—and, be it also noted, having been carried on in subcultures through many generations showed both the Pfeiffer's test *in corpore* as also the Bordet-Durham test *in vitro* with "cholera serum."

From the slanting surface of an Agar culture—incubated for two days at 37° C.—the growth was scraped off and distributed in sterile salt solution, and then added to the sea water in a tank in which oysters fresh from the oyster-beds had been previously deposited. Several of these oysters as also the sea water had for control been previously carefully examined by the culture test for the presence of vibrios. In the case of the oysters, after well brushing off under a stream of water from the tap the exterior of the shell, the latter was dried with a clean towel, opened with a sterile knife, and of the liquor and the mashed-up substance of the oyster a number of peptone salt cultures were made. Of the sea water 90 cc. were placed in a sterile flask, to it were added 10 cc. of a 10 per cent. peptone, 5 per cent. salt solution, and the whole was incubated at 37° C. In neither case, the interior of several oysters as also the sea water, were any vibrios discovered.

After the addition of cholera culture to the sea water in

the tank, as previously stated, oysters kept therein for four days and for nine days respectively—the tank being daily well irrigated with fresh filtered sea water—yielded in peptone salt cultures from their interior liquor and body substance positive results,[1] that is to say, yielded cultures of vibrios, but though in many respects they resembled the cholera vibrios added to the tank water, yet in some important points they differed markedly from them as also from one another, and retained these differences constant through subcultures.

In another series in which oysters were kept for four days in cholera-infected sea water the peptone culture yielded vibrios which possessed distinct differences, retaining them in subcultures, not only from the original vibrios employed for the experiment, but also from those obtained from the previous two sets of oysters. The conclusion which these observations justify seems to be that in the bodies of oysters vibrios, which had an undoubted cholera origin, become markedly altered and become possessed of certain apparently permanent characters not possessed by the vibrios previously. I cannot here enter into the details of these observations, as these will be published in the Reports of the Medical Officer of the Local Government Board, and must content myself with the statement that I think a permanent alteration of the characters of the cholera vibrio had been established. If this be so, then the differences noted in many of the vibrios discovered in various waters (Spree, Danube, Elbe, Seine, &c.) after the visitation by cholera of the respective countries, as also those discovered by Pestana in cholerine, need not indicate that these vibrios were not originally cholera

[1] The water of the tank was examined in the above-named manner and was found to yield positive peptone cultures after four and after ten days respectively.

vibrios; their cultural and other differences may, just as was the case with the above oyster-vibrios, have been acquired and established through the environment, through their sojourn for some time under abnormal conditions.

R. Pfeiffer, in a series of well-known papers published in the *Zeitschrift f. Hygiene und Infekt.* during 1894 and 1895, has established the important fact that the blood-serum of guinea-pigs highly immunised by repeated intraperitoneal injection of living cholera vibrios (*see* a former page) possesses potentially specific immunising or germicidal action *in corpore*, that is to say, when in a certain proportion mixed with an otherwise fatal dose of cholera vibrios and injected into the peritoneal cavity of a guinea-pig, it kills the vibrios, and no disease follows, the animal remains alive and well and is "passively immunised." This was then extended by Pfeiffer also for the obtaining of "cholera serum," *i.e.* of immunising serum, from the highly immunised goat, and was shown to hold good also for "typhoid serum," *i.e.* for a potential specific germicidal action of blood-serum of animals highly immunised by intraperitoneal injection of cultures of the typhoid bacillus against an otherwise fatal dose of the typhoid bacillus. Several observers, I myself, have been able to confirm—as indeed is easily done—Pfeiffer's fundamental discovery.

Bordet (*Annales de l'Institut Pasteur*, June, 1895) and recently Durham (*Proceedings of the Royal Society*, January 23, 1896) show that also *in vitro* "cholera serum" shows a definite separating action, inasmuch as when added in definite proportion (sometimes alone, sometimes with normal serum—Bordet, alone—Durham) to a suspension of living cholera vibrios contained in a test-tube it makes the vibrios become matted together in clumps, settling at the bottom of the test-tube while the suspending

fluid becomes clear, and that after some time the motility of the vibrios becomes impaired and ceases, although living colonies can still be cultivated from them. Durham shows this action to take place also when "typhoid serum" is added to a suspension of typhoid bacilli.

We shall speak of Pfeiffer's germicidal action of the cholera serum *in corpore* as of Pfeiffer's test, of the Bordet-Durham separation test *in vitro* as of the Bordet-Durham test.

As stated just previously, the fundamental fact discovered by Pfeiffer as to the pronounced germicidal action of "cholera serum" or "typhoid serum" on cholera vibrios and typhoid bacilli, respectively, is well established. Now, Pfeiffer shows by numerous experiments that the "cholera serum," that is, the blood-serum of animals highly immunised by living vibrios of an undoubted cholera origin, possesses this pronounced germicidal action *in corpore* on all samples of vibrios—several hundred—which he and others got hold of from undoubted cases of Asiatic cholera, but that it fails to exhibit this action on vibrios of doubtful derivation, like the various water vibrios, the vibrio Nordhafen, &c.—that is to say, on vibrios which are not directly and notoriously derived from undoubted cases of Asiatic cholera—and he therefore feels justified in concluding that any species of vibrio which submitted to Pfeiffer's test succumbs is a cholera vibrio, any species which does not succumb to Pfeffer's test is not a cholera vibrio. The same is applied by Pfeiffer to the typhoid test *mutatis mutandis*. Bordet and Durham imply through their test *in vitro* a somewhat similar conclusion; but though their test was of positive differential value in the case of the cholera serum and cholera vibrio it was not so unequivocal, according to Durham, in the case of colon serum and colon bacillus. Koch has shown (*Zeitschr. f. Hygiene*, vol. xii.) that if in

any case of suspected cholera the flakes of the intestinal fluid or evacuation contain the vibrios in the typical distribution and in almost a pure condition such a case can without further hesitation be declared as cholera asiatica; those who have had sufficient experience of microscopic and cultural experiments of numerous cases of cholera can but confirm Koch's statement. Subsequent cultivations confirm the primary diagnosis. It must be obvious that if there be sporadic, not typical cases, from which by the culture test vibrios are isolated which in many respects resemble, in others slightly deviate from, the typical Koch's vibrio, an unfailing test by which these vibrios could be shown to be or not to be the true cholera vibrios would be invaluable, and in much higher degree would such a test be invaluable in the case of vibrios which like the above-quoted water vibrios cannot be shown to have been directly derived from cholera cases, and which owing to slight cultural differences are declared not to be cholera vibrios. Pfeiffer maintains that his test does furnish this important and unfailing evidence: the vibrios, no matter what their slight cultural differences be, that are derived from true cholera cases, give his test, therefore are true cholera vibrios; the vibrios, however, no matter how similar they be in morphological and cultural respects to the Koch's vibrio, that are not derived from cholera cases, do not give his test, are therefore not cholera vibrios. Without wishing in the least to deny Pfeiffer's justification in formulating so definitely his conclusions, nor wishing to accept unconditionally and in full —for reasons presently to be stated—Pfeiffer's statement, it is at the outset only fair to draw attention to the following hitherto unexplained facts of the Massowah vibrio.

Pasquale had a few years ago sent to Pfeiffer and to Metchnikoff cultures obtained from cases assumed to be

cholera that had occurred in Massowah. Pfeiffer accepted this Massowah vibrio as the cholera vibrio—notwithstanding its slight deviations in cultural respects from the typical Koch's vibrio, no doubt influenced by the knowledge gained that the vibrios derived from undoubted cases of cholera do not all coincide in all cultural characters—and his earlier experiments and statements on cholera were admittedly made with, and refer to this Massowah vibrio. Metchnikoff also accepted, after study, the Massowah vibrio as the true cholera vibrio; many of his experiments and observations on animals and human beings were made with this vibrio. Now, unfortunately this Massowah vibrio does not give Pfeiffer's test, and therefore is declared by Pfeiffer not to be cholera vibrio at all. This is a difficulty, though like all such difficulties it need not deter us from altering an initial wrong conclusion. But there are other and greater difficulties. Pfeiffer cannot deny the possibility that vibrios originally derived from true cholera, but living afterwards under abnormal conditions of temperature, soil and others, for considerable periods, could so alter as to change some of their original cultural characters as also their physiological reactions.

This, for instance, seems to me to have been the case with Sanarelli's water vibrios, with Pestana's vibrio, and I have already given direct evidence of such being the case with my oyster vibrios. There is nothing extraordinary or new in such an assumption; it is borne out by laboratory observations on a number of microbes, altering their characters permanently, cultural and chemical, by the influence of medium, temperature, the animal body, &c. One could therefore well assume or at any rate admit the possibility—it would be no exaggeration even to say the probability—that cholera vibrios living in water might or would so alter that the nature of their behaviour under Pfeiffer's test might

or would be altered. As a matter of fact, I have found that of guinea-pigs immunised by repeated intraperitoneal injection with one variety of living cholera vibrios—derived from an undoubted typical fatal case of Asiatic cholera in one locality in England in 1893—a certain percentage did not prove themselves resistant against a subsequent intraperitoneal injection with a fatal dose of living cholera vibrios derived from an undoubted and typical fatal case of Asiatic cholera that occurred in another locality in England in 1893. The animal that so died had acute peritonitis and only few vibrios in the peritoneal exudation, but the intestine was full of grumous fluid that contained the cholera vibrios in almost pure culture (Reports of the Medical Officer of the Local Government Board for 1894).

All these results seem to me to show that the apodictic announcement that such and such a vibrio is not a cholera vibrio because it does not succumb to the "cholera serum" obtained by immunisation with a particular cholera vibrio is not sufficiently established, although it may be conceded that a vibrio which does answer in positive fashion to Pfeiffer's test is a cholera vibrio. For this last reason Pfeiffer's test is undoubtedly of exceedingly great value both with reference to cholera and typhoid, but it should not extend its differential value to the negative cases.

(*m*). *Vibrio Metchnikovi.*—Gamaleïa[1] describes an acute fatal disease—gastro-enteritis cholerica—affecting fowls in Odessa during the summer months; the disease in its symptoms and its fatality is very similar to fowl cholera, but it differs in this essential respect that it is not caused by the bacillus of fowl cholera; it is caused by a vibrio present in large numbers in the blood. In its morphology, motility, size, and shape, and formation of S-shaped and spiral forms,

[1] *Annales de l'Institut Pasteur*, No. 9, 1888.

as well as in its cultural character it resembles, but is not quite identical with, Koch's cholera vibrio. Inoculated subcutaneously into pigeons or guinea-pigs it proves very virulent, producing acute disease and death; fowls can be infected by ingestion. On *post-mortem* examination of the infected animals the intestines are found greatly congested and contain in their cavity grumouss anguineous fluid. The spleen is not enlarged.

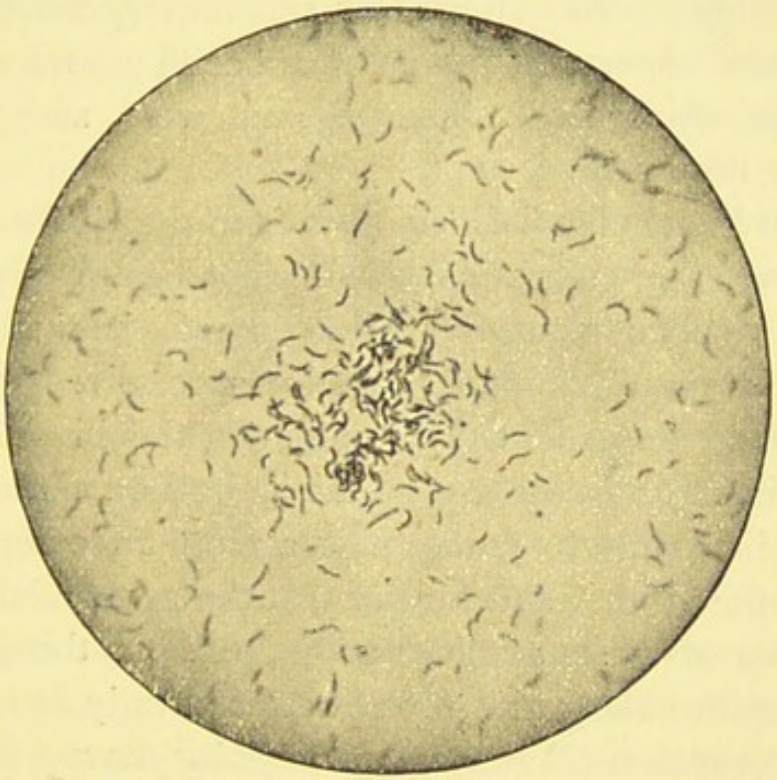


FIG. 183.—FILM SPECIMEN OF A CULTURE OF VIBRIO METCHNIKOVI.
× 1000.

The vibrio is copiously present in the intestinal fluid and in the blood.

Gamaleïa made the statement that the vibrio Metchnikovi and the cholera vibrio are mutually protective for the pigeon; that is to say, that a pigeon that has survived disease caused by the injection of a non-fatal dose of one vibrio is protected against a subsequent injection of a fatal dose of the other.

But Pfeiffer[1] has shown that while the vibrio Metchnikovi is virulent for the pigeon the vibrio of cholera is not, and further that a pigeon that had received first a large dose of cholera vibrios succumbs to a further injection of the vibrio Metchnikovi just like any normal pigeon. That pigeons are insusceptible to subcutaneous and intermuscular injection can be easily shown; I have injected into the pectoral muscle as much as 2–3 cc. of recent active broth culture, and on searching by the culture test and film specimens twenty-four hours afterwards for comma bacilli no trace of them could be discovered. The pigeons were and remained perfectly normal.

Vibrio Metchnikovi differs then from the vibrio of cholera as regards the guinea-pig, the former being very virulent injected subcutaneously. Metchnikoff has shown that guinea-pigs can be immunised by successive inoculations of non-fatal doses of culture, and that the blood-serum of such animals also possesses germicidal action and can confer passive immunity to guinea-pigs against an otherwise fatal dose of the vibrio; and further that the germicidal action of the serum of an immunised animal exhibits this germicidal action against the vibrio Metchnikovi already *in vitro*.

(*n.*) *Spirillum Obermeyeri of relapsing fever.*—Obermeyer (*Centralbl. f. d. med. Wiss.*, 1873, No. 10) discovered in the circulating blood of patients affected with this fever, during the febrile stage, innumerable spirilla actively motile: they disappear from the blood immediately preceding the end of the febrile stage.

The spirilla are very thin and about 20–30–40 μ. long; their movement is that of rapidly progressing spirals. Koch has demonstrated by photography of dried and stained specimens the presence of the flagella in the spirilla. Weigert

[1] *Zeitschrift f. Hygiene*, vii. 3.

has shown that, unlike other bacteria, they are barren of a cellulose sheath, since dilute liquor potassæ dissolves the whole substance of the spirilla. By drying and staining cover-glass specimens it has been shown that the spirilla are uniform spirals, and do not show anything that might be interpreted as being made up of shorter elements, comma bacilli or vibrios. The spirals when long are often plicated, but their turns are always close, and more or less in the manner of a corkscrew. Immediately preceding the febrile stage they appear in the blood, grow more and more numerous during the fever, and disappear again completely from the circulating blood before the fever quite ceases. During the non-febrile stage they most probably are present in the spleen and marrow of bone—Birch-Hirschfeld found many of them in the necrotic foci of the spleen—where perhaps they undergo germination and reproduction. It is feasible to assume that when during the febrile stage they reach the acme of their development they gradually break down, leaving spores in the shape of granules behind: these are carried into the spleen and bone marrow where they accumulate. During the non-febrile stage these spores germinate here again and gradually grow into the spirilla, which when ripe and motile gradually find their way again into the blood. Such a view would well harmonise with the facts of the case and also with what has been shown of the plasmodium malariæ.

As a matter of fact the spirilla in the blood often show bright granules in their interior, which might well be spores.

Koch has shown that in artificial culture the spirilla are capable of growing out into long spiral filaments matted together, but no real artificial cultures have been as yet produced. That the spirilla are the real microbes of relapsing fever is proved by the experiments of Vandyke Carter

(*British Medical Journal*, October, 1881), who was the first to produce typical relapsing fever in the ape after injection of blood of a patient taken during the febrile stage and containing the spirilla. The disease produced in the ape was

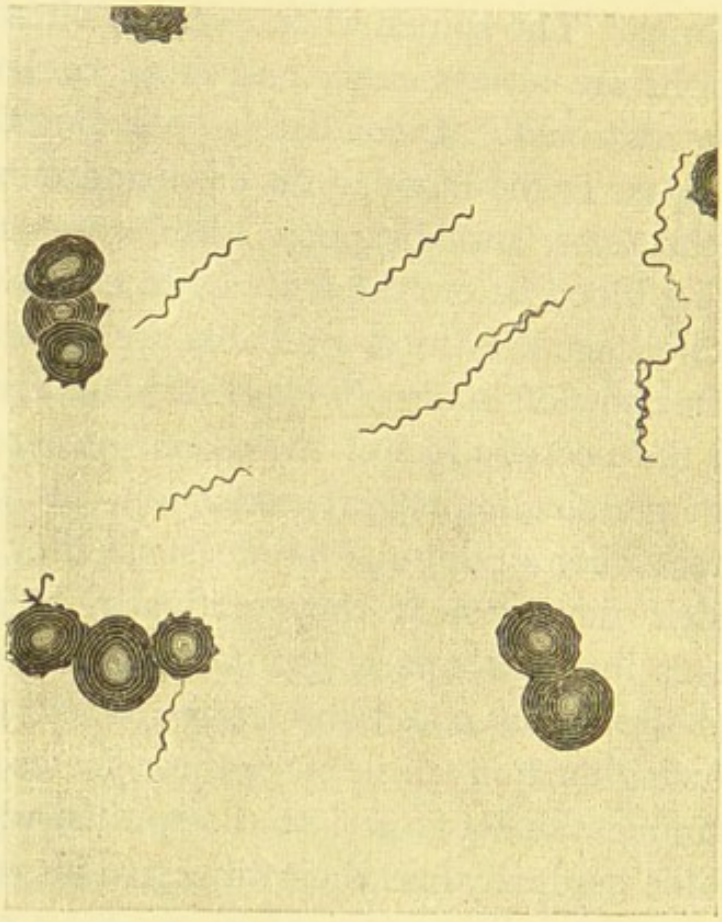


FIG. 184.—BLOOD OF RELAPSING FEVER (HUMAN).
Blood-corpuscles and spirilla Obermeyeri.
Magnifying power 700. (After Koch).

true relapsing fever, and the animal's blood contained during the febrile stage the identical spirilla in large numbers. Koch, Heydenreich, and Metchnikoff have confirmed this. Motschutkowsky (*Deutsches Archiv f. klin. Med.*,

Band xxiv.) has produced relapsing fever in the human subject by inoculation of blood containing the spirilla.

Metchnikoff (*Virchow's Archiv*, Band cix., 1887) maintains that the disappearance of the spirilla from the system, *i.e.* recovery, is due to phagocytes, that is to say, that the

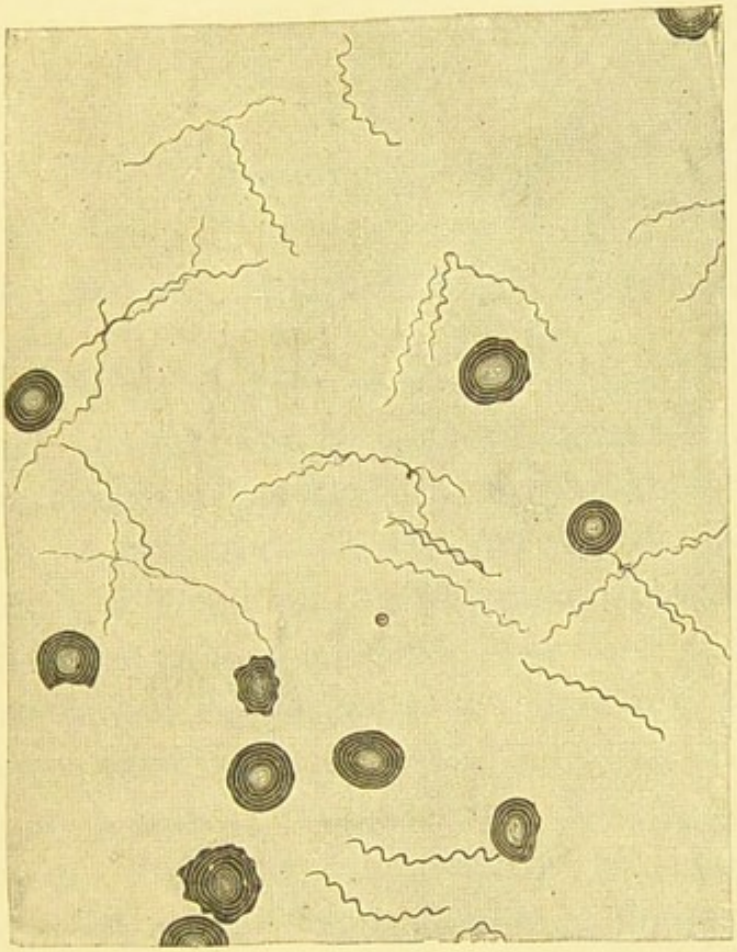


FIG. 185.—BLOOD OF APE INOCULATED WITH BLOOD SHOWN IN PRECEDING FIGURE.

Blood-corpuscles and spirilla.
Magnifying power 700. (After Koch).

white cells of the spleen swallow and destroy the spirilla and thus purge the system of them. It is a fact that the spirilla are found enclosed within the white cells of the spleen, but it does not follow that Metchnikoff's view is correct, for

Baumgarten has justly pointed out that as in other diseases so also in relapsing fever the enclosure of the spirilla by leucocytes may be only a result of the microbes having previously been killed by other agencies, and that after this they have been taken up by the white cells just like other dead formed matter.

CHAPTER XVII

YEAST FUNGI: TORULACEÆ, SACCHAROMYCES

YEAST, *torula* (Pasteur), or *saccharomyces*, is not a bacterium, but belongs to an altogether different order of fungi —the *Blastomycetes*. It consists of spherical or oval cells, very much larger than the largest micrococci, and as in the case of these each cell consists of a membrane and contents. The contents are either homogeneous or finely granular protoplasm; in the latter case there are generally present one, two, or more small vacuoles.

There are a great many species of *Torula*, varying from one another morphologically chiefly in their size, and physiologically by their action on various fluids (*see* below).

The cells multiply in suitable media by gemmation, a minute knob-like projection appearing at one side of the cell, and enlarging till it reaches nearly the size of the original or mother-cell. It finally becomes altogether constricted off from this latter, or having reached its full size remains fixed to the mother-cell, and each cell again producing by gemmation a new cell. In this way aggregations of four, six, eight, or more cells are formed, which may be arranged either as a chain when the production proceeds

in a linear manner, or as a group if the gemmation takes place laterally.

Under varying conditions of growth, *e.g.* on transplanting ordinary yeast growing in sugar-containing fluids on to potato, but sometimes also in the same nutritive fluid, it is observed that some of the yeast cells enlarge twice, thrice, and more times; they then form in their interior two, three, or more small cells by endogenous formation; these new cells are

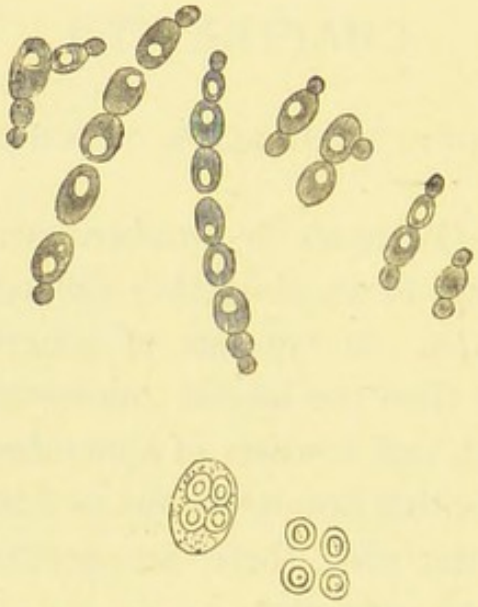


FIG. 186.—TORULA, OR SACCHAROMYCES.

In the lower part of the figure an ascospore and four isolated spores (after Rees) are shown.

Magnifying power about 700.

regarded as spores[1]—the mother-cell being an *ascospore*—and become free by finally bursting the membrane of the mother-cell. On sowing these new cells into sugar-containing fluids they multiply by the process of gemmation.

Classifying them according to physiological function there are various species of torula or saccharomyces. They all

[1] T. de Seynes, *Comptes Rendus*, 1866; Rees, *Bot. Zeitschr.* 1869; Hansen, *Carlsberg Laborat.* 1883.

have the power to split up sugar into alcohol and carbonic acid, but this power is not possessed by all to the same degree.

(*a*) *Saccharomyces cerevisiæ* (*torula cerevisiæ*).—This is the ordinary yeast used in the production of beer. The individual full-grown cells vary in diameter from 0·008 to 0·01 mm.; they form beautiful long chains. They produce ascospores.

(*b*) *Saccharomyces vini* is very common in the air, and produces alcoholic fermentation of grape-juice; it is therefore the proper yeast of wine-production. Its cells are elliptical, slightly smaller than the former; it forms ascospores.

(*c*) *Saccharomyces pastorianus* is of various kinds (Hansen): in some the cells are about 0·002 to 0·005 mm. in diameter, in others larger. Some form ascospores, others do not. Most of them can be found in wine-fermentation and in cider-fermentation, but only after the first alcoholic fermentation is completed. They are very common in the air. I have sown a saccharomyces, which was contained in ordinary water, on solid nourishing media (gelatine and gelatine and broth). It grew up copiously and formed groups of a distinct pink colour. When growing in the depth of the nourishing medium it grew as a colourless torula, no ascospores were formed, multiplication taking place by gemmation only.[1]

(*d*) *Saccharomyces mycoderma* (*mycoderma vini*).—This yeast is found forming the scum or pellicle on the surface of wine, beer, and fermented cabbage (*Sauerkraut*); its cells are oval, about 0·006 mm. long and 0·002 broad. It forms chains; the ascospores are two or three times larger. It has nothing to do with the alcoholic fermentation, and is not to

[1] *Quart. Journ. of Micr. Science*, 1883.

be confounded with *mycoderma aceti*,[1] which is a bacterium and the efficient cause of acid fermentation in wine and beer.

The saccharomyces mycoderma does not grow well in the depth of liquids, but when sown into a liquid of acid reaction and containing but little sugar Cienkowsky saw the

FIG. 187.—SACCHAROMYCES MYCODERMA, OR OIDIUM ALBICANS. (After Grawitz.)

From an artificial cultivation in dilute nourishing material.

d. Branched mycelium.
fα. Torula stage.
fβ. Mycelial stage.

cells elongating into cylindrical elements; each of which by gemmation produced a new cell which also elongated, and so on till a linear series of cylindrical cells was formed, separated from one another only by a thin septum; a mass of filaments very much resembling a mycelium was thus

[1] Nägeli, see chapter viii. 2.

formed. The cylindrical cells give origin by gemmation to spherical and elliptical torula-cells.

Such a growth, in which the torula-cells are capable of forming a sort of mycelium, was formerly called *oïdium*, and as *oïdium albicans* is recognised as the cause of "thrush"; the well-known white patches which occur on the mucous

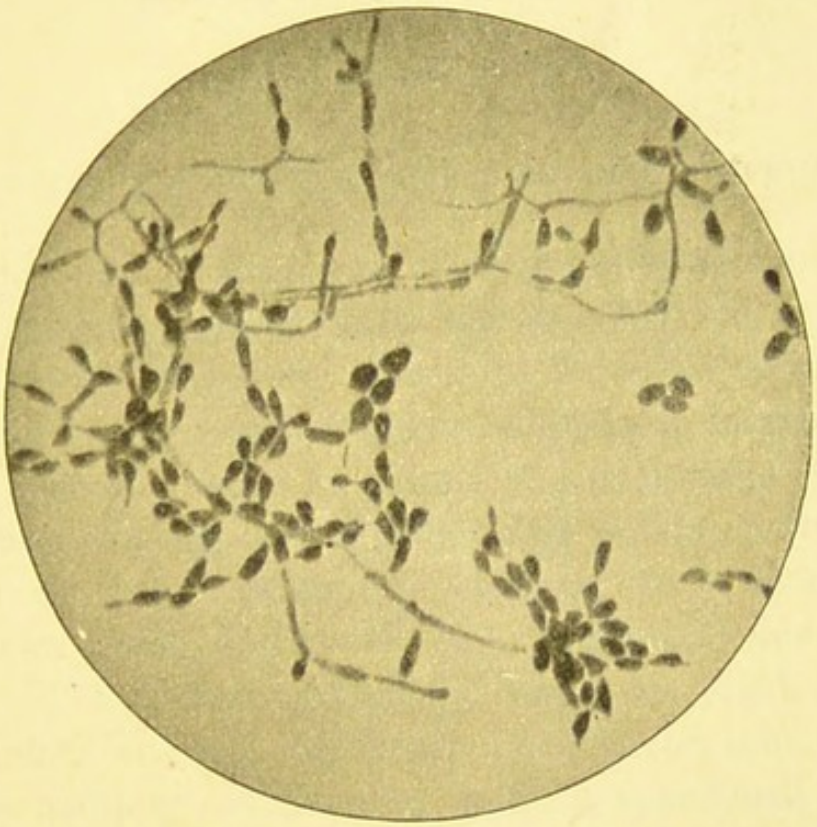


FIG. 188.—FILM SPECIMEN OF THRUSH FUNGUS.
(Bousfield.)
× 700.

membrane of the mouth and pharynx in suckling infants and debilitated patients.

Grawitz [1] has proved by observations on artificial cultures that this fungus is identical with the oïdium variety of *Saccharomyces mycoderma*; the cells are spherical or cylindrical, the former about 0·003 to 0·005 mm. in diameter, the latter

[1] *Virchow's Archiv*, vol. lxx.

up to 0·03 or 0·05 mm. long. As above described it forms mycelium-like filaments from which, by lateral and terminal gemmation, spring spherical or oval torula-cells. It also forms ascospores containing four to eight spores.

It grows well on gelatine at 20° C. and on Agar at 37° C., forming white round colonies from which extend radially fine threads; it does not liquefy the gelatine.

CHAPTER XVIII

MOULD-FUNGI: HYPHOMYCETES OR MYCELIAL FUNGI

Of this class of fungi only those are of special interest to the pathologist which in some way or other are connected with disease. The fungi consist of branched and septate threads or hyphæ; each filament or hypha is composed of a row of cylindrical cells, consisting of a membrane and clear protoplasm, the individual cells being separated from one another by a thin transverse septum; they increase in number by fission, and in this way the filaments increase in length. The growing ends of the hyphæ are filled, not with transparent, but with highly-refractive protoplasm. Some cells, by budding out laterally, produce cylindrical threads, which subdivide into a series of cylindrical cells, these by division and lengthening forming a new branch-hypha. The filaments form by their branches an interlacing feltwork, called thallus or mycelium. The mycelial fungi which interest us belong to the order known to botanists as the *Ascomycetes*. They are characterised by the fact that one or other branch of the mycelial hyphæ produces at its end a series of spherical or oval cells—the conidia-spores or *conidia*. In addition to this some of the hyphæ form peculiar large mother-cells, or *sporangia*, in the interior of which

spores are formed by endogenous formation. When these sporangia are cylindrical or club-shaped, they include eight spores, and are called *asci*: the spores being *ascospores*. All conidia or spores by germination grow into the mycelial threads which become septate or subdivided into a row of cylindrical cells; these by division cause the lengthening of the mycelial threads.

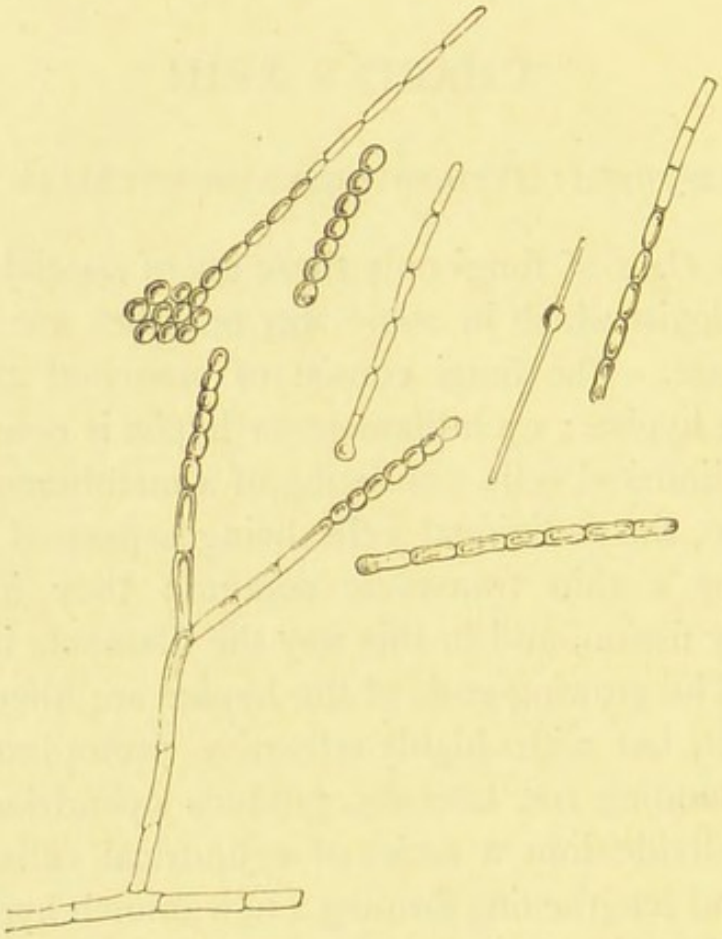


FIG. 189.—OÏDIUM LACTIS.
Mycelium and spores.

(*a*) *Oïdium lactis*.—Here the mycelium is composed of septate branched filaments of various thicknesses. Some branches of the mycelium at their ends or laterally at a septum produce by division a series of spherical or oval conidia-spores, about 0·007 to 0·01 mm. long. These ultimately become isolated, and then germinate into a short cylindrical

filament, which subdivides by transverse septa into a series of cylindrical cells; these by continued growth and division give origin to the ordinary septate branch-hyphæ. The formation of conidia proceeds at the ends of these in the same manner as before. The oïdium lactis forms a whitish mould on milk, bread, paste, potato, &c.

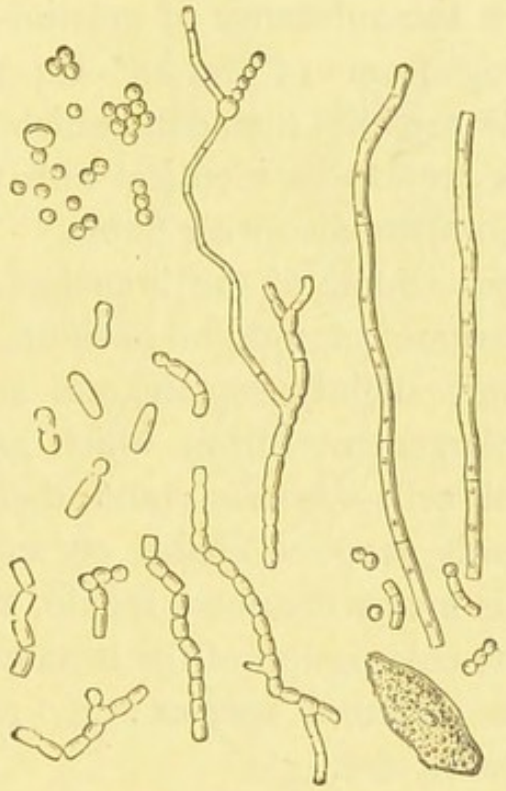


FIG. 190.—FUNGI FROM A FAVUS-PATCH (NEUMANN).

Favus, Herpes tonsurans, and Pityriasis versicolor of man and animals, are, according to the researches of Grawitz,[1] due to a fungus in morphological respects identical with oïdium lactis. In favus it is known as *Achorion Schoenleini*, in Herpes tonsurans as *Trichophyton tonsurans*, in Pityriasis versicolor as *Microsporon furfur*. Grawitz has shown by artificial cultures on gelatine that the spherical or oval

[1] *Virchow's Archiv*, vol. lxx. p. 560.

conidia germinate into shorter or longer cylindrical filaments, which by subdivision form septate mycelial hyphæ. These and their branches give origin in their turn to spherical or oval spores or conidia. They, as well as the hyphæ, differ in size in the various species.

Malcolm Morris and G. C. Henderson,[1] on the other hand, maintain that by artificial cultivation of the spores of Trichophyton in the substance of gelatine-peptone, at temperatures varying from 15° to 25° C., these grow into branched septate mycelial filaments, which by their mode of fructification are seen to be identical with the mycelium of *Penicillium*. Compare also with Babes.[2]

(*b*) *Aspergillus*.—Some of the branches of the mycelium of this fungus assume an upright position, are thicker and not at all, or only slightly, septate, and at their end form flask-shaped enlargements, from which grow out radially short cylindrical cells—*basidia*; and these again at their distal or free ends produce chains of spherical spores or conidia. This is a very common mould, and according to differences in the colouration of the mycelium and spores is subdivided into different species: *A. glaucus*, *candidus*, *flavescens*, *fumigatus*, *&c.*

Besides this mode of spore-formation (asexual), there is another (sexual), which according to De Bary consists in this: some terminal branch of the mycelium becomes twisted like a spiral, this is considered the female organ of fructification or *carpogonium*; from the same thread branches grow towards the carpogonium; one of these branches becomes fused with the terminal portion of the carpogonium called the ascogonium, while the others—the *pollinodia*—branch and surround the carpogonium like a capsule: the

[1] *Journal of the Royal Microscopical Society*, April 11, 1883.
[2] *Archives de Physiologie*, 8, 1883, p. 466.

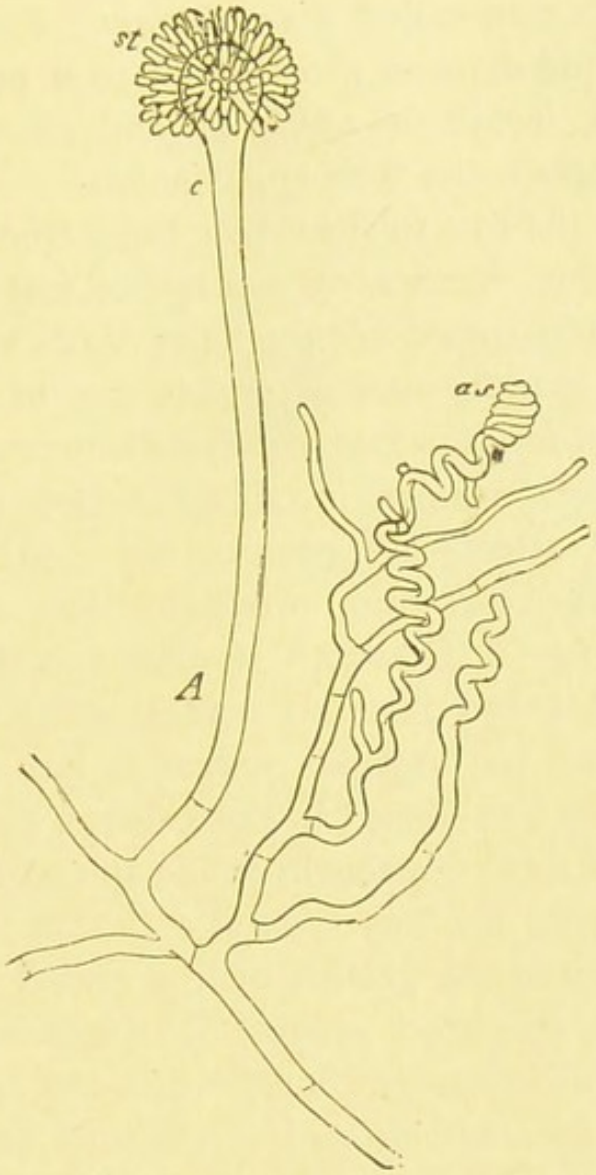


FIG. 191.—ASPERGILLUS GLAUCUS (AFTER DE BARY).

A. Hypha, the end of which, *c*, bears
st. The basidia.
as. Ascogonium.

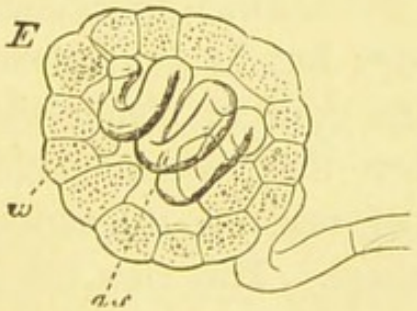


FIG. 192.—E. PERITHECIUM, HIGHLY MAGNIFIED.

as. Ascogonium.
w. Cells of the pollinodia.

whole organ is now called a *perithecium*. Finally the ascogonium by rapid division gives origin to a number of oval septate tubes, inside of which by endogenous formation numerous spores make their appearance.

Grohe[1] was the first to show that the introduction of the spores of some species of aspergillus into the vascular system of rabbits sometimes produces death, with symptoms of metastasis into the various organs due to localised foci, where these spores grow into mycelial filaments. Lichtheim[2] showed that such mycoses in rabbits cannot be produced by the spores of *Aspergillus glaucus*, but easily by those of *Aspergillus flavescens* and *fumigatus*, less in degree by *Aspergillus niger*. Grawitz[3] studied this process more minutely, and found that, no matter whether the spores are injected into the vascular system or into the peritoneal cavity, there are established in the kidneys, liver, intestines, lungs, muscles, and occasionally in the spleen, marrowbones, lymphatic glands, nervous system, and skin, minute metastatic foci, due to the growth of the spores into mycelial filaments with imperfect organs of fructification, but no spore-formation. Grawitz thought that the spores of ordinary moulds (penicillium and aspergillus) are capable of assuming these pathogenic properties if cultivated at higher temperatures (39° to 40° C.), and in alkaline media. These fungi, as is well known, grow well at ordinary temperatures and in acid media, and are then innocuous when introduced into the animal body; but by gradual acclimatisation they can also be made to grow at higher temperatures and in alkaline media, when they assume pathogenic properties, becoming capable of resisting the action of living tissues and of growing in them. This view has been proved to be

[1] *Berl. klin. Woch.* 1871. [2] *Ibid.* 9 and 10, 1882.
[3] *Virchow's Archiv*, vol. lxxxi. p. 355.

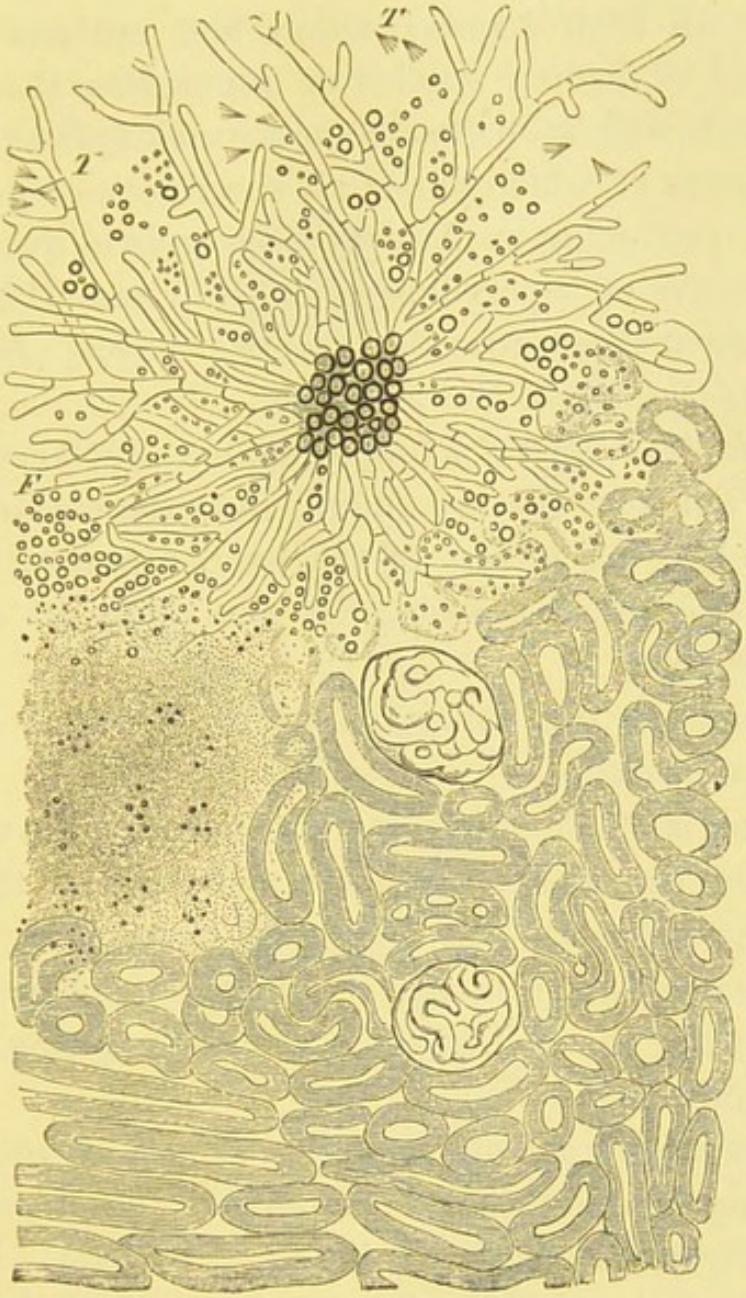


FIG. 193.—FROM A SECTION THROUGH THE KIDNEY OF A RABBIT DEAD THIRTY-SIX HOURS AFTER THE INJECTION OF SPORES INTO THE JUGULAR VEIN.

F. Fat droplets. *T.* Tyrosin crystals.

In the upper part of the figure is a metastatic focus composed of Aspergillus spores and mycelium. In the lower half of the figure the urinary tubules and two Malpighian corpuscles are seen. (After Grawitz.)

incorrect by Gaffky,[1] Koch,[2] and Leber.[3] Those spores that do exert such pathogenic properties are not at all

[1] *Mittheil. a. d. kais. Gesundheitsamte*, 1880.
[2] *Berl. klin. Woch.* 1881. [3] *Ibid.* 1882.

dependent on such acclimatisation, and are not ordinary moulds, but a distinct species of aspergillus (Lichtheim), which grows well at higher temperatures (38° to 48° C.), and the spores of which under all conditions of growth are capable of producing in rabbits the mycosis in question.

Several cases of aspergillus infection in man (Pneumonomycosis) have been recorded, *see* R. Boyce, *Journal of Pathol. and Bacteriol.*, No. 2, 1892.

(*c*) *Penicillium.*—In this fungus hyphæ, which are not septate, grow out from the mycelium; from the end of each of these arise like the fingers of the hand a number of short branched cylindrical cells, which give origin to chains of spherical spores.

The following two fungi belong to the order of fungi called *Phycomycetes.*

(*d*) *Mucor* is characterised by this, that from the mycelium hyphæ grow out which are not septate, and at the end of these a large spherical cell originates, *sporangium*, in which by endogenous formation a large number of spherical spores are developed; the wall of the sporangium giving way, the spores become free.

An important case of general "*mycosis mucorina*" in man, ending in death, has been recently described by Dr. Paltauf (*Virchow's Archiv*, vol. 102, 3, p. 543). From the alimentary canal of the patient an invasion of the internal organs by the mycelium and spores of a kind of mucor occurred, leading to the formation of metastatic inflammatory foci in the Peyer's glands, lungs, pharynx, larynx, cerebrum, and cerebellum. In these organs were found foci of inflammation caused by mycelial threads and sporangia, belonging to the group of mucor. Mucor rhizopodiformis and corymbifer were shown by Lichtheim to be pathogenic when injected into the vessels of the rabbit.

(*e*) *Saprolegnia ;* colourless tubular threads, forming gelatinous masses on living and dead animal and vegetable matter in fresh water. The cylindrical or flask-shaped ends

FIG. 194.—SAPROLEGNIA OF SALMON DISEASE.

A sporangium filled with zoospores ; in connection with them several young mycelial threads.

of the threads—*zoosporangia*—form in their interior numbers of spherical or oval spores —*zoospores*, possessed of locomo-

tion (one flagellum at each pole) and which finally escape from the threads. These zoospores after some time become resting, surround themselves with a membrane, and finally germinate into a cylindrical mass which becomes transformed into the mycelium. Besides this asexual there is, however, a second or sexual mode of fructification, consisting in this: At the end of a mycelial thread a cell grows up into a spherical large ball, the *oogonium*. From the same thread, thin threads—*antheridia*—grow towards the oogonium, with the protoplasm of which they merge. This latter then differentiates into a number of spherical masses, the *oospores*, which become invested with a membrane. These become free and then germinate and grow into a mycelium. Saprolegnia grows on the skin of living fish, and causes here severe illness often terminating in death. Thus the salmon disease, as Professor Huxley has shown,[1] is caused by this parasite. The zoospores of this salmon saprolegnia are, however, as Huxley has shown, as a rule non-motile. The hyphæ of the fungus traverse the epidermis in the diseased patches of the salmon, and they bore through the superficial layer of the derma, a stem-part being situated in the epidermis, and a root-part in the derma; each of these elongates and branches out. "The free ends of the stem-hyphæ rise above the surface of the epidermis and become converted into zoosporangia, more or fewer of the spores of which attach themselves to the surrounding epidermis and repeat the process of penetration." In saprolegnia associated with the salmon-disease Professor Huxley observed only the asexual mode of fructification.

(*f*) *Actinomyces, or ray fungus.*—Bollinger[2] first showed that various tumours in cattle leading to chronic suppuration,

[1] *Proceedings of the Royal Society*. No. 219, 1882.
[2] *Centralbl. f. d. med. Wiss.*, 1877, No. 27.

e.g., in the jaw, the tongue, pharynx and skin, are one and the same disease, due to a parasite which he constantly found

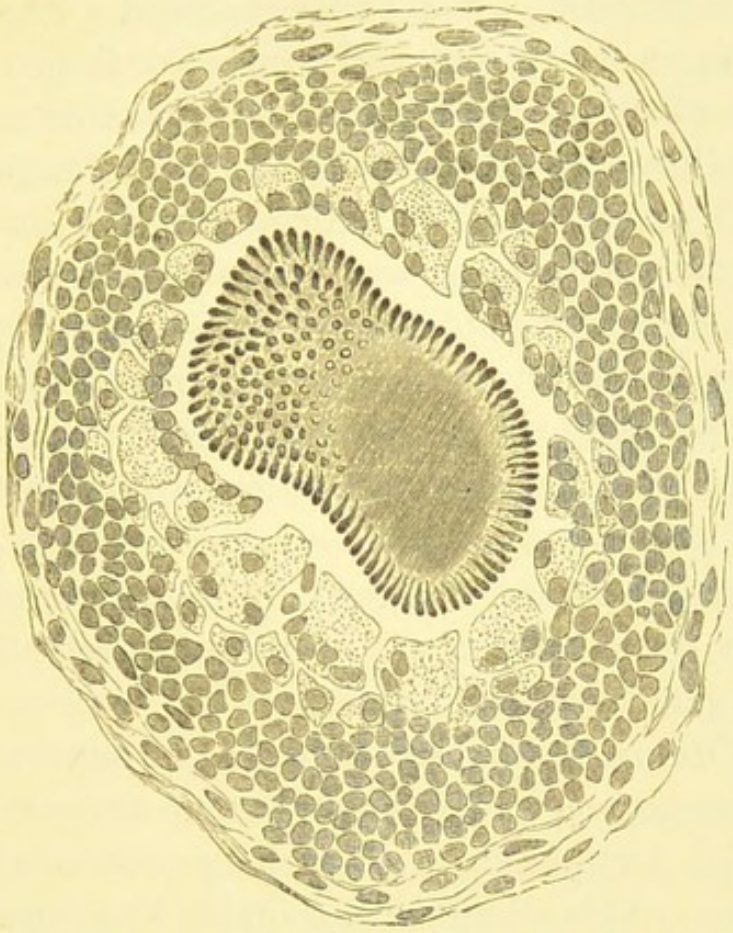


FIG. 195.—FROM A SECTION THROUGH THE TONGUE OF A COW DEAD OF ACTINOMYCOSIS.

A nodule is shown composed of round cells; in the centre is the clump of actinomyces surrounded by large transparent cells. Magnifying power 350.

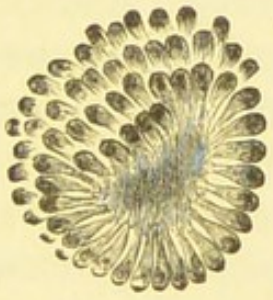


FIG. 196.—A CLUMP OF ACTINOMYCES MORE HIGHLY MAGNIFIED, 700.

in the tumours in the shape of yellow granules, and which, when examined under the microscope, consisted of radially

arranged fibres and clubs which Professor Harz designated *actinomyces*, or *ray fungus;* the disease it causes is therefore called *actinomycosis.* Israel[1] next observed a disease in the human lung, in which he found a mycelial fungus; this was afterwards identified by Pomfick as the same ray fungus seen by Bollinger in the tumours of cattle. Pomfick himself published[2] several cases of actinomycosis in man. Since these observations a large number of cases in cattle, pigs, and in man have been published, in which tumours, abscesses, and suppurations, &c., were found in one or the other of the following organs: in the jaw, skin, tongue, pharynx, larynx, lung, intestine, liver, brain, and which proved to be due to the same parasite—actinomyces.

In cattle the disease manifests itself by firm tumours in the jaw, in the alveoli of the teeth, and particularly by a great enlargement and induration of the tongue—"*wooden tongue.*" On making sections through this latter organ there are found present in all parts microscopic tumours of small-cell growth. In the centre of each tumour is a clump of actinomyces. This clump is surrounded by a zone of largish cells, with one to four nuclei. The periphery of the tumour is made up of a fibrous capsule, with spindle-shaped cells. Occasionally the tumours are to be seen also in the skin and in the lung; in the latter organ they appear as whitish nodules, easily mistaken for tubercles.

As the central fungus by active growth enlarges, so the tumour enlarges by new infiltration with round cells spreading into the surrounding tissues. In a later stage the central portion softens and becomes purulent: an abscess is thus formed which, opening on to the surface, or into the nearest cavity, soon discharges copious pus; when the abscess opens

[1] *Virchow's Archiv*, Band lxxiv.
[2] *Die Actinomykose des Menschen*, Berlin, 1882.

on to the free surface, *e.g.*, jaw, skin, pharynx, larynx, lung, intestine, an ulcer is established, which, as the infiltration in the periphery proceeds, enlarges. In the discharge of the abscess a number of yellowish minute granules can be found; these granules looked at under the microscope are a mass of the ray fungus. It has now been established that carious teeth may represent the point of entrance for the fungus; in these cases the alveolar process of the jaw becomes the place for the growth of the fungus, leading to the formation of a hard tumour, gradually becoming converted into an abscess and ulcer. The infection, *i.e.* invasion by the fungus, then spreads to the lymph glands and skin nearest to the affected jaw, and here produces tumour, then suppuration and ulcer; or it invades the tonsils and the pharynx, either primarily or after it has once taken root in the jaw, tongue, or cheek. Or it appears primarily in the larynx, trachea, and lung; in these cases the fungus has evidently been introduced by the air during inspiration. In the case of the lung extensive interstitial inflammation is set up, leading to abscesses perforating into a bronchus. Or it invades primarily the alimentary canal and leads here to abscess and copious suppuration, and even to perforation of the part; in the case of the alimentary canal the fungus may have entered with the food. From the alimentary canal the disease spreads to the mesenteric glands and the liver; in this latter organ it produces abscess, which may open through the peritoneum into the peritoneal cavity, or, if previously an adhesion with the abdominal wall had been established, may perforate outwards. In all these instances the discharged pus contains the yellow granules, *i.e.* groups of the ray fungus.

In the case of the skin the fate of the tumours is suppuration and formation of abscess, and this opening on the surface leads to the formation of a sore. The primary

infection of the skin by actinomyces has been proved (E. Müller, *Mitth. aus d. chirurg. Klinik*, Tübingen, Band iii., 3) in a case in which a wood splinter in the skin had evidently been the means of providing an entrance for the fungus. Both in man and cattle these various ways of infection with actinomyces have been observed in many cases.

The various ways above mentioned in which the fungus invades the organism at once suggest that it has its usual habitat in the outside world, *i.e.* that it is an organism which is introduced into the animal or human body from the outside, and is not directly derived from an infected animal or man. It is a prevalent opinion that the natural habitat of the ray fungus is on cerealia, that it lives on these parasitically, and through and from these enters the animal body through wounds, abrasions, &c. Johne (*Centralbl. f. d. med. Wiss.* 1881, No. 15) has shown that actinomyces occurs normally in the pits and the loculi in the tonsils of the pig; in these instances there are always present bits of ears of barley covered with what appeared to be ray fungus. Jensen (*Deutsche Zeitschrift f. Thiermed.*) observed an epidemic of actinomycosis in cattle fed on barley; and Piana described actinomyces nodules in the tongue of cattle, where in the midst of some of the nodules there were present portions of vascular fibre tissue of corn surrounded by ray fungus. Finally, Soltmann (*Breslauer ärztl. Zeitschrift*, 1885, No. 3) made the remarkable observation of an actinomyces abscess in man in the region of the dorsal vertebral column, which was caused by the penetration (during swallowing) of an ear of barley; the abscess opened and the ear was discharged. Fischer (*Centralbl. für Chirurgie*, No. 22, 1890) describes a similar case: a labourer on chewing barley pierced his tongue with a portion of the awn. Eight days

later a swelling appeared on the punctured spot, and after a fortnight a tumour of the size of a filbert could be distinctly felt. After eighteen days an incision was made into the tumour, and the examination of the scanty pus and the tissue of the tumour revealed the presence of numerous yellow granules—actinomyces. Also the fragment of the awn was removed from the interior of the tumour, and on examining it under the microscope was found covered with clumps of actinomyces. So that from all this the conclusion appears justified that actinomyces is a fungus having its habitat on cerealia, and with and by them is introduced into cattle and man.

As mentioned above, the tumours and abscesses occurring in one or the other organ contain peculiar minute granules and clumps, visible already to the unaided eye, generally of a yellowish, occasionally of a yellow-greenish tint. Under the microscope they appear made up of a central mass of fine granules, or of a distinct trellis-work of fine branched threads; next is a zone of coarser granules, which granules do not look unlike cocci; but when this or the central zone is teased out it can be shown that the granules are not really granules, but in reality are densely aggregated and twisted branched fine fibres, the "granules" being only due to optical sections of the fibres; at the periphery of the mass are glistening densely and radially aggregated flask-shaped or club-shaped bodies called the "clubs" (Fig. 198). The central mass is occasionally found in a state of calcification: this is not seldom the case in cattle.

That these clubs are an important and characteristic feature in the morphology of the fungus is shown by the name of ray fungus and by the fact that, what is commonly observed—at any rate in cattle it is common—all the actinomyces nodules and abscesses contain one or more central

mass or masses of these radiating aggregations of clubs. But also in the human disease the clubs are with few exceptions present, though there are cases described in which the fungus was said to have been represented only by a dense felt-work of branched fine threads.

Examining sections of hardened actinomyces nodules, after suitable staining (rubin, 2 per cent. watery solution,

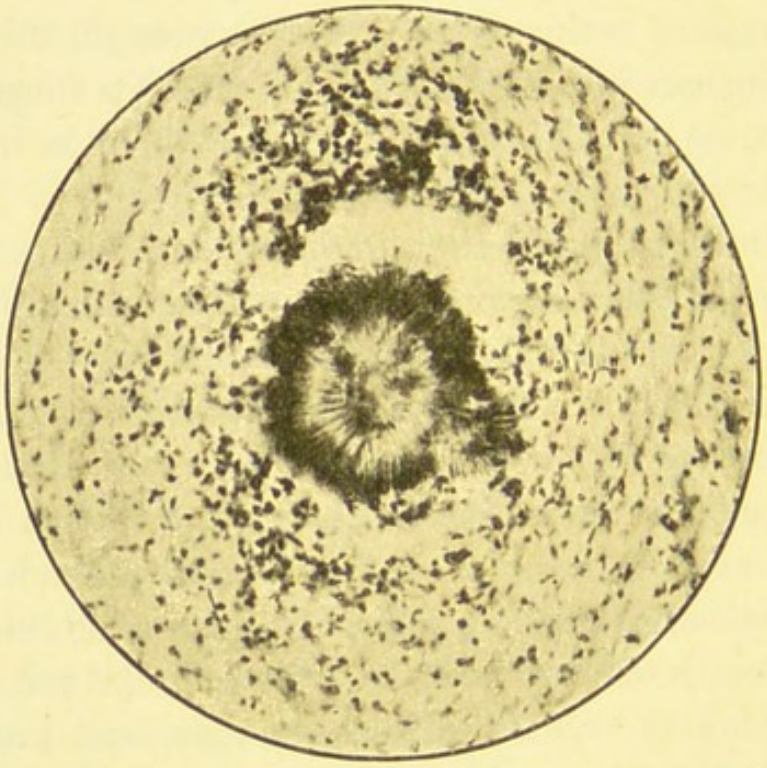


FIG. 197.—From a section through a nodule of the tongue of an ox, showing the central mass of the ray fungus. Low magnifying power.

for several hours, then washed in water and stained in methyl-blue anilin water for fifteen to thirty minutes) the ray fungus appears in the middle of the nodule as an irregular, spherical, or, more commonly, particularly when large, a lobed mass, composed of a central, faintly stained, homogeneous, or faintly granular mass; around this is a zone deeply stained in blue, and owing to its being composed of densely aggregated and twisted branched threads

looking not unlike cocci. The peripheral part is made up of conical or cylindrical or club-shaped corpuscles of different length and thickness, deeply stained pink, closely placed side by side, and all radiating by longer or shorter thin, pink, filamentous stalks from the next, the blue or "granular" zone; each of the "clubs" possesses a faintly stained homogeneous sheath. In human actinomycosis,

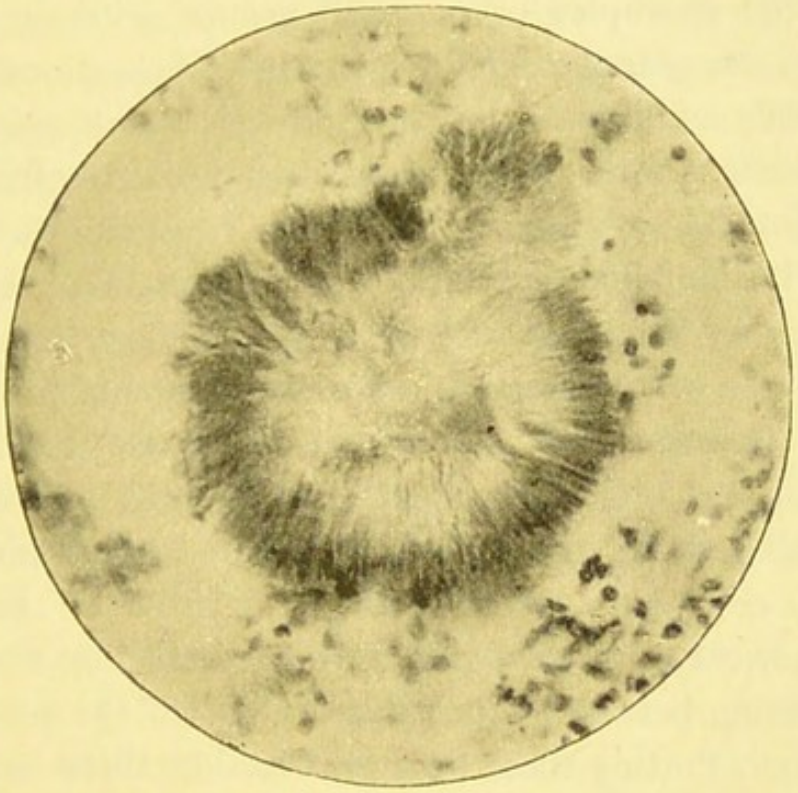


FIG. 198.—From a similar preparation, more highly magnified.

and also in actinomycosis of cattle, the central mass is not seldom recognisable as a dense felt-work of fine branched threads; from the periphery of the mass longer threads project, each or only some of which possess a terminal enlargement; in other cases the terminal club-shaped enlargement is the only distinct portion.

Now, some observers consider the clubs as indicating an involution or a degeneration phase of the threads, and,

further, the above granules of the second zone as indicating a coccus phase of the threads, and for this reason consider the ray fungus as belonging to the species of cladothrix, a polymorphous fungus, in which the threads may break up into or develop from cocci and shorter or longer rods or bacilli. Now, I quite agree with Crookshank in not accepting this view, for I find constantly in actinomycosis of cattle some of the smaller, *i.e.* younger, tumours contain fine clubs in isolated examples or in small groups, without any filamentous or granular centre; in the preparations stained successfully as above I find appearances which place me in full agreement with Crookshank (*Transactions of the Med. Chir. Soc.*, 1889): single clubs very conspicuous by their deep red staining attached to a short single or branched stalk free or enclosed within a nucleated cell. Further, there is, free or enclosed within a larger mass of protoplasm, a small homogeneous mass from which are budding out two, three, or four clubs of different lengths and with very short stalks, these structures being stained bright pink stand out very conspicuously from the blue ground. Further, I find spherical or oval globules recognisable by their deep pink staining becoming constricted off from the free end of the clubs. Putting these features together there can be no difficulty in recognising a striking likeness between the ray fungus and a mycelial fungus: the fine branched threads being the mycelium, the clubs being the growing ends of the hyphæ, such as are common to most hyphomycetes; these clubs, with their power of sprouting and giving off conidia (the above spherical or oval globules), would render this view easily intelligible. Further, the central part is the only part which in any way can be said to represent the part which is actually degenerating, since it often contains lime deposits. This view of considering the clubs as the

sprouting parts and conidia-bearing ends, the threads as analogous to the mycelium of a mould-like fungus (Bollinger, Israel, and others) is the view which stands better in harmony, I think, with the actual facts than the view that the ray fungus belongs to a species of cladothrix (Boström, Paltauf, Afanassiew). Israel has shown that the ray fungus can be artificially cultivated, but Boström was the first to have succeeded in artificially producing good cultures of this fungus. On blood-serum, on Agar at 33–37° C., the fungus forms whitish granules, which rapidly enlarge; they show a yellow or reddish, round, knobbed centre, from which start fluffy nebulous branched masses. After five to six days the growth has reached its height. The presence under the microscope of the mycelial branched threads and of the clubs was established in these cultures. Paltauf and Afanassiew have confirmed these observations.

O. Bujwid has cultivated the actinomyces fungus anaerobically (*Centralbl. f. Bakt. u. Parasit.*, vol. vi. p. 630) and succeeded in obtaining an actively growing mycelium and clubs.

Actinomyces "granules" planted in the depth of glycerin-bouillon at 37° C. grow well, the yellowish granules increase in size and number and from their margin a fine mycelium is seen to project. On the slanting surface of grape-sugar gelatine actinomyces (taken from a previous culture) grows well at 20° C., the tube having been sealed up after inoculation; it forms at first minute whitish-yellow dots which gradually—in the course of a few weeks—enlarge to yellow or yellow pink, dry, firm, tough patches and warts which by enlarging coalesce so as to form a coherent, uneven, knobby membranous expansion, the gelatine gradually liquefies and the growth sinks in, and after liquefaction has extended to the deep layers the growth falls to the bottom of

the culture tube; the liquefied gelatine is limpid, thick like syrup and of a brownish colour. When a part of the growth is examined under the microscope, having been previously stained, then well separated and teased out with needles, it is seen to be composed of a delicate mycelium

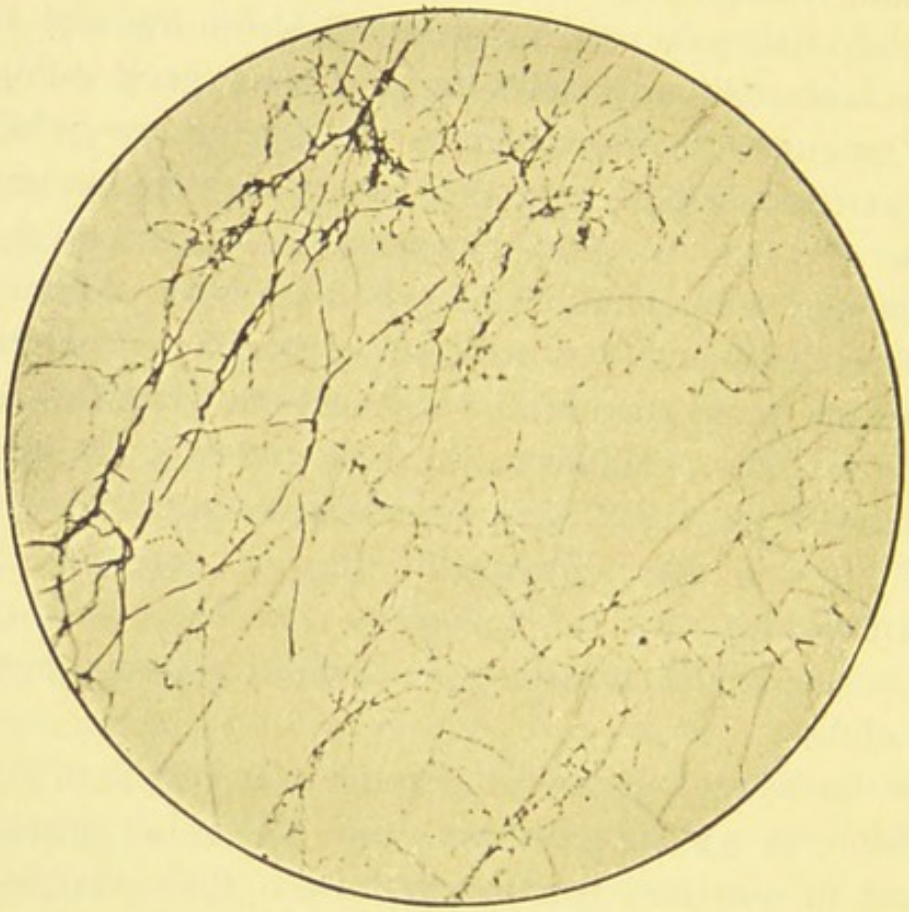


FIG. 199.—From a teased-out specimen of the actinomyces growth on sugar gelatine.
× 1000.

of fine threads, some uniform, others containing within the sheath granules, rods, and cylinders (*see* Fig. 199).

An important fact established by Israel, Boström, Rotter, and others is this, that the ray fungus of man can by inoculation produce typical actinomycosis in cattle, and there is therefore the greatest probability that the inverse also holds good.

The chronic necrotic disease occurring in India, and known as the Madura disease (*Mycetoma*), or the fungus disease, has been investigated by Kanthack and found to be caused by a fungus resembling in many respects actinomyces. This was found to be the case in the yellow or pale variety, as also in the black or melanoid kind (Pathological Society of London, January 19, 1892).

Boyce and Surveyor, however, find different parasites in the two forms of the disease (*i.e.* in the white and in the black variety). After carefully describing the naked-eye and low-power appearances of the "roe-like particles" in the white and black variety, they find on microscopic examination that the fungus neither in the white nor black variety is comparable to actinomyces, but to a mycelial hyphomycetes which is different in the two varieties of mycetoma. (*Phil. Transactions*, 1895, B, part i. p. 1 and *passim.*)

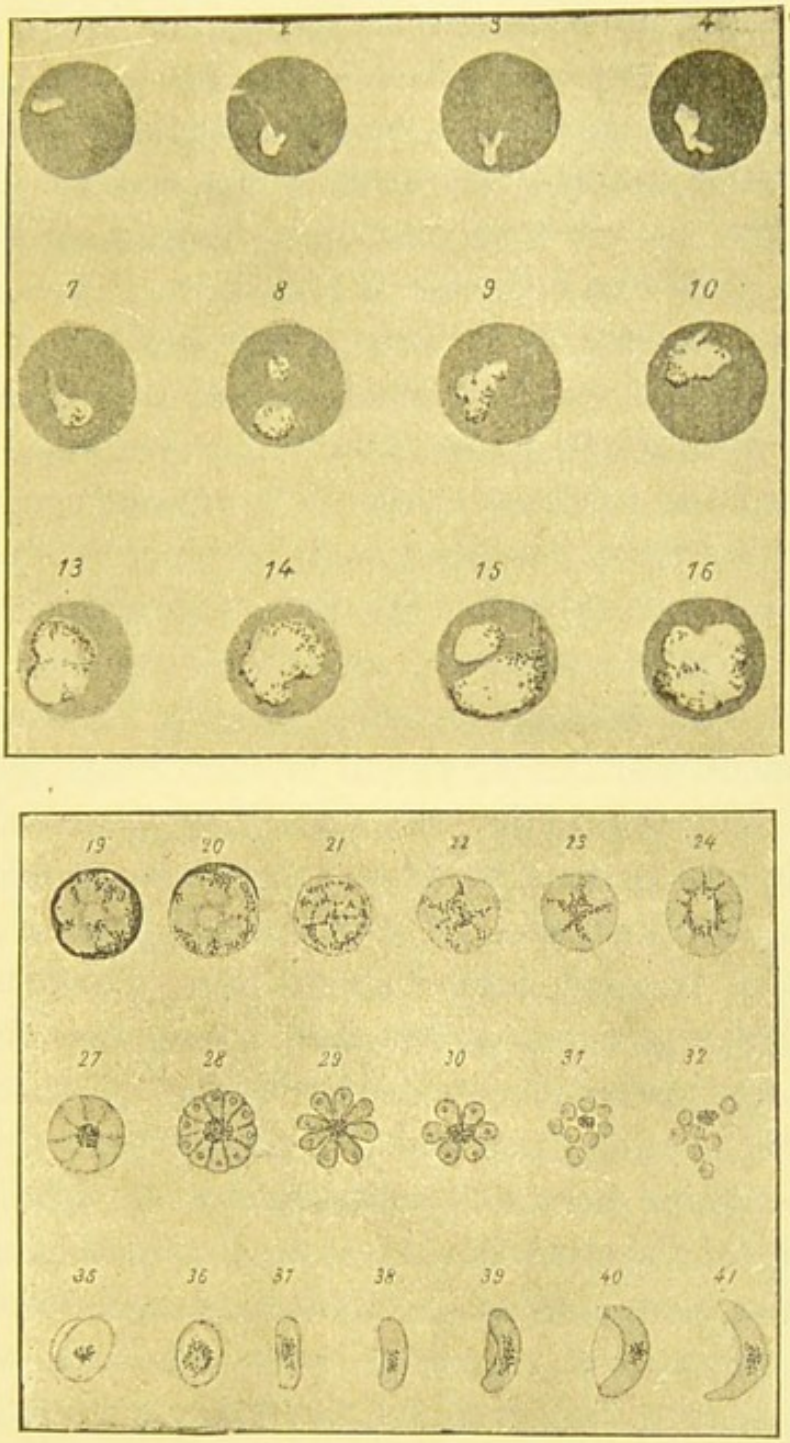
CHAPTER XIX

PROTOZOA CAUSING DISEASE

1. *Plasmodium Malariæ.*—Laveran (*Comptes Rendus*, 1882, No. 17) and Richard (*ibidem*, No. 8) were the first who discovered in the blood of malaria cases in the febrile stages peculiar bodies, spherical or crescentic, consisting of a pale, homogeneous substance and enclosing clumps of pigment granules; these bodies are possessed of cilia by which they are enabled to perform rapid movement. Laveran considered these bodies as the true cause of malaria and identified them as protozoa. This discovery was a few years later (1885) confirmed and considerably amplified by Marchiafava and Celli. The credit of the important discovery of the malaria parasite belongs therefore unquestionably to Laveran, and the observations of Marchiafava and Celli[1] have amplified by a good deal our knowledge of them, they called the parasite *Hæmoplasmodium Malariæ* or *Plasmodium Malariæ.*

Marchiafava and Celli showed that during the beginning of the febrile stage the parasite invades the red blood-corpuscles as small, globular, pale, homogeneous corpuscles

[1] Untersuchungen über die Malaria-Infection, *Fortschritte d. Med.*, 1885, p. 339 and 787.



FIGS 200 and 201.

Reduced from Golgi's Plate III. in the *Fortschritte der Med.*, vol. iv.

1—16 show the plasmodium malariæ within the red blood discs, gradually enlarging at the expense of the substance of the blood disc; pigment granules in 13—16 derived from hæmatin.

19—32 show the successive changes of the plasmodium towards final segmentation in sporules.

35—41.—Laveran's corpuscles from atypical cases of malaria.

measuring not more than a fifth to a seventh of the diameter of a red blood-corpuscle; in this host the parasite performs active amœboid movement, hereby changing continually its shape; but it gradually increases in size and consumes the substance of the red blood-corpuscle, leaving black pigment granules—iron-free melanin—in the disc. These pigment granules, as the parasite grows to the size of the original red blood disc, are now contained within the body of the parasite, in which they appear uniformly distributed. When the disc of the red blood-corpuscle is entirely consumed by the growth of the parasite this latter appears free in the blood plasma, its substance filled with the melanin granules; some of these free parasites have cilia by which they move actively—these are the corpuscles seen by Laveran. Next, the pigment granules aggregate in the central part of the parasite and the peripheral, pale, homogeneous portion gradually undergoes a more or less regular mode of segmentation, in the course of which small globular particles or sporules become constricted off from the main body; when this segmentation has been completed the young gemmæ or sporules all disappear from the blood, so also the pigmented central parts, and are stored up in the spleen, liver, and bone marrow; this terminates one febrile attack. The next febrile attack is caused by the sporules again invading the blood-corpuscles of the general circulation, and herein undergoing the same series of changes as just described. So that each febrile stage comprises the invasion of the blood-corpuscles by the sporules, the germination, amœboid movement and growth of these latter within and at the expense of the former, then the gemmation and segmentation into a new crop of sporules, and finally the disappearance of these from the general circulation. Golgi[1]

[1] *Fortschritte d. Medizin*, 1889, No. 3.

by his numerous researches was able to show that the various forms of malarial fever are due to various species of the parasite, at any rate that in the different forms of intermittent fever the time in which the parasite passes through all the above-mentioned phases of its development is different, and stands in a definite relation to the form of the fever. Thus Golgi found that in the febris quartana the parasite from its first appearance in the red blood-corpuscle, that is, from the onset of a febrile attack, through the complete segmentation of the full-grown parasite into the sporules, and to the disappearance of these from the general circulation, *i.e.*, till the end of the febrile stage, requires three days, whereas in the febris tertiana it requires only two days. Besides, there are certain slight morphological differences between the parasite in the febris quartana and in that of the tertiana, as also differences in the mode of segmentation (*see* Figs. 200 and 201). As to the parasite in the fever of irregular type, Golgi shows that also in this the time occupied for passing through its phases is irregular, either too rapid or too slow. The crescentic form of the parasites mentioned by Laveran and Marchiafava and Celli are present only in fever of irregular type, and are really an atypical form in the development of the parasite. So also the flagellate forms seen by Laveran are atypical forms.

Whether in these different forms we have really to deal with different species of the same group of parasites, as Golgi inclines to think, or rather with differences in the life-history of the same species caused by unknown conditions, *e.g.*, individual person, different tissue, season, locality, &c., is not decided.

Canalis (*Studi della Infezione malarian*, Torino, 1889) studied the atypical forms of malarial fever, characterised by longer or shorter febrile intervals. He found in these cases an endoglobular form of the plasmodium malariæ, which has

been signalised already by Golgi, viz., a crescentic form; but also here the commencement of the attack is characterised by the amœboid endoglobular forms, and the life cycle of the parasite becomes completed by the division of it into sporules.

Danilewsky, Grassi and Feletti, Kruse, Pfeiffer, Celli and Sanfelice, and others describe the occurrence of similar parasites in the red blood-corpuscles of a number of different animals, frogs and birds (see *Fortschritte d. Med.*, Band ix., Nos. 12 and 13, 1891).

2. *Amœba Coli* of dysentery.—Lösch (*Virchow's Archiv f. pathol. Anatomie*, 1875, Band lxv., p. 196) was the first who discovered the amœba coli in great numbers in a case of ulcerated large intestine in the human subject. This case, in all its clinical and pathological symptoms, resembled true dysentery.

Kartulis (*Centralbl. für Bact. und Parasit.*, vii., 2) has shown that in the cases of tropical dysentery which he examined there were present in the characteristic sanguineous stools numerous *amœbæ* (amœba coli of Lösch) showing active amœboid movement, and he gives good reason for considering these the cause of the dysentery, though others who had met with similar amœbæ in intestinal diseases in Russia (Massiatin, *Centralbl. für Bact. und Parasit.*, vi., Nos. 16 and 17) did not think so.

Further, Kartulis has shown that in twenty cases of abscess of the liver complicating dysentery he found in every one of them the same dysentery amobæ; they could be seen in sections through the wall of the abscess, but in the pus of the abscess cavity he did not find them.

A considerable amount of literature exists at present on the occurrence of amœbæ in certain forms of dysentery, chiefly those that run a chronic course, and on their absence and the presence of various species of bacilli in other

forms of acute dysenteric inflammation of the large intestine. While some have confirmed Kartulis (Osler, Councilman, Maggiora, and others) others have missed the amœba, but describe various species of bacteria as connected with the disease; from the careful bibliography collected by Maggiora (*Centralblatt f. Bact. und Parasitenkunde*, xi., Nos. 6 and 7) there can be little doubt that what is clinically spoken of as dysentery is not one single disease in etiological respects, since some dysenteric affections are, others are not, caused by the amœba coli.

3. *Flagellate protozoa.*—Many species of flagellate infusoria are known to inhabit the body of invertebrate and vertebrate animals; of these the group known as Monadinæ are in so far of interest as some of them have been found in vertebrates in connection with disease. The genus *Trichomonas*[1] has been found by L. Pfeiffer in the oral and pharyngeal mucus of pigeons affected with the chronic necrotic thickening of the mucous membrane, called also "diphtheria,"[2] and which this observer considers to be connected with the cause of the disease. But Löffler (*see* the chapter on Diphtheria) has shown that the disease in the pigeon is due to a specific bacillus, and in this he is fully confirmed by Babes.[3] Pfeiffer in the monograph just quoted still maintains his original assertion, that the disease is due to trichomonas invading and ultimately destroying the epithelial cells. Pfeiffer, however, differs, as regards the life-history of this protozoon, from all other observers and writers on protozoa (Leuckart, Bütschli, Dallinger and Drysdale), inasmuch as he describes the formation of spores within the substance of the trichomonas.[4] It ought also to be mentioned

[1] Leuckart, *Die Parasiten des Menschen*, 2te Auflage, p. 311.
[2] L. Pfeiffer, *Die Protozoen als Krankheitserreger*, 1890 (Jena), p. 85.
[3] *Zeitschr. f. Hygiene*, Band x. [4] *Loc. cit.* p. 85, fig. 26.

that the seemingly identical trichomonas is frequently found in the pharyngeal mucus of perfectly normal pigeons. I have had the opportunity of examining a case of this so-called diphtheria in the pigeon, and found the presence of the parasite in the pharyngeal mucus, but on comparing with it a perfectly healthy pigeon the same trichomonas was found abundantly also here in the pharyngeal mucus. Davaine[1] mentions the genus Circomonas, much smaller than Trichomonas, being minute club-shaped, ciliated protozoa, possessed of no envelope, having a pointed prolongation at one end and a long, fine flagellum at the other (*Circomonas intestinalis hominis*), as occurring in the stools of cases of acute Asiatic cholera, and once he also found them in the stools of a patient in typhoid fever. Lambl already in 1859 described them as occurring in the stools of children in diarrhœa, and Lösch found them also in the stools in cases of dysentery. The writer has had the opportunity of finding in a mouse, spontaneously dead, the peritoneal cavity and almost the whole of the intestine distended by, and filled with, a grumous milky fluid, in which, besides leucocytes and micrococci, there were present trichomonas and innumerable circomonas; in fact, the main part of the corpuscular elements was made up of circomonas, many of them very rapidly moving

A certain species of flagellate monadinæ was first described by T. Lewis in 1877 as occurring in the blood of normal horses, dogs, and rats; by Evans in 1880 as occurring in the blood of horses in Madrid; by Wittich and R. Koch in 1881 as occurring in the blood of normal badgers. These protozoa are known as the *Herpetomonas Lewisii*: the body is cylindrical, often spiral; the flagellum extends as a delicate membrane all along the body of the creature; anteriorly the

[1] *Traité des Entozoaires*, p. xxiii.

body terminates as a pointed rigid process. A hæmatozoon which, according to Lewis and Crookshank,[1] is identical with that occurring in the healthy rat has been discovered by Evans, but first assumed to be a spirillum and considered by this observer as the cause of the *surra disease*, a deadly malady affecting in India, in an epidemic form, horses, mules, and camels. Crookshank[2] gave good photographs of them; he believes them to belong to the genus Trichomonas. Lingard in his exhaustive reports to the Government of India on surra disease had besides making a thorough investigation into the clinical and pathological aspects of the disease described the nature of the contagion, the life-history of the parasite in its relation to the various phases of the disease.

4. *Psorospermia or Coccidia.*—(*a*) *Coccidium oviforme.* The class of protozoa known as sporozoa—unicellular parasites of fixed form of body, surrounded by a capsule, forming within their body a number of spores, each surrounded by a cuticle, the spores becoming free after the bursting of the capsule, and giving rise to a new parasite—comprise a group which is important to the pathologist, oval psorospermia or coccidia. These are capsulated, uninuclear, oval, protoplasmic corpuscles, in the interior of which out of the protoplasm a number of spores are developed; many of these coccidia are endo-epithelial parasites, and as such are the causes of a chronic hypertrophy of the epithelium. The coccidium best studied is the Coccidium oviforme, causing in the liver of the rabbit a chronic disease of the epithelium of the bile ducts, by which the bile ducts become greatly distended, their epithelium much hypertrophied and their coats thickened; in consequence of this whitish-grey nodules appear in the

[1] *Journal of the Roy. Micr. Society*, Nov. 10, 1886.
[2] *Ibidem, loc. cit.*

liver composed entirely of the hypertrophied folded and fringed wall of the bile ducts.

Coccidium oviforme occurs also in the epithelial cells lining the mucous membrane of the intestine in the rabbit. The coccidia are oval corpuscles about 33–37 μ in length, 15–20 μ broad; each possesses a distinct capsule or cuticle, which at one (thinner) pole contains a minute opening or micropyle. The body of the parasite is a granular protoplasm, in its fully formed state completely filling the space within the capsule, and containing an oval, clear nucleus. In this condition they are numerously found amongst the epithelium, and also free in the cavity of the intestine and the hypertrophied bile ducts respectively; but the majority of forms seen in the epithelium are almost spherical, less oval than the above, and possess a thinner capsule; in some the capsule is hardly recognisable. they contain a more coarsely granular protoplasm, and within it a clear, spherical nucleus. These are found in great numbers in the epithelium, replacing at points almost completely the epithelial cells, some being distinctly situated within the body of the epithelial cells. The same is the case in the epithelium of the enlarged bile ducts in the liver nodules.

It is not at all easy to decide what is the exact relationship between these smaller, granular, indistinctly capsulated, spherical bodies and the large, granular, oval, distinctly capsulated coccidia. According to Leuckart, the former would represent young coccidia just germinated from the spores; but this can hardly be correct, considering that in all nodules, particularly the large ones, the small spherical, indistinctly capsulated coccidia abound, and, further, considering that spores are not formed in the coccidia within the animal body. It is therefore more probable that the small spherical coccidia are derived by division from the

large oval forms, and in their turn, on their ripening, grow into these latter.

It can be shown that the coccidia first appear in the epithelium of the intestine, and from here they find their way into the epithelium of the hepatic duct and gradually into the bile ducts within the liver. Here their multiplication produces saccular, tubular, and cystic enlargements of the interlobular bile ducts, the wall of which becomes thickened by connective tissue, and folded in many ways. In this manner numerous whitish irregularly shaped firm nodules and cysts are formed in the liver, which, when cut into, show a cavity with the thick white wall folded inwards.

The columnar epithelial cells lining the hypertrophied bile ducts, which harbour the coccidia, are, in fact, the soil at the expense of which the coccidia grow and ripen; these latter in their turn, and for their own purpose, cause a continuous multiplication of the epithelial cells.

Leuckart in his work, *Die Parasiten des Menschen*, 2te Aufl. i., gives an exhaustive account of the life-history of the coccidium oviforme, the mode of passing into new animals, and the changes and distribution of it. In the human subject nodules of the liver have been observed which were caused by coccidia, probably coccidium oviforme. Gubler, Leuckart, Dressler, and Perls (*see* Leuckart, *loc. cit.* p. 281) have observed such cases. Besides the rabbit coccidium oviforme has been found in the intestines of the dog, cat, sheep, guinea-pig, and pheasant.[1]

[1] *Miescher's coccidia tubes* occur in the muscles of the mouse occasionally; they are noticed as fine white lines which under the microscope are tapering cylindrical granular masses, the latter in reality densely packed crescentic or kidney-shaped pale corpuscles, about 0·01 mm. long and considered to be spores. (Leuckart, *loc. cit.*)

(*b*) Another disease in which the presence of psorosperms,[1] or at any rate of parasites morphologically related to them, causes a chronic thickening and hypertrophy of epithelial structures has been described by L. Pfeiffer (*Die Protozoen als Krankheitserreger*, Jena, 1890) as occurring in the fowl. This animal is occasionally found to show on its skin in various parts of the body large and small prominent nodules which consist of greatly hypertrophied epidermis (stratum Malpighii) with corresponding infiltration of leucocytes and distension of the blood-vessels of the subjacent corium. The disease in question—*epithelioma contagiosum* of the fowl—is not at all of rare occurrence, and when it occurs on a farm it generally spreads to other fowls. I have myself met with several such instances. Sections made through the nodules show an enormous local hypertrophy of the stratum Malpighii of the epidermis; and amongst the epithelial cells, in many places within these cells, are found oval bodies, mostly capsulated, which are distinctly of the nature of psorosperms. On staining the sections in fuchsin, and then washing in alcohol or dilute nitric acid, the epithelium is decolourised and leaves the psorosperms stained pink; in this manner the psorosperms, with their pink protoplasm, their capsule and clear nucleus, can be easily recognised. It is not a question of finding such a psorospermic corpuscle here and there; the epithelium is pervaded by them in almost continuous masses. They are found isolated within the epithelial cells, destroying the substance of these latter; or, when the epithelial cells are almost destroyed over extensive areas, the psorosperms are found to have entirely replaced them. There is not the

[1] The following account is an abstract of my Report on "Psorosperms in their Relation to the Etiology of Cancer" in the *Reports of the Medical Officer of the Local Government Board for* 1893–1894, pp. 479, &c.

slightest difficulty in recognising these psorosperms and in differentiating them from the epithelial cells. This disease in the fowl is, like the psorospermosis of the liver in the rabbit, essentially a chronic hypertrophy of the epithelium caused by the process of growth and multiplication of the psorosperm parasite.

(*c.*) *Cancer parasites.*—There are recognised in the human subject a number of chronic diseases which consist essentially in a chronic hypertrophy, with ultimate destruction, of the epithelium of the skin, and of various mucous membranes. The principal diseases known as such are: Darrier's disease, molluscum contagiosum, Paget's disease of the nipple of the breast, and last, but not least, various forms of epithelioma and cancer of the skin, mucous membranes, &c. The number of observers who have searched for and found in these various chronic epithelial disorders parasite-like bodies comparable to psorosperms is legion; but it is equalled, if not surpassed, by the number of other observers, who, though they have searched for these alleged psorosperms in these diseases with equal care and perseverance, have utterly failed to identify them.[1] While, however, it is one of the simplest and easiest things to demonstrate, by a microscopic examination of the above-mentioned nodular disease of the rabbit, not only the existence of the psorosperms but their relation also to the hypertrophy of the epithelium, it is quite another affair to obtain anything like clear evidence of similar conditions in the above-named human diseases. In the first place, amongst the number of observers who affirm that they have discovered psorosperms in epithelioma of the human subject there are scarcely two who describe the same parasite;

[1] An excellent summary of all these researches is given by Strœbe in the *Centralblatt für Allg. Pathol.*, &c., 1894, Nos. 1, 2, and 3.

while not one amongst them is able to do more than draw attention to certain morphological appearances in the epithelium which are put forward as denoting the presence of something extraneous to the typical epithelial cells. No one has in regard of cancer ever succeeded in isolating the parasite, as is easily enough done in the psorospermosis of the rabbit; and, notwithstanding all assertions to the contrary, the evidence amounts only, as I have said, to the description of certain bodies which, after certain methods of staining, can be demonstrated within or between the epithelial cells in the above human affections. Amongst these bodies, however, none are typical psorosperms. Under these circumstances the critics of those who affirm a parasitic cause of cancer have an easy task; they can with perfect justification demand, where—if such-and-such indefinite and mysterious bodies do occur in cancerous epithelium—is the proof that they are of a parasitic nature. To this the others can only reply that they are most probably parasites, because they are not typical epithelial cells. But how do you know that they are not part of the cell or nucleus? again ask their critics. Because they stain differently from epithelial cell substances or epithelial nucleus, answer the upholders of parasitic cause. But surely, retort their critics, you have no right to call those bodies psorosperms merely because you are unable to think of them as derivatives of the substance of the epithelial cells or their nuclei. And so on, and so on.

I have I think thus given sufficient account of the general nature of a great part of the controversy; at least it is not necessary for the present to go further into details, as will duly appear later on.

Before coming to the discussion of the alleged parasites of cancer and of other chronic epithelial diseases in man that I have referred to, it is necessary to have a clear view

of the elements of the problem that has to be dealt with; indeed, to every one who has devoted any considerable amount of attention to pathological histology in general, and to the changes of the epithelium in health and disease in particular, it will be clear that insistence on this necessity is by no means uncalled for. By going carefully over the descriptions and illustrations of the "cancer parasites" put forward by different observers, it is quite obvious that a considerable number of them have thought it sufficient, having made a few sections of cancer material, and having stained and mounted them, to at once declare without hesitation—and regardless of the histology, normal and pathological, of epithelium—that such-and-such a particle or corpuscle present in the substance of the epithelial cell or in its nucleus is a parasite. As to this, I illustrate my meaning as follows :—

Podwyssozki and Sawtschenko published in the *Centralblatt f. Bakt. und Parasit.* vol. xi., Nos. 16, 17, and 18, a paper on cancer psorosperms, and they add two coloured plates (Plates VII. and VIII.) illustrating the presence of the alleged sporozoa within and between the epithelial cells. Now, any one who, after staining them, has examined sections of well-preserved epithelial structures, growing and proliferating under normal and under pathological conditions, will recognise in the figures given by these authors appearances very commonly met with : viz., bodies, variously shaped and variously sized, contained within the cells; bodies, indeed, which take the stain differently from the typical nucleus of the epithelial cell. These bodies are commonly and justly considered to be derivatives of the nuclear substance, particularly of that portion commonly called chromatin. Appearances such as are shown by the authors in their Figures 1-7, 11-15, 16-20,

&c., are to be met with within the epithelial cells in sections through normal glands, as also in sections of the skin and of oral mucous membrane, in normal and (better still) in pathological states. The epithelium of cancer is not required for demonstration of these bodies, though in cancer—owing no doubt to extensive multiplication of the epithelial cells—they are met with sometimes, but by no means always, as copiously as these authors would imply. Why, therefore, they should consider these bodies to be coccidia, is not easy to understand.

Another and perhaps more striking illustration of the same tendency is afforded by Soudakewitsch in *Centralblatt f. Bakt. und Parasit.* vol. xiii. page 415, Plate 1. He describes there, as parasites, intracellular nucleus-like bodies, which most histologists, with experience of normal and pathological epithelium, would have no difficulty in identifying as chromatic and other derivatives of epithelial nuclei.

To quote one more instance: In vol. i., p. 198, of the *Journal of Pathology and Bacteriology*, Drs. Ruffer and Walker describe and figure epithelial cells, in which the main part of the cell substance is occupied by a vacuole, while within this vacuole lie three round clear cells, each with several nuclei. Most histologists recognise these at once as vacuolated epithelial cells containing common leucocytes, such indeed as are commonly found in epithelium under pathological conditions, and normally in certain localities, *e.g.*, fauces, tonsils, and tongue. Such vacuolated epithelial cells containing leucocytes have been familiar to histologists for many years; they were the very cells about which, seventeen or eighteen years ago, a considerable amount of discussion arose. The question then under debate was: whether they are endogenously developed

within the inflamed epithelium (Stricker, Rindfleisch, and others); or whether, as Cohnheim and others maintained, and as is now universally believed, they are immigrants into the epithelium and into the epithelial cells themselves. As a matter of fact, they are found easily in the epithelium in gonorrhœa and in the conjunctiva in catarrhal (blennorrhœal) inflammation.

Messrs. Ruffer and Walker meeting, in cancer, with vacuolated epithelial cells enclosing several leucocytes, seem to assume that there has originally been a parasite within each such epithelial cell, which has been eaten up by the leucocytes. And in this way it could be shown that, in a considerable number of instances, belief has arisen in the existence of coccidia, psorosperms, and other parasitic forms in cancer epithelial cells. Those enunciating such belief would seem to be wanting in a clear understanding of the elements of the problem with which they are concerned; for in dealing with purely morphological questions, such as the presence or absence of certain bodies in the hypertrophied epithelium constituting cancer and similar chronic epithelial diseases, it is obvious that a critical apprehension of what appertains to cancerous epithelial new growths *and to no other epithelium in health or disease* is necessary as an elementary condition for proper study of the subject. And let me here at once say, in making this statement, that I am in no way disinclined to regard molluscum contagiosum, Paget's disease, and cancer as belonging to the group of infectious diseases that are of parasitic origin; all that I wish to insist on is that many, if not most, of the assertions as to coccidia, psorosperms, and similar parasites in cancer and in allied diseases are not founded on admissible evidence. See Mr. D'Arcy Power's demonstration[1] of some

[1] *Journal of Pathology and Bact.* viii. No. 1, p. 124.

of the so-called "cancer parasites" in epithelium experimentally inflamed, but not of the nature of cancer.

From the various considerations I have adduced, it must be evident that a number of assertions as to the parasitic nature, in cancer, of various bodies in the substance of the epithelial cells, of nuclear-like bodies between the epithelial cells, of encysted nucleated bodies, of hyaline nucleated cells, of multi-nucleated cells within epithelial cells, and of stained particles within the epithelial nuclei, take no account of the presence of similar bodies and of similar change in epithelium that is not cancerous; and until such comparative study of the epithelium in health and in disease has been undertaken statements to the above effect cannot claim that value which their authors attribute to them.

But I have not completely exhausted the list of difficulties which beset a theory of parasitic cause for cancer. Not least among those that remain is the circumstance that few observers describe the same kind of parasite. One might almost say that there seem to be as many kinds of cancer parasites as there are writers thereon; and, further, that the morphological characters of most of these alleged parasites do not conform with the characters of any of the known psorosperm species.

An exception is however to be made in the case of L. Pfeiffer. His numerous researches[1] testify not only to his thorough and extensive knowledge of the subject of undoubted parasitism of sporidia in the animal kingdom, but also in a very high degree to his systematic investigations of the characters and occurrence of these and similar bodies. Whatever, therefore, he has to say on this subject should

[1] *Die Protozoen als Krankheitserreger*, Jena, 1890; *Untersuchungen über den Krebs*, Jena, 1893; and "Der Parasitismus des Epithelcarcinoms," *Centralblatt f. Bakt. und Parasitenk.*, vol. xiv., page 118.

command universal and respectful attention. We may differ from him, and may hold that his generalisations, notably with reference to the same kind of parasitism in variola, vaccinia, varicella, herpes, variola ovina, and in other vesicular diseases, are by no means satisfactorily established by the evidence he adduces; nevertheless he makes a genuine scientific and systematised attempt at throwing light on what is shrouded in darkness, and what is considerably complicated by the many less extensive investigations and somewhat hasty assertions of a great number of young pathologists.

Omitting Pfeiffer's studies of the nature, character, and distribution of coccidia, klossia, and eimeria, and of the sporidia (myxo-, sarco-, and micro-sporidia), let us turn to his observations on amœbo-sporidia in cancer in the human subject. According to him the parasite of cancer is found either within the epithelial cells in the form of an intracellular encysted spore, or around the epithelial cells as a free and growing cell-like germ which, when enlarging, resembles a nucleated protoplasmic cell; such cells as are always found numerously infiltrating the connective tissue around the true cancer epithelial masses. That is to say Pfeiffer considers the various intracellular bodies as spores, and the leucocytes infiltrating the connective tissue as the growing cancer parasites. Views of this kind, which, on the one hand, cannot be accepted as based on anything like evidence, but rather as a sort of *ipse dixit*, cannot, on the other hand, be directly disposed of. In just the same way it is open to any one to affirm that the white blood cells found in inflammation, &c., are amœbo-sporidia, though other persons may prefer to call them inflammatory cells, exudation cells, or leucocytes. They all are, whatever we call them, living independent organisms which grow and

multiply. If it be contended that they, being normal constituents of the healthy body, cannot be the parasites of cancer, it might be answered that the amœbo-sporidia of Pfeiffer are not identical with them; that the normal white blood cells are one species, and that the amœbo-sporidia are a different pathogenic species. I am merely showing the kind of argument that Pfeiffer could bring forward if he chose to go beyond his *ipse dixit*. And nothing is gained by studying Pfeiffer's photograms (l. c., Plate 1, Figures 1—4), representing sections through epithelial cancer of the pectoral muscle and the lip of man, which are submitted by him as illustrating amœbo-sporidia. What is represented by him under a low magnifying power (× 60) may be anything; whereas the appearances shown (Figure 4) under a magnifying power of 600 are nothing else than a cluster of nuclei, which may be those of epithelial cells or of leucocytes.

Pfeiffer's researches, indeed, though systematic and exhaustive so far as they refer to the nature and distribution of coccidia and allied psorosperms, and to the various sporidia in the animal kingdom, are extremely fragmentary with reference to cancer in man; there is practically no satisfactory evidence of the presence of what Pfeiffer calls spore forms in the cancer epithelial cells, or of the presence of amœba forms around them.

One of the most striking facts in the large mass of the literature on the subject of coccidia and psorosperms in cancer is the absence of any well-authenticated sickle-like bodies; that is to say, of those characteristic bodies which, according to the unanimous testimony of all those who have investigated the life-history of the various parasitic and non-parasitic coccidia and psorosperms (Leukart, Bütschli, Eimer, L. Pfeiffer, and others), constitute one of the most

typical phases in the life cycle of a coccidium—the phase, namely, of commencing germination of the spores. True, there have been a few observers (Soudakewitsch, for instance), who assert the occurrence in cancer of sickle-like bodies, but most other observers deny their relation to cancer, and regard them as altered nuclei. There is no difficulty, whatever, in finding in cancer, as also in other chronically and acutely changed epithelium, nuclei of the epithelial cells which resemble, or rather possess the shape of, crescentic or sickle-like bodies; nuclei, that is, which are so changed that they appear swollen or hydropic, with their chromatin collected at one side in the form of a crescentic body. So that the only typical phase, *i.e.*, sickle-like germs, of a coccidium or psorosperm, the constancy of which would represent a certain morphological evidence for the presence of coccidia or of psorosperms in cancer, is absent. As a consequence, therefore, the acceptance of other phases of the alleged coccidia in cancer constantly met with in the writings of parasitologists is beset with very grave difficulties.

Briefly reviewed the following are the conditions which have been described as indicating psorosperms or zoospores in cancer:—

1. There is, in the first place, the occurrence of encysted nucleated protoplasmic bodies among the epithelial cells of cancer, which resemble, to a limited extent, similar bodies in, for instance, the coccidia in the rabbit's liver. With reference to these encysted cells it has to be said that such forms do undoubtedly occur in cancer. I have examined a considerable number of sections through cancer—of the lip and tongue, of the penis, of the liver, of the omentum, of the breast, of the bladder, and of the œsophagus—and have met with such encysted cells. But I have met the

same bodies in stratified epithelium of a variety of tissues in normal, and particularly in pathological, conditions which have nothing to do with cancer. The same is shown by D'Arcy Power. Hence I am justified in denying their parasitic nature, not only because they occur in normal stratified epithelium, but also, and chiefly, from the fact that in every respect they resemble epithelial cells wherein the main body of the protoplasm has shrunk around the nucleus, leaving a peripheral portion surrounding it like a capsule. Whether this apparent encysting of nucleated epithelial cells is connected with and dependent on the structural differences of the marginal and central portions of the cell protoplasm, as is suggested by Heidenhain's researches, I am unable to say; but as evidence at least tending in this direction, I may state that, however partial or extensive coagulation-necrosis occurs in stratified epithelium, such apparent encysting of the main coagulated mass of the epithelial substance does occur. To this class of appearances belong epithelial cells in which the nucleus is shifted to one side and compressed, owing to the presence within the central part of the cell of an almost homogeneous spherical body; and in this remnants of granular matter may be often recognised. These clear intracellular globules are represented by some observers as the cancer parasites; but, apart from their remarkable dissimilarity to anything resembling a sporozoon, the possibility of their being indicative of and due to a hydropic or colloid change of the cell protoplasm must not be lost sight of. Moreover, the presence of fluid colloid fatty matter or other material within the cell protoplasm is not at all of rare occurrence in epithelial and other cells in various pathological states.

2. The presence of well-outlined, more or less clear, intracellular bodies showing a more or less distinct peri-

pheral radial striation with a central deeply stained granule or granules (Soudakewitsch, Ruffer). These strikingly resemble altered nuclei of the epithelial cells: that is, in a hydropic condition with accumulation in the centre of the main part of the chromatin, remnants of the mitoma still attaching the chromatin to the nuclear membrane. I do not miss these forms in cancer, in fact they are by many considered to be the most characteristic forms. But in some cancers that I have examined they are not numerous; and I do not see that the evidence as to their parasitic nature is at all satisfactory, the less since bodies closely resembling such forms are to be met with in epithelial structures under other conditions, as already stated. It is quite possible that in cancer the changes of the nuclei in epithelium are of a chemical nature different from those obtaining in other conditions, and that hence they are more easily met with in cancer than in other diseases; but this is no reason why such changes should be considered as indicating the presence of parasites. In passing, it may be mentioned that these bodies do not occur in the coccidia, say, of the rabbit's liver.

3. The presence of "spores" and "spore-like" bodies in the cell substance and in the nuclei of the epithelial cells in cancer, either isolated or in groups. To these, Sjöbring, Soudakewitsch, Ruffer, and Walker have devoted particular attention; and they undoubtedly represent good types of the so-called cancer parasites which can be easily studied. But, as I have stated, such "spores" cannot be distinguished from masses of cell protoplasm or of nuclear substance respectively, separated from the main cell substance, and owing to chemical change taking dyes often differently. Besides such spore-like bodies are met with in epithelium under conditions (sheep-pox, foot-and-mouth disease, chronic

inflammation of skin, &c.) that have nothing whatever to do with cancer. It must, however, be left open whether this interpretation is or is not a correct one; though amongst the figures given by the different observers as showing intracellular spores (Sjöbring, Ruffer, and Walker) there are some that cannot be distinguished from vacuoles in the protoplasms of epithelial cells.

4. As to the presence of rounded transparent cells with one, two, or more nuclei amongst, or even within, the epithelial cells, there is nothing to distinguish them from ordinary leucocytes. They are met with in cancer, and they are met with in the normal epithelium of the palate, tonsil, and back of the tongue. To assume with L. Pfeiffer that these are amœbo-sporidia seems quite gratuitous. The same applies to the occurrence of kerato-hyaline cells which are said to be a phase of the cancer coccidium.

5. Perhaps the most important bodies that have been adduced as cancer parasites are those described by Korotneff in *Centralblatt f. Bakt. und Parasitenk.*, vol. xiii., p. 373. Under the name of *rophalocephalus carcinomatosus*, Korotneff describes a pedunculated and band-like protoplasmic mass, consisting of a spheroidal or pear shaped nucleated "head," and, directly continued from it, a band-like longer or shorter protoplasmic mass. The whole is without a sheath and is situated partly within and partly without the epithelial cells of a cancer; and the band-like stalk is several times the diameter of the individual epithelial cells. In this form the "parasite" is considered as an adult one, whereas smaller uni-, duo-, or pluri-nucleated masses without stalks are considered young or growing forms—gregarina forms. This parasite was found not only in carcinoma labii but also in carcinoma mammæ, maxillæ, &c. Kurloff in *Centralblatt f. Bakt. und Parasit.*, vol. xv., p. 341, adduces confirmatory

evidence as to the existence of these forms: namely, adult band-like masses with a nucleated head, and also smaller spherical, oval, or pear-shaped masses (*i.e.*, growing forms) in a case of carcinoma of the skin of the hand.

These band-like masses with nucleated pear-shaped head are from their shape and their size so unlike anything else amongst the epithelium that it is quite out of the question to refer them to anything belonging to the epithelial or to other known cells, and the question therefore arises as to their nature and as to their relation to carcinoma. Both Korotneff and Kurloff show that by special staining they can be easily demonstrated; and in this I agree, so far as their presence in some carcinomata is concerned. I have seen identical bodies in sections of a carcinoma of the œsophagus, which were stained first in fuchsin or rubin and then in methyl-blue. They were conspicuous not only by their shape but also by their deep pink stain (fuchsin or rubin), which they take up and retain with great persistence. They appeared as partly intracellular, more generally intercellular, filamentous, or band-like deeply stained masses with a spheroidal or pear-shaped nucleated enlargement; and in this form they are easily recognised. Besides such forms there occur similar deep-pink spheroidal or oval nucleated intracellular masses; and it is difficult to decide whether all these or only some of them, notably the larger ones, are merely truncated enlargements of the former. The smaller of such forms are, most probably, young growing forms, as is maintained by Korotneff and Kurloff; but it must be obvious that the "rophalocephalus" may appear in the specimen in longitudinal, in oblique, or in transverse section, and as a matter of fact gradations between the long band-like adult (so called) forms and the spherical or oval nucleated bodies can be easily observed. I myself cannot

say whether the smaller uni-, bi-, or multi-nucleated intracellular corpuscles referred to by these authors are in reality what they appear to be, namely, young and growing forms, or whether they are merely the "heads" of the band-like forms seen in optical or in real transverse section. If the former view, viz., that of Korotneff and Kurloff, be the right one, some of these young amœba-like nucleated bodies (gregariniform) have been described by other observers, notably by Sawtschenko and Ruffer.

It may not be amiss to mention here that it seems to me that some, at any rate, of the "fuchsin bodies" first described by Russell, *British Medical Journal*, 1891, belong to this category; and it may also be mentioned that other of Russell's fuchsin bodies are red blood-corpuscles, for in some carcinomata red blood-corpuscles deeply stained with fuchsin can be found between the deep epithelial cells. That they are red blood-corpuscles can be recognised by their size and shape, and by the fact that the capillaries of the papillæ contain them. But some of the larger "fuchsin bodies" of Russell seem to me to be undoubtedly the above parasite-like bodies.

There can be then no doubt that there occur in cancer certain bodies which can be distinguished as separate and different from epithelial cells, from nuclei, or from leucocytes; and the question is, Of what nature are they? The two Russian authors consider some of them as rophalocephalus carcinomatosus in its adult stage, while the smaller nucleated bodies they consider as young growing gregarinous forms. It seems to me, however, that it is not necessary to accept this view of a new species, one which by the way does not coincide in its characters with any known gregarinæ. It appears more probable that the band-like pedunculated knobbed nucleated mass of protoplasm represents simply a

large amœba that has thrown out a long stalk, and that when the amœba, after the nature of amœbæ, divides it gives origin to smaller nucleated protoplasmic masses. If only the latter were present in the sections, it would, owing to their special staining and their intracellular position, not be difficult to mistake them for epithelial cells or leucocytes; but the presence of the large pedunculated knobbed masses is of the utmost importance as proving that we are dealing with something quite different from either epithelial cells or leucocytes.

I have searched for these pedunculated amœbæ in a large number of carcinomata; but, excepting a single case of carcinoma of the œsophagus, I have not come across them. As already stated, however, Korotneff has in this respect been more fortunate.

To sum up, then, we have to exclude from the evidence adduced by the various authors, as indicating cancer parasites, the following bodies:—

(*a*) Nucleated epithelial cells which have undergone a kerato-hyaline change. These are observable as spheroidal or oval corpuscles, generally situated away from the deepest epithelial cells; that is, from the cells immediately in contact with the connective tissue matrix. They are of about the size of ordinary epithelial cells, stain like keratin of the superficial cells, and possess a relatively small deeply stained shrunken nucleus, such as is found in many other examples of chemically or acutely inflamed epithelium.

(*b*) Spherical transparent cells with one, two, or more nuclei, which in aspect, size, nuclei, and in their mode of staining, cannot be distinguished from ordinary leucocytes. They are found between the epithelial cells, or have immigrated into the substance of the latter—as in the case of vacuolated epithelial cells enclosing leucocytes. This

appearance is found, not only in the epithelium of cancer but in many other normal or pathological conditions of epithelium. There is nothing to distinguish these small bodies from leucocytes.

(*c*) Small and large particles, vacuoles singly and in clusters, situated in a more or less distinct cavity in the cell substance. They occur singly or several together in an epithelial cell; but similar cell enclosures occur in many other epithelial structures besides those of cancer, and the fact that they occasionally stain differently from the main cell substance may merely indicate a chemical change which this part of the cell substance has undergone: it is not a proof of their being spores of parasites.

(*d*) Encysted nucleated epithelial cells. These are not uncommon in cancer epithelium, but they also occur in other epithelial structures. The envelope is not a real capsule, but owes its origin to a separation and shrinkage of the main part of the protoplasm around the nucleus, whereby a peripheral part remains detached and resembles a capsule. Further, in epithelial cells in which the central part has undergone a hyaline change (hydrops, colloid), the nucleus of the epithelial cells is pressed to the side.

(*e*) Nuclear well-defined bodies, containing one or even several small granules, with, in the periphery next to the surrounding membrane, a more or less distinct radial striation. These bodies are nuclei of epithelial cells, the epithelial cell substance having become destroyed and the nucleus become swollen and hydropic. The granules and striæ are remnants of chromatin. Such bodies occur not only amongst the epithelial cells of cancer, but also in other rapidly growing epithelium.

(*f*) Nuclear bodies situated within the epithelial cell next to the normal nucleus; the latter slightly swollen and

staining differently from the former. Such occur in many epithelial and other cells — as paranuclei — both in normal and in pathological conditions. These secondary "nuclei" are probably derivatives of the chemically changed chromatin substance of the original nucleus.

All that, therefore, remains and cannot be placed to the account of either epithelial cells or their nuclei, or of leucocytes, are the large pedunculated protoplasmic bodies with a nucleated knobbed enlargement, contained within epithelial cells, that were first seen and described by Korotneff, as rhophalocephalus carcinomatosus. These seem to me to be large amœbæ-like bodies, which, by reproduction, bring forth small nucleated protoplasmic amœbæ, generally also contained within epithelial cells. These small amœbic offsprings, just like the parent amœba, are conspicuous by their staining, and by their apparent direct connection with the pedunculated large amœbæ. Whether many of the nucleated cells enclosed within the epithelial cells of cancer, seen and described by other observers (Soudakewitsch, Ruffer, and others) as conspicuous by their staining, are or are not the young amœbæ in question, cannot be easily determined.

Lastly, it has to be mentioned that the above pedunculated amœbæ have been found by myself in one case only, that of cancer of the œsophagus; I could not find them in many other cancers. Korotneff, however, asserts that he has found them in a variety of cancers. It is quite possible that this condition, viz., that of the pedunculated form, may be more difficult to meet with, or may be more rare; the form of smaller, rapidly dividing amœbæ being more frequent. But at all events even these latter forms are in many cancerous epithelial growths only sparingly to be met with; in some I have missed them

altogether, while in others several sections had to be examined in order to find one or the other nucleated bodies resembling them. From this it would appear hazardous to assign to them a definite causative relation to the rapid growth and multiplication of the epithelial cells constituting carcinoma.

CHAPTER XX

ANTAGONISM AMONGST BACTERIA

THAT the chemical products of some species of microbes, while acting inimically on the further multiplication of this species, are not inimical to that of another species has been proved by various observations, but it has also been proved that an inimical action is undoubtedly exerted by the growth of particular species on that of others. It is well known that a number of species of bacteria can exist and thrive under conditions under which other bacteria cannot so exist; take, for instance, the water bacteria, *i.e.*, the bacteria inhabiting common drinking water; these are capable of living and of multiplying on the very small amount of nutritive material present in ordinary drinking water, nay, micrococcus aquatilis and bacillus erythrosporus (Flügge) and others, as mentioned above, multiply even in distilled water (Meade Bolton, Niessen, Percy Frankland); whereas numerous species of bacteria non-habitually in water cannot do so under the same conditions; therefore the water bacteria will persist and even multiply, whereas others added to the water, or accidentally finding entrance into the water, will perish, some sooner, some later. Numerous observations have been put on record by Meade Bolton, Wolffhügel and Riedel, and others to show in what way and to what

extent various bacteria—the bacillus anthracis, cholera spirilla, the typhoid bacillus, micrococcus tetragenus, and staphylococcus aureus gradually die off when kept in ordinary drinking water, *i.e.*, water very poor in nutritive materials. (The results of Meade Bolton are published in the *Zeitschrift für Hygiene*, i. 1, p. 76; those of Wolffhügel and Riedel in the *Mittheil. aus dem k. Gesundheitsamte*, Berlin, i. p. 455. See also G. and P. Frankland's *Handbook on Water Examination*.)

It need hardly be said that if even small amounts of nutritive material be added to water these bacteria will have a better chance of survival and of multiplication, and this chance will be proportionate to the amount of nutritive material added. Similarly De Giaxa (*Zeitschrift f. Hygiene*, vi. 2, p. 162) made observations with reference to the conditions of existence of various bacteria in sea water, and his results are parallel to those made on ordinary drinking water. It need not be specially insisted on that neither ordinary nor sea water in themselves have any killing power on bacteria, but that where such an inhibitory power is observed it is due to the want of sufficient nutritive material, and that the greater the dependence of bacteria on organic material, and the poorer the water in such material, the more unfavourable is such water for the existence and multiplication of those bacterial species.

Next we have to consider the relations between two or more species simultaneously present in the same medium with sufficient nutritive material. Here more rapid multiplication will naturally depend, *cæteris paribus*, on the greater assimilative power; the greater this is, the more predominating will the species become. Thus, for instance, if in any organic material, say dead animal tissues, saprophytic bacteria are present together with bacillus anthracis, this

latter has not much chance of growing and multiplying; and hence in any part of an animal dead of anthrax, at first full of the bacillus anthracis, as soon as putrefaction has actively set in, the anthrax bacilli will be gradually killed off by the saprophytes, so that such material becomes deprived of producing anthrax infection. The same obtains with other highly specialised bacteria, *e.g.*, the streptococci, the typhoid fever bacillus, and others. While this process of killing off of the more specialised and less assimilative bacteria by the more rapidly growing and more assimilative bacteria is essentially a survival of the fittest in the struggle for existence, there is another factor to be considered that not immaterially helps to bring about that result: it is the inimical influence the chemical products of the saprophytic bacteria have on the more sensitive and more highly specialised pathogenic bacteria. If, for instance, a filtered solution is made of a putrid albuminous substance, the putrefactive bacteria being all removed, and of this solution a considerable amount is added to an otherwise favourable nutritive material, *e.g.*, alkaline broth, it will be found that this mixture is unfavourable for the growth of some species, in some cases more than in others. To the same class of inimical influences belongs the influence of fæcal matter on various species of bacteria, *e.g.*, anthrax bacilli, cholera spirilla, typhoid fever bacillus investigated by Kitasato. His results on the death of cholera spirilla in fæcal matter are instructive. They are published in the *Zeitschrift f. Hygiene*, v. p. 487.

Of a similar character are the observations recorded by Garré (*Correspond. f. schweizer. Aerzte*, xvii. 1887), who showed that nutritive gelatine which has served already for the growth of bacillus fluorescens putidus—a common saprophyte in water and putrid fluids—is no more capable of

serving the growth of some bacteria : bacillus of Friedländer, typhoid bacillus, pink torula, staphylococcus pyogenes aureus ; while others are capable of growing in such gelatine, though slightly retarded : cholera spirillum ; and still others grew normally : Finkler's spirilla, bacillus anthracis. Towards the former, therefore, the bacillus putidus has a decided *antagonistic* action, while with the latter it is symbiotic. But this antagonism, when existing, is not necessarily mutual, for while the typhoid bacillus renders the gelatine also unfit for the growth of the bacillus fluorescens putidus—these two species being mutually antagonistic—it is not so with the bacillus of Friedländer or the staphylococcus aureus. Diphtheria bacilli grow well in broth previously exhausted by proteus vulgaris.

To the same category belong the observations of Soyka and Bandler, who studied the manner in which certain bacteria are capable of growing in media previously exhausted by other bacteria (*Fortschritte der Med.* 1888, p. 76).

Cash has made similar observations with bacillus anthracis and certain micrococci when growing simultaneously. He found that the growth of the bacillus anthracis does go on to a certain extent, but that the virulence of it is impaired by the growth of the micrococci. This subject deserves a more exhaustive study than it has hitherto received ; it is mainly of importance to ascertain whether and to what extent an inimical influence is exerted by one species on the other capable of growing simultaneously in the same medium. There is good reason for supposing that hereby, in some cases at any rate, one species is capable of attenuating the virulence of another. Thus in the cultures which Pasteur used as attenuated cultures for producing protective inoculation in fowl cholera it was not, as Pasteur believed, the prolonged exposure to air that produced the attenuation of

his cultures, but the impurity of his cultures (Kitt), and likewise the attenuated condition of the culture fluids that Pasteur used for protective inoculations against swine erysipelas was probably caused by the impurity of the culture fluid (Schütz), there being present in Pasteur's fluid, besides the true bacillus of swine erysipelas, a contaminating micrococcus.

Watson Cheyne, von Emmerich (*Archiv. f. Hygiene*, vi. 1887), and others showed that the streptococcus erysipelatos possesses such an attenuating influence on the bacillus anthracis, for by inoculating simultaneously pure cultures of the two microbes into rabbits they were able to show that the bacillus anthracis was unable to produce fatal anthrax, though when separately inoculated they exerted their full virulence. It depends, however, to a considerable degree how much of the one and how much of the other microbe is injected in order to produce this inhibitory effect, for if too little of the streptococcus be injected the bacillus anthracis will exert its full virulence, or *vice versâ*. This whole subject is obviously a very important one from a practical point of view—from the point of view of finding antidotes against the action of pathogenic bacteria—and it deserves greater attention than it has hitherto received.

The writer has himself made some experiments with regard to injecting simultaneously two species. In one series the bacillus of fowl enteritis was grown in broth with the swine fever bacillus; in the other, the bacillus of swine erysipelas with that of swine fever, but neither in the amount of multiplication nor in the virulence of the swine fever bacillus could any change be noticed. He has, however, succeeded in neutralising the fatal effect on mice of the grouse bacillus, if at the same time the aerobic malignant œdema bacillus (*see* later) be injected.

Bouchard, Charrin, Woodhead and C. Wood have shown that there exists a strong antagonism between the bacillus pyocyaneus or its products and the bacillus anthracis; so much so that upon the injection of the former, simultaneously with or immediately after the latter, into an animal susceptible to anthrax, this latter disease does not take place at all, whereas a control animal not treated with the pyocyaneus succumbs to anthrax.

It is well known that certain infectious diseases, of which infection occurred simultaneously in the same body, do not take place simultaneously, but that the one probably has to wait, as it were, till the other has gone through its course. In other cases one disease has clearly an inimical influence on another. Take, for instance, the observations repeatedly made by surgeons that erysipelas has a curative influence on certain tumours; Fehleisen had by direct experiment with pure cultures of streptococcus erysipelatos proved that certain sarcomata can be made to disappear and a cure effected by producing erysipelas in the skin of the part.

But there is a converse side to this, namely the question whether, and if so, to what extent, one condition, one species of bacteria or its products, enhances the power of multiplication and the action of another. Monti (*Ac. d. Linc.*, October 6, 1889) pointed out that the culture of the diplococcus pneumoniæ—which, as is well known, gradually (by age and by continued subcultures) loses its virulent action on animals—regains the virulence if injected simultaneously with broth culture of the common saprophyte proteus vulgaris, from which the bacilli themselves are previously removed or killed by heat. This increased virulence of the pneumococcus may be achieved either by injecting this and the proteus culture at the same place, or at distant places simultaneously or soon after one another. Similarly I found that cultures of strepto-

coccus of erysipelas, which had lost their action on rabbits, regained virulence if injected mixed, *i.e*, simultaneously, with broth culture (four days old) of the proteus vulgaris ; and it made no difference whether the latter culture was or was not previously sterilised. The virulence on the guinea-pig of the bacillus diphtheriæ is, as I have shown, greatly enhanced by a simultaneous inoculation of the bacillus pyocyaneus.

CHAPTER XXI.

THE RELATION OF SAPROPHYTIC TO PATHOGENIC ORGANISMS.[1]

THERE is hardly any question which to the pathologist and sanitary officer can be of greater importance than the relation of saprophytic to pathogenic or parasitic organisms. To the pathologist the life-history of a micro-organism, outside and within the animal body, must ever remain an important field of inquiry; to the sanitary officer all conditions affecting the life and death of those organisms which produce, or at least are intimately bound up with, infectious diseases, such as the distribution and growth of these micro-organisms outside the animal body, the agencies which affect it in a favourable and unfavourable sense, are the points which he has particularly to consider in dealing with the spread and prevention of infectious maladies. Now, it is known of many micro-organisms, both those that are associated with putrefactive processes as well as those that are bound up with infectious disease, that temperature, the character of the medium in which they grow, presence and absence of certain chemical compounds, &c., are capable of materially affecting them. I need not for this purpose enumerate all that is known already

[1] Part of this chapter is copied from an interim report by myself to the Medical Officer of the Local Government Board, 1884.

by direct experiment, but will only limit myself to reference to the researches of Schröter, Cohn, and Wernich on that group of micro-organisms known as pigment bacteria, *i.e.* bacteria which only under certain conditions, notably temperature and soil, produce definite pigments (Cohn's *Beiträge zur Biologie d. Pflanzen*); to those of Hansen (Carlsberg Laboratory) on yeast; to those of Neelsen on the bacilli producing the blue colour of milk, the bacillus syncyanus (*Beitr. zur. Biol. d. Pflanzen*, iii. 2, p. 187); to the works of Toussaint, Pasteur, Chauveau, Koch, and others on the bacillus anthracis; Arloing, Thomas, and Cornevin on the bacillus of symptomatic charbon; of Koch on the bacillus of tuberculosis; of Israel on actinomyces, and many others; and particularly would I refer to the many valuable suggestions and considerations expressed by v. Nägeli in these respects in his book, *Die niederen Pilze*, München, 1877 and 1882.

While from these observations it would appear that both saprophytic and parasitic micro-organisms are capable of suffering modifications in their morphological and physiological behaviour, sometimes small, sometimes great and pronounced, it is nevertheless still an open question whether an organism which under ordinary conditions is only associated with septic changes in dead organic material, and which cannot under these ordinary conditions grow and multiply within the living body, can, under certain extraordinary circumstances, acquire the nature of a parasite, become endowed with the power of growing and multiplying within the body of a living animal, creating there a pathological condition, inducing there an infectious disease.

It is a common laboratory experience that many specific microbes, owing to medium, temperature, &c., or to successive subcultures, while retaining their general morphological characters nevertheless gradually change their physiological action, becoming more and more attenuated, and ultimately

lose their specific action altogether. Examples illustrating this have been mentioned on various previous occasions and they are familiar to every bacteriologist: the attenuated anthrax vaccines obtained by Pasteur by growing bacillus anthracis in chicken broth at 42·5° C., and successfully used for protective inoculation, the attenuation of the bacillus of fowl cholera by Pasteur, of the bacillus tuberculosis grown on Glycerin Agar, of the pneumonococcus, of the bacillus of malignant œdema, of streptococcus of erysipelas, and many other microbes. Similarly it is a common experience that a specific microbe which possesses a low virulence, or which has altered or lost its specific pathogenic action, can by altering the soil (artificial medium or animal body) become more virulent or recover its former virulence respectively. All these phenomena are constantly met with in all bacteriological work, and very few microbes are exempt from such changes.

Now, the questions that to the sanitarian are of great importance are these: (1) can a parasitic microbe which although at first derived from a virulent animal source, but existing under abnormal conditions inside or outside the animal body, alter its physiological nature so as to cease to be any longer capable of being a pathogenic or parasitic microbe? (2) can such a degraded microbe, *i.e.* once pathogenic but now living as a saprophyte again, under altered conditions resume its virulence? and (3) can a true saprophyte, that is a microbe not at any time connected with pathogenicity, owing to certain peculiar conditions under which it has been living when introduced into the animal assume the nature of a parasite?

From what has been stated previously, laboratory experience justifies us in answering questions (1) and (2) in the affirmative, but it is more difficult to give a decided answer to question (3), for it is quite possible to imagine, it

has indeed been shown by experimental investigation, that there are a good many microbes, not derived from, or associated with, any infectious disease of man or animals, but generally carrying on a saprophytic existence, which under certain conditions are capable of producing decided pathogenic action in the animal body. This question has to be considered under two aspects: (*a*) is there any evidence to show that a true saprophyte can, owing to alteration in the conditions of its growth in outside nature, acquire pathogenic action? and (*b*) can a true saprophyte, previously non-pathogenic, become pathogenic in the animal body owing to conditions within the animal body? It must be clear that for the sanitarian the first aspect is of the first importance, for if a true saprophyte could so alter in outside nature as to be capable of eventually starting an epidemic of infectious disease his views of the specific nature of infectious diseases will have to undergo a complete alteration. We will illustrate this by the following instances: G. Roux and Bordet have asserted that the bacillus coli, which as we have shown is a common saprophyte in the human and animal intestine, when sojourning in sewage—into which it naturally and commonly finds its way—is capable of becoming changed into the typhoid bacillus. This assertion was made on quite insufficient bacteriological evidence; moreover it was made at a time when the distinction between bacillus coli and bacillus of typhoid fever was not as easy or as well established as it now is. We can now dismiss this statement, viz., the conversion into and interchange of bacillus coli and bacillus of typhoid, as contrary to bacteriological experience.

Another instance of this kind is that adduced by Buchner on the experimental conversion of bacillus subtilis or hay bacillus into the bacillus anthracis ("Ueber die experim.

Erzeugung des Milzbrandcontagiums," *Sitzungsb. d. math.-phys. Classe d. K. Bair. Akad. d. Wiss.* 1880, iii. p. 369). We have in a former chapter described in detail the morphological and cultural characters of these two microbes and have shown them to be sufficiently striking to be readily distinguishable one from the other.

Buchner states that the bacillus anthracis when passed through a large number of successive cultures at a temperature of 35° to 37° C. gradually loses its pathogenic properties. In a Report to the Medical Officer of the Local Government Board for 1881–1882 I have shown that, even assuming that Buchner has had in all his cultures the true bacillus anthracis, but for which there is no definite proof, as Koch has so ably pointed out in his critical review of Buchner's work (*Mittheilungen aus dem k. Gesundheitsamte*, Berlin, 1881, Bnd. I.), Buchner, having tested his cultures on white mice only, has fallen into a serious error, for, as I have shown (Reports for 1881–1882), a culture of bacillus anthracis may have become quite harmless to white mice, but be still virulent to other animals. In fact, therefore, Buchner's result does not require for its achievement more than one culture, provided this has been kept for several days or weeks without spore-formation, as was the case in Buchner's experiments.

As regards Buchner's statement that by successive cultivation of bacillus anthracis at 35° to 37° C. this assumes the morphological and physiological characters of hay bacillus, I agree with Koch in regarding this as a complete error. If the cultures are kept safe from contamination, nothing of the sort ever happens. It is of course clear that if by any accidental contamination, say at the time of inoculating a fresh tube, a motile septic non-pathogenic bacillus, with which, or with the spores of which, the air sometimes

abounds, is introduced, every new culture established from this one will abound in this bacillus, and as it grows quicker and more easily than the bacillus anthracis, the next cultivations become barren of all the bacilli anthracis and only the non-pathogenic motile bacillus will be found present. This criticism has been applied by Koch to Buchner's experiments, and I must fully endorse it.

But there is a much more serious statement of Buchner's —serious, because if true in nature it is dreadful to contemplate to what amount of anthrax man and brute may become subject—viz., he maintains to have succeeded in transforming the hay bacillus into bacillus anthracis, by carrying the former through many generations under ever varying change of soil. It is needless to detail here all these experiments of Buchner, since I do not attach any great value to them, and I should not have troubled myself much about them, were it not that one meets in mycological literature, particularly on the part of botanists, an acceptance of Buchner's statement that hay bacillus can change into the pathogenic bacillus anthracis (*see* Zopf, *Die Spaltpilze*, Breslau, 1883).

I have repeated Buchner's experiments on rabbits, guinea-pigs, and white mice. I have grown the hay bacillus in various kinds of broth, in gelatine broth mixtures, in hydrocele fluid, in peptone fluid, in Agar-Agar and peptone, at temperatures varying between 30° and 38° C., and I have, to put it shortly, never seen that it shows the least tendency to change its general morphological characters, or that it ever assumes the morphological or physiological characters of the bacillus anthracis. I consider this a perfectly hopeless task, and I feel sure any one might as soon attempt to transform the bulb of the common onion into the bulb of the poisonous colchicum.

A further instance in which the transformation of a common saprophyte into a specific or pathogenic organism has been experimentally achieved, or I should rather say has been stated to have been achieved, is the *jequirity bacillus*. In 1882 the well-known ophthalmologist M. L. de Wecker in Paris drew attention to the therapeutic value of the seeds or beans of *Abrus precatorius*, a leguminosa common in India and South America. The people of Brazil use it under the name jequirity as a means to cure trachoma, or granular lids. De Wecker after many experiments found that a few drops of an infusion made of these seeds causes severe conjunctivitis, in the course of which, no doubt, trachoma is brought to disappearance and cure, and it is accordingly on the Continent and in this country now used for this therapeutic object. [I was informed by the late Dr. T. Lewis, formerly of India, then pathologist at the Netley Army Medical School, that the people in some parts of India know the poisonous properties of these seeds, and use them for inoculating cattle subcutaneously; in consequence a severe inflammation is set up, and the animals die of some sort of septicæmia. This is done for the sake of simply obtaining the hides of the beasts.]

Sattler, in a very important and extensive research (*Wiener medic. Wochenschrift*, N. 17–21, 1883, and *Klin. Monatsbl. f. Augenheilk.* June 1883), ascertained that when an infusion of the jequirity seeds is made of the strength of about half per cent. this infusion after some hours to a few days contains numerous bacilli, motile, capable of forming spores, and in most respects identical with the bacillus mesentericus. The bacilli are about 0·00058 mm. thick, and from 0·002 to 0·0045 mm. long. They form a pellicle on the surface of the infusion, and in the bacilli of this pellicle active spore formation is going on. The bacilli grow and multiply well

at a temperature of about 35° C., but also, only slower, at ordinary temperature. Sattler cultivated artificially the bacilli on blood-serum gelatine and meat extract peptone gelatine, both solid media, and continued their growth through several successive cultivations. Both the infusions of the jequirity and the bacilli taken from these artificial cultures inoculated into the conjunctiva of healthy rabbits produce severe ophthalmia, leading to the production of great œdematous swelling of the conjunctiva and eyelids, and temporary closure of the latter, and to the secretion of purulent exudation. Both the exudation and the swollen lids are said to contain infective bacilli and their spores. Sattler ascertained by many experiments that none of the bacilli and the spores distributed in the atmosphere had those specific properties, viz., to excite ophthalmia, as long as they grow in other than jequirity fluid, but having had access, *i.e.* having entered the jequirity infusion, assume here this specific power. There is no doubt that Sattler worked the whole problem with great care, worked out all points connected with it in great detail, and for this reason his work was considered to have for the first time unmistakably established that a harmless saprophyte, to wit the bacillus mesentericus, owing to the particular soil in which it grew, assumes definite specific or pathogenic properties. To me this jequirity bacillus had a great interest, since I was particularly anxious to get hold of such an organism, in order to see whether and how far it can again be made harmless. For if ever there was a good case, a case in which a previously harmless saprophyte had by some peculiar conditions become specific, this was a case; and therefore it must be here possible by altering its conditions of life again to transform it into a harmless being. The theoretical and practical importance of such a case must be

evident to every one who has at all devoted any thought to the relation of micro-organisms to disease. The whole doctrine of the specificity of infectious diseases, I might almost say, is involved in such a case, for if in one case it can be unmistakably proved that a harmless bacterium can be transformed into a pathogenic organism, *i.e.* that an infectious malady can originate *de novo*, then we should at once be relieved of searching for the initial cause in the outbreak of an epidemic. But in that case we should be forced to contemplate, as contained in the air, in the water, in the soil, everywhere, numbers of bacteria which, owing to some peculiar unknown condition, are capable at once to start any kind of infectious disorder, say anthrax (Buchner), infectious ophthalmia (Sattler), and probably a host of other infectious diseases, and thus to form the starting-point of epidemics. And the only redeeming feature, if redeeming it can be called, in this calamity would be the thought that the particular bacterium would by-and-bye, owing to some accidental new conditions, again become harmless.

These were the reasons, and good reasons I think they were, which prompted me to inquire into the jequirity bacillus and jequirity ophthalmia, and after a very careful and extensive series of experiments, to be described presently, I have proved beyond any doubt that the jequirity bacillus, *per se*, has no more power to create an infectious ophthalmia than Buchner's hay bacillus had of creating anthrax.

The following experiments prove this conclusively :—

The seeds of jequirity (*Abrus precatorius*) are crushed and powdered, the perisperm is removed, and of the rest an infusion is made of about the strength of half per cent. with distilled water, previously boiled and contained in a flask previously sterilised (by heat) and plugged with sterile

cotton-wool. The infusion is made while the water is still tepid. After half an hour the infusion is filtered into a fresh sterile flask, plugged with sterile cotton-wool, the access of air being limited as much as possible. This is effected by keeping the cotton-wool in the mouth of the flask around the end of the glass filter. The filtered fluid is of a slightly yellowish-green colour, and is almost neutral and limpid. A small quantity is withdrawn with a capillary glass pipette freshly drawn out, and from this several test-tubes containing sterile nourishing material (peptone solution, broth, Agar-Agar and peptone) are inoculated; and from the same pipette, and at the same time, several eyeballs of healthy rabbits are inoculated, by placing a drop or two of the infusion under the conjunctiva bulbi. The test-tubes are placed in the incubator and kept there at 35° C. After twenty-four hours all eyeballs are intensely inflamed, the eyelids closed and swollen, and a large amount of purulent secretion is present in the conjunctival sac, but all the test-tubes remain perfectly limpid; no growth has made its appearance, and they remain so.

In a second series the infusion prepared in the above manner is used fifteen minutes after it is made and used as above, for inoculation of test-tubes and eyeballs. The fluid in the test-tubes after incubation remains limpid, the eyeballs all become inflamed. In both series the amount of fluid inoculated into the test-tubes is more than twice as great as that injected into the eyeballs. From this it is quite clear that the fluid used for inoculation of the test-tubes was barren of any micro-organisms, and nevertheless it possessed a powerful poisonous principle. I do not mean to say that the infusion as a whole contained in the flask contains no organisms, but that the small quantity of the fresh infusion that was used for the inoculation of the test-tubes and eyeballs

contained none is absolutely certain. When such a flask is placed in the incubator, after twenty-four to forty-eight hours or later there are found in it large quantities of bacilli, the spores of which must have entered from the air during the process of preparing the infusion. The bacilli are such as described by Sattler; they soon form spores in the usual way. Such an infusion is very poisonous, just like the fresh one. Sattler has shown, and this is easily confirmed, that the spores of these bacilli stand boiling for a few minutes without losing their power to germinate. Consequently, if such a poisonous infusion full of bacilli and spores be boiled for half a minute the spores are not killed; proof for this: that if with a minute dose of this spore containing boiled infusion any suitable sterile nourishing material in test-tubes be inoculated, and then these test-tubes be placed in the incubator at 35° C., after twenty-four to forty-eight hours the nourishing fluids are found teeming with the jequirity bacilli; *but no amount of this material produces the least symptom of ophthalmia. Every infusion of jequirity loses its poisonous activity by boiling it a short time*, $\frac{1}{2}$ to 1 minute, and hence the above result.

In this respect the poisonous principle of jequirity infusion comports itself similarly to the pepsin ferment, which, as is well known, is destroyed by short boiling.

If an infusion is made as above, and after fifteen minutes it is filtered and then subjected to boiling for $\frac{1}{2}$ to 1 minute, it will be found to have become absolutely non-poisonous, but not sterile: placing it in the incubator after twenty-four to forty-eight hours, vast numbers of the jequirity bacillus are found in it. But no amount of this fluid is capable of producing the slightest symptom of ophthalmia.

A large percentage of the rabbits, whose conjunctiva has been inoculated with the fresh unboiled poisonous infusion,

die after several, three to eight, days. The eyeballs and eyelids are intensely inflamed, as stated above, the skin and subcutaneous tissue of the face, neck, chest, and even abdomen, are found enormously œdematous, the pericardium, pleura, lungs, and peritoneum very much inflamed, their cavities filled with a large quantity of exudation. The exudations of the conjunctiva, pericardium, peritoneum, the œdematous skin and subcutaneous tissues contain no infective property, and as a rule no bacilli or spores of any kind, if examined in the living animal or immediately after death.

There is one point which requires careful consideration; it is this: Sattler states that he has cultivated the bacillus, taken from a poisonous jequirity infusion, through several successive generations on solid material, and with the new cultures he was able to produce the jequirity ophthalmia. I have no doubt whatever that this is really the case, but it bears an interpretation different from the one Sattler gave it. Sattler, and many others, would, of course, say this: if any micro-organism taken from a soil that possesses infective properties be carried through many successive artificial cultivations, all accidentally adhering matter would hereby become so diluted that it may be considered as practically lost; that is to say, the organisms of the further generations have become altogether free of that matter. If the organisms of these further generations still possess the same poisonous property as the original material, then we must conclude that this poisonous principle is identical with the micro-organism. I do not agree with this whole chain of propositions although I agree with some parts. If a micro-organism be carried through several successive cultivations, always using for inoculation of a new culture an infinitesimal dose, then, no doubt, carrying on the cultivations through

four, five, or six successive cultures, any accidentally adhering original matter becomes practically lost, and if then the organism still possesses the same specific action as the original material, then no doubt the conclusion that organism and poison are in this case identical becomes inevitable. But this is not the case with the jequirity bacillus. Taking from a poisonous jequirity infusion full of the bacilli one to two drops, and inoculating with it a test-tube containing about four to five cc. of nourishing fluid, and using this at once *without previous incubation*, we find that even a few drops of this so diluted fluid still possess poisonous action. Precisely the same result is obtained when taking from a perfectly fresh jequirity infusion, *i.e.* before any organisms have made their appearance, one to two drops, and diluting them with four to five cc. of distilled water, and using of this diluted fluid one to two drops for inoculating the conjunctiva of healthy rabbits: severe ophthalmia will be the result. Carrying on the cultivation of these bacilli started from a poisonous infusion, for a second generation in fluid medium, no trace of any poisonous action can be now detected, any quantity of such a cultivation is incapable of producing ophthalmia. Sattler used in his cultivations solid nutritive gelatine on the surface of which he deposited his drop of poisonous jequirity infusion containing the bacilli; after some days' incubation, the bacilli having become greatly multiplied and having liquefied the top layer of the gelatine, he took out from this second culture a drop, and transferred it to a new culture-tube of solid material, and so he went on: every one of these cultures possessed poisonous action. Clearly it would, since he always used part of the original fluid deposited on the surface of the solid nourishing material. Part of this (being gelatine) became by the growth liquefied, but considering that Sattler started with infusions of considerable concentration

—he left the seeds for many hours and days in the infusion —it is not to be wondered at that this would bear a considerable amount of dilution, and still retain its poisonous properties. From all this we see, then, that the jequirity bacillus *per se* has nothing to do with the poisonous principle of the jequirity seeds, but that this principle is a chemical ferment in some respects (in its inability to withstand boiling) similar to the pepsin ferment.

Messrs. Warden and Waddell published in Calcutta during 1886 a most valuable memoir, detailing a large number of observations on the jequirity poison, which are in complete harmony with my own observations. They have definitely proved that the active principle is a proteid —*abrin*—closely allied to native albumen; that its action is similar to that of a soluble ferment, that it can be isolated, and that it is contained not only in the seeds but also in the root and stem of *Abrus precatorius*.

Sidney Martin has also published important facts concerning the chemical nature of abrin, according to him it is allied to an albumose. Ehrlich considers it as a tox-albumin and he has shown the remarkable fact that by small and repeated doses of abrin an animal (rabbit) can be immunised against a fatal dose (*see* later).

The second question which we put, *viz.*: Can a true saprophyte become pathogenic in the animal body owing to conditions within the animal? is more difficult to answer, and is intimately bound up with the further question, viz.: What is and what is not a specific or pathogenic microbe?

Specific or Pathogenic Microbes.—If under a specific microbe is understood a microbe that is connected with and is the *causa causans* of a definite infectious disease belonging to the group of communicable diseases occurring in nature and affecting man or animal or both, then the number of

pathogenic microbes is a limited one, and the number of such microbes known is less extensive than the number of known infectious diseases, since of some of them the specific microbe has not been discovered yet, *e.g.*, hydrophobia, syphilis, measles, whooping cough, &c., &c.; if, however, under a pathogenic microbe is understood one that is capable of living, under one condition or another, parasitic in the animal body and causing therein disease or a diseased condition, then their number is practically unlimited and no line of demarcation can be drawn between specific or parasitic and non-specific or saprophytic microbes. It is well established that microbes like bacillus prodigiosus, bacillus subtilis, proteus vulgaris, bacillus coli, and others, *i.e.* microbes living generally as saprophytes, when injected subcutaneously into the guinea-pig in small quantities, such as in the case of specific microbes capable of producing a specific disease, cause no disturbance, because they are not capable of living and multiplying in the subcutaneous tissue. I have shown that if these same saprophytes be injected in considerable quantities into the peritoneal cavity of a guinea-pig (*see* a former chapter) they are capable of living herein, of multiplying and causing acute peritonitis and death; after death the peritoneal exudation is found teeming with the living microbe, and they can be demonstrated in a living state in the blood and in the cavity of the inflamed intestine. Under this aspect of pathogenicity many a species occurring in nature ordinarily as saprophytes and connected with no infectious disease does cause acute fatal peritonitis and is capable of multiplying within the peritoneal fluid, whereas I have likewise shown that a notoriously specific microbe, like the diphtheria bacillus (from a gelatine culture), while virulent in the subcutaneous tissue of the guinea-pig, when injected in large doses into the peritoneum

fails to cause disease, it becoming soon killed herein. If then we are to judge of the nature of a microbe by its behaviour in the peritoneal cavity of the guinea-gig, *i.e.* whether pathogenic or non-pathogenic, we should have to include among the pathogenic class a large number of microbes which are generally pure saprophytes, while we should have to exclude from that class microbes which, as a matter of fact, are notoriously specific. If, on the other hand, we distinguish the microbes by the presence or absence of poisonous substances in their bodies (intracellular poisons or proteins) or elaborated (or secreted) by them while growing and multiplying—toxins—we do not get much further either; because, as I have shown and as has been mentioned in a former chapter, some notoriously specific microbes have not these intracellular poisons (anthrax, fowl cholera, diphtheria), while other notoriously saprophytic bacteria possess them (vibrio of Finkler, bacillus prodigiosus, bacillus coli, &c.). Again, if we judge by this whether a microbe does or does not produce in the course of its growth and multiplication toxins, *i.e.* poisonous metabolic substances, we would not get at a true definition either, because some microbes connected with putrefactive changes (proteus vulgaris) produce well-specialised toxic principles, while other microbes, connected with infectious diseases, do not, as far as one can judge from experiments, produce any specialised toxin, *e.g.* the whole group of microbes which cause in the rodent hæmorrhagic septicæmia; then there are other microbes, true specific or pathogenic, which although producing highly specialised toxin, *i.e.* toxin which injected into the animal causes the same disease as when we inject the living microbe (tetanus, diphtheria), do not as a rule live in the blood or in the tissues: the bacillus diphtheriæ, the bacillus tetani, lives chiefly at the seat of inoculation, where it produces

its toxin; in the blood or tissues it as a rule does not seem capable of existing.[K] Further: there are microbes which are capable of producing specialised toxins when growing in one kind of medium but not in another; bacillus anthracis is a good case in point: it would be extremely difficult—in fact it has not succeeded hitherto—to obtain from a broth peptone culture of bacillus anthracis, however luxuriant, anything like the specific toxin that was obtained by Wooldridge in cultivations in fluid alkali albumen, by Hankin in fibrin, by Sidney Martin in albuminous fluid. Or take the cholera vibrio. This microbe does not produce toxic substances to any appreciable degree in broth culture, but in albuminous fluids (van Ermengem) it produces it in a concentrated form. I have found the same to be the case with the vibrio of Finkler, bacillus coli, and others. From all this it follows that the presence or absence in the microbic bodies of principles poisonous to the animal body or of poisonous principles produced in culture is no guide in distinguishing a pathogenic from non-pathogenic microbes. Equally unsatisfactory is the distinction into microbes which can and such as cannot grow and thrive in the tissues of an animal, the former being generally considered pathogenic, the latter non-pathogenic. We have already mentioned the fact that some notorious saprophytes not connected with any specific disease—*e.g.* bacillus prodigiosus, bacillus coli, proteus vulgaris, vibrio Finkler-Prior, bacillus subtilis, and others—can live and multiply in the peritoneal cavity of a guinea-pig, provided they are injected therein in comparatively large quantities, whereas a notorious specific or virulent microbe, *e.g.* the bacillus diphtheriæ taken from a gelatine culture, cannot live in the peritoneal cavity even if introduced in large quantities; it rapidly in the course of a few hours degenerates and dies, whereas of the same culture a

much smaller dose injected subcutaneously causes tumour and fatal issue in thirty to thirty-six hours. But also in the subcutaneous tissue of the guinea-pig the above saprophytes can live and multiply, provided they are injected in large doses; a swelling appears, which is as a rule only of a temporary nature, lasting for from a few days to a week, according to the dose, and sometimes leading to abscess or purulent infiltration and necrosis. While the tumour lasts the microbe injected can be demonstrated in a living state by the culture test. In very large doses they may even produce rapidly general infection and death. On the other hand we see that even in the case of well-recognised specific microbes the subcutaneous injection may produce no appreciable result or only a slight and transitory tumour and recovery.

We have mentioned in several instances such results having been obtained by using attenuated cultures; it is this result which represents the essence of protective inoculations. A temporary tumour can be therefore produced by a specific microbe of attenuated virulence or by an otherwise virulent microbe if it be injected in too small a dose, or into an animal which is not very susceptible; according to any or all of these factors the effect of inoculation may be *nil*, or very slight and rapidly passing, or it may be moderate and slowly passing off, or it may be conspicuous and leading to death. So that the microbe introduced, although specific and pathogenic, may degenerate and be killed rapidly in the tissue, or it may multiply slightly, or it may multiply rapidly and easily and cause general infection.

From these considerations it follows then both in the case of true saprophytic as of true pathogenic microbes that all gradations of their capability to live and thrive in the animal tissues may be shown to be demonstrable, these

gradations in both classes of microbes depending (*a*) on the initial character (or virulence), some species acting in smaller doses than others, (*b*) on the quantity injected, (*c*) on the greater or lesser reactivity, smaller or greater resistance of the tissue in particular or the animal in general.

This brings us now to the consideration of the subject of what constitutes this resistance or immunity, what is the cause of this natural resistance or spontaneous insusceptibility, observed at the outset.

It must be clear from what has been just stated that the greater or lesser immunity or resistance of the tissue or of the animal are relative quantities. Under spontaneous or natural resistance is meant the natural capability of a tissue to withstand the growth and multiplication of a microbe and to destroy or kill the latter. And this spontaneous immunity must be distinguished from acquired or secondary immunity. We have shown that while one tissue is capable of destroying the microbes brought in contact with it, another tissue of the same animal does not achieve this result, or while an animal in one condition is found resistant it is found susceptible under another condition, or again while an animal is susceptible towards a microbe in an unaltered virulent condition it is possessed of resistance against an altered or attenuated condition of the same microbe, and lastly, while a particular tissue or the animal is insusceptible and found resistant against a small dose it is found susceptible against a large dose. We will mention here a few examples illustrating these points :—

(*a*) The cholera vibrio, the vibrio of Finkler, the bacillus prodigiosus, the bacillus coli, and many other microbes when injected into the peritoneal cavity of a normal guinea-pig in sufficient doses live and grow well and produce acute peritonitis and death, while when introduced in the same amount into the subcutaneous tissue or into the blood they soon

degenerate and die off; the diphtheria bacilli of a gelatine culture injected into the subcutaneous tissue of a guinea-pig —one-fifth of a culture per one kilo animal weight—produce typical tumour and death in thirty to thirty-six hours; when from the same stock one-third or one-fourth of the culture per 300—400 grms. guinea-pig is injected into the peritoneal cavity, nothing happens, the bacilli very soon degenerate and are killed.

When hay bacillus, staphylococcus taken from septic fluids, bacillus mesentericus, bacillus coli, or other saprophytes are injected into the vascular system of a healthy animal, no disease follows, the microbes soon disappear; but if in such an animal a focus of inflammation, necrosis, or ulceration has been previously established this focus becomes easily the soil for the growth and multiplication of those saprophytes (Wyssokovitch, *Zeitschr. f. Hygiene*, i.). Of the same nature are the observations constantly to be made of micrococci of various kinds, pneumococcus, and of bacillus coli or proteus vulgaris being found present, as a secondary invasion, in inflamed portions of various organs—liver, lung, spleen, kidney—in which the inflammation had been caused by some other antecedent disease.

Even in the case of an animal highly susceptible to a particular specific microbe it will be noticed that not all tissues of such animal are favourable for the life and growth of the microbe. While in the so-called blood diseases: septicæmias of different kinds, anthrax, fowl cholera, &c., the microbe lives and thrives well in the blood and blood-vessels of all tissues, this is not the case in many other instances, *e.g.* diphtheria, tetanus, cholera, tubercle, typhoid fever, and others.

(*b*) A normal frog is insusceptible to anthrax infection, but if it be kept heated to the temperature of a warm-blooded animal it is susceptible (Petruschki). A normal

frog or a normal adult white rat is insusceptible to anthrax infection, but if it be subjected to narcosis with ether-chloroform it becomes susceptible (Klein and Coxwell). Fowls are insusceptible to anthrax, but if cooled they become susceptible (Pasteur).

Charrin and Roger (*La Semaine Méd.*, 1890, No. 4) show that while normal rats are, as is known, very little susceptible to anthrax, they become highly susceptible if by working at a treadmill they are made fatigued, and H. Leo (*Zeitschrift f. Hygiene*, vii. 3) finds that by the presence of much sugar in the blood and tissues the susceptibility to anthrax and tubercle is not increased, while for glanders it becomes greatly enhanced. Phloridzin is administered in small doses with the food, sugar thereby becoming present in the tissues. Rats thus prepared resist anthrax as much as unprepared rats, guinea-pigs first prepared with phloridzin and then inoculated with tubercle do not show more intensive or more rapid tuberculosis. While normal white mice are almost insusceptible to glanders, they become highly susceptible to such infection if prepared with phloridzin. Maya and Sanarelli give an account (*Fortschr. d. Med.*, ix. No. 22) of a large number of experiments, in which by introducing acetylphenylhydrazin into an animal insusceptible to a particular disease this animal becomes thereby susceptible. This substance is known to produce destruction of the red blood-corpuscles (Gottstein) and hæmoglobinæmia; pigeons and rats thus prepared proved susceptible to anthrax.

(*c*) A normal guinea-pig when injected subcutaneously with a moderate dose of cholera vibrio not of high virulence from the outset, or that had owing to subculture for many generations lost its virulence, fails to show any result, but when the dose is transmitted through the peritoneal cavity of

the guinea-pig for some successive transmissions it becomes virulent even for subcutaneous injection; the same applies to bacillus coli, bacillus of typhoid, vibrio of Finkler, and others. Particularly bacillus coli can in this way so much increase in virulence that small doses injected subcutaneously cause acute septicæmic infection.

Some very striking phenomena are shown in these respects by the bacillus coli. The typical bacillus coli cultivated from the intestinal contents of a guinea-pig does not possess towards the guinea-pig greater pathogenic power (as shown by subcutaneous or intraperitoneal injection) than the typical bacillus cultivated from the human intestine.

Now, occasionally on intraperitoneal injection of a fatal dose of one or the other microbe (bacillus prodigiosus, staphylococcus aureus, vibrio Finkler, or vibrio choleræ) after death of the animal (in sixteen to twenty-four hours according to the dose and virulence) the peritoneal fluid contains besides the microbe injected also an abundance of a rapidly motile cylindrical bacillus which in culture proves to be the typical bacillus coli (Klein, Gärtner). That this could have been derived from the interior of the intestine only seems clear, but whether it got through the diseased but uninjured wall of the intestine (there has been established severe peritonitis by the microbe injected) or whether during the intraperitoneal injection the intestine has been injured by the cannula of the syringe it is difficult to say, at any rate there is no visible puncture of the intestine to be found. But it must be obvious that if on injection into the peritoneal cavity of say a pure culture of bacillus prodigiosus there should after the death of the animal be found in the peritoneal exudation, besides the bacillus prodigiosus, a bacillus which possesses all cultural characters of the bacillus coli the conclusion that this latter has got into the peritoneal

cavity from the interior of the intestine is the only one that can be admitted. Now this peritoneal accidental bacillus coli after cultivation proves highly virulent for the guinea-pig, a small dose injected subcutaneously produces acute hæmorrhagic septicæmia easily transmissible from guinea-pig to guinea-pig.

Bacillus anthracis of one source or another possesses different degrees of virulence (as has been mentioned in the chapter on Anthrax), thus if a comparatively large dose of anthrax bacilli in the blood of a mouse be injected into a sheep perhaps only transitory illness will be the result, the sheep possessing a certain amount of resistance against the mouse-bacilli, but if a few drops of blood of sheep dead of anthrax be used for subcutaneous injection of a normal sheep fatal anthrax will be the result.

(*d*) That the amount, *i.e.* the number, of the microbes introduced plays an important part has been mentioned on various previous occasions; here are a few more examples: A guinea-pig is susceptible to virulent anthrax if only a few bacilli are injected subcutaneously (Watson Cheyne, Lubarsch), while for the rabbit to achieve this result a considerably greater number is required, and in the case of a dog not even large doses suffice to produce infection.

The bacillus of fowl cholera taken from a drop of the blood of a fowl dead of the disease, injected subcutaneously into a rabbit or a pigeon, produces acute fatal infection, in the guinea-pig such a dose produces no result. A small particle of a glanders nodule of the horse injected subcutaneously into a guinea-pig or a field mouse produces fatal infection, in a rabbit it produces local abscess, and in a normal white mouse produces no result or only a slight transitory tumour.

These, as stated above, are only a few examples amongst

the large mass of observations that have been made on the relation of the degrees of natural immunity of different tissues and different animals in their normal and abnormal states against saprophytic and specific microbes in different conditions (of virulence) and introduced in different amounts.

Now, what is the cause of this spontaneous immunity? At the outset it is necessary to keep this question separate from that of acquired immunity, that is immunity which is produced by one or more previous mild or transitory attacks; it has been known as long as infectious diseases have been recognised as such that in some at any rate one attack, mild or severe, protects against a second severe attack, and the inoculations against small-pox (brought from the East), Jenner's vaccination against small-pox, "vaccination" against anthrax, fowl cholera, swine erysipelas (Pasteur), are based on that experience. The important fundamental observations that have been made in this field, subsequently to Pasteur's work, by Salmon, Roux and Yersin, Klemperer, Behring and Kitasato, Behring, R. Pfeiffer, and many others show that a specific immunity or resistance of different degrees can be produced against a specific microbe or its toxin according to the strength (virulence) and amount of the living microbe or its toxin previously introduced. Moreover this same principle of "active immunisation" by previous toxin injections holds good also for other than microbic toxins: Ehrlich produced this active immunity against an otherwise fatal dose of Ricin and Abrin respectively by previous repeated administration of subfatal and gradually increasing doses of these toxins, Calmette and Fraser the same against snake venom. The point that at present concerns us is the meaning and cause of spontaneous or natural resistance or immunity of one or another tissue or the animal body against one or another kind of

microbe, be this either a saprophyte or a pathogenic microbe.

Spontaneous or natural immunity.—The first who attempted an explanation based on experiment was Metchnikoff, who ascribed to the leucocytes, lymph- or white blood-corpuscles the power of taking up, destroying, and neutralising the microbe introduced into the tissue and thereby protecting the tissue and the body from infection, inasmuch as the microbes thus destroyed cease to exist, to multiply, and to produce their toxic effects. This view was based on the fundamental observation of Metchnikoff that when in a normal frog anthrax bacilli are introduced into the dorsal lymph sac, leucocytes soon rush, as it were, and are attracted to the place, eat up the bacilli, and thus protect the animal against infection, preventing the bacilli from living, growing, multiplying, and causing disease. This process of "phagocytosis," as it was called, is therefore an essential feature in natural immunity; it is in the first instance a purely mechanical process, effected by the amœboid movements and capability of the leucocytes to embody and swallow up and digest and destroy the invading enemy. In a large number of instances of known immunity—of greater or lesser degree—Metchnikoff and his pupils have sought and found this process of mechanical phagocytosis, and have explained to their own satisfaction every case of immunity of one or another animal or its tissues against one or another kind of microbes, be they true saprophytes or true parasites.

This theory relies on the following facts: (1) leucocytes are well known to be capable in the course of their amœboid movements of embodying and swallowing particulate matter, (2) leucocytes generally accumulate at, *i.e.* are attracted to, a locality into which foreign particles *en masse* are introduced or injected, and (3) it is notorious that in cases of immunity

of tissues or the animal the leucocytes present do contain in their interior the bacteria, some more, some less degenerated. The first and third points may be taken to represent phagocytosis in a mechanical sense, the second point may be considered as leucocytosis owing to positive chemiotaxis.

Now, while this represents the positive side of immunity, the reverse, viz., the greater or lesser inability of the leucocytes to rush to the bacteria, the greater or lesser inability to take them up, and the greater or lesser inability to destroy them, represent the negative side of complete or imperfect immunity; that is to say: if the leucocytes are of this nature, no immunity is present, the introduced microbes are not interfered with, they live, thrive, and multiply and cause infection and the disease. This is in essence the sum total of the views and observations that Metchnikoff and his school have put forward as sufficient to explain immunity complete and incomplete. For a fairly complete literature and history of this view see Lubarsch, *Centralbl. f. Bakt. und Parasit.*, vol. vi., No. 20.

First as to the phenomenon of leucocytosis: it is notorious that in many instances when microbes are injected into the subcutaneous tissue of an insusceptible animal, or are introduced in an attenuated form or in too small a number to maintain themselves in the struggle for existence against the living tissue, such leucocytosis does take place; this is the case when, for instance, a dose of anthrax bacilli is injected subcutaneously into a normal adult rat, or a dog, or into the lymph sac of a normal frog, or if a small dose of bacillus of symptomatic charbon is injected into the subcutaneous tissue of the little susceptible rabbit, or if a fair dose of moderately virulent bacillus typhosus or of vibrio choleræ is injected subcutaneously into the guinea-pig. But this is by no means universally the case. Take, for instance, the case

of bacillus prodigiosus, or bacillus coli, bacillus typhosus, or cholera vibrio, in its relation to the subcutaneous tissue of the guinea-pig. If a small dose of either of these living microbes (taken from the slanting surface of an Agar culture), say one-fifteenth or one-twentieth of a culture of an ordinary not exceptionally virulent stock distributed in sterile salt solution or sterile bouillon, be injected subcutaneously into a normal guinea-pig of about 300 grammes, the result is *nil*, no tumour is noticed, no leucocytosis; if the dose be larger, say one-tenth to one-eighth of a culture, there is noticed next day a more or less distinct swelling and leucocytosis, with general constitutional disturbance; the swelling increases for a day or two, then diminishes and becomes firmer, and may ultimately lead to suppuration and ulceration of the skin. While the tumour grows and increases, and even when it has begun to decrease and to become firmer, the microbes injected can be recovered by culture in a living state. If the dose be still more increased, say a quarter to a third of a culture, the result is more pronounced, the tumour and leucocytosis are greater and in some cases in two or three days may be followed by general infection and death.

So that the capability or incapability of a microbe to persist in a tissue, and to maintain its life and multiply therein, stands in no necessary relation to the existence or non-existence of a leucocytosis.

Moreover, even in the case of a particular microbe, *e.g.* bacillus anthracis, its introduction into the subcutaneous tissue of an insusceptible animal, say an adult rat or dog, is by no means necessarily followed by leucocytosis, and yet no infection ensues; this is noticed in the case when a small dose is injected. A further important fact to be mentioned in this connection of leucocytosis preceding

phagocytosis is this : after the introduction of a particular microbe, say bacillus anthracis, into the tissue, say the dorsal lymph-sac of a normal frog, the ensuing leucocytosis develops comparatively slowly and late ; if, for instance, a dose of bacillus anthracis or its spores, or of bacillus prodigiosus, be injected into the dorsal lymph-sac, numbers of these bacilli or the spores are rapidly absorbed into the blood of the general circulation, and can be there demonstrated by culture already ten minutes after the injection (Klein). If the animal be killed ten, thirty minutes, two or six hours after injection, the heart opened and a drop of blood rubbed over the slanting surface of gelatin or Agar respectively, and incubated, twenty-four to forty-eight hours later a large number of typical colonies of bacillus anthracis or prodigiosus respectively will be found on the gelatine or Agar respectively.

The "fight" ensuing between the bacilli and their spores introduced into the dorsal lymph-sac and the leucocytes, which are supposed to rush to the seat of the battle, *i.e.* the dorsal lymph-sac, must be considered a very hollow affair, if before the defending army can reach the seat of war a host of the invaders have already escaped all over the country as it were. Besides, before the defenders can amass their legions at the seat of battle, many hours must elapse, and it has been shown that many of the invaders are already dead in the lymph-sac before there is any sign of attack by the defenders, any sign of phagocytosis (Fischel, *Fortschr. d. Med.* ix. 2), and that the lymph of the lymph-sac free of leucocytes destroys the bacilli (Sanarelli, *Centr. f. Bakt. und Parasit.* ix. 14). More than that, Kanthack and Hardy show conclusively that prior to any phagocytosis, *i.e.* prior to the accumulation of leucocytes which are able to take up the microbes, the cells which are present or which aggre-

gate in the first place are certain cells which do not take up the microbes, which do not act as phagocytes, the latter coming only late into the field. Those non-phagocytic first-comers are the eosinophyle granular cells, which have the power to destroy the anthrax bacilli with which they come in contact, probably by secreting some matter obnoxious to the bacilli, and that only after this work of injuring the bacilli had been accomplished, the later comers, *i.e.* the ordinary pale leucocytes, commence to take the bacilli up, to act as phagocytes. These observations of Kanthack and Hardy are very clear and easily verified, and appear to me of the utmost importance, inasmuch as they prove a first process of a change of the bacilli, followed by a second process of scavenging by leucocytes.

One of the weak points in Metchnikoff's theory of phagocytosis being the primary cause of spontaneous immunity is the notorious fact that while in some cases of immunity such mechanical phagocytosis—*i.e.*, swallowing of the microbes by leucocytes—cannot be demonstrated, there are other cases not connected with immunity at all, in fact, just the reverse, in which a mechanical phagocytosis is a conspicuous phenomenon : we have in former chapters repeatedly mentioned that if a fairly large dose of active and otherwise virulent diphtheria bacilli (taken from the slanting surface of gelatine), such as would more than suffice to produce tumour and death if injected into the subcutaneous tissue, be injected into the peritoneal cavity of a normal guinea-pig, as a rule no disease or no death follows, the diphtheria bacilli soon disappear from the peritoneal cavity, in fact their degeneration and breaking up can be demonstrated already a few hours after injection. But occasionally in a percentage if the dose be too large, or if instead of gelatine Agar culture is used, disease and death

do follow. This is particularly the case if recent Agar or serum cultures be used instead of gelatine culture, or if in addition to the gelatine cultures diphtheria toxin—from an active broth culture—be injected. In such a case the animal, presumably on account of the toxin present (evidently not present in the gelatine growth), soon sickens, and is found dead in thirty-six to forty-eight hours or later. On examining the peritoneal fluid in such a case it will be seen that very few diphtheria bacilli are demonstrable, either in cover-glass specimens or by culture; in the latter case a drop of the fluid yields only a few colonies. But if we look to the omentum next to the large curvature of the stomach we find masses of lymph, which examined under the microscope show aggregations of leucocytes all filled with diphtheria bacilli, some well preserved, others in fragments; between the leucocytes are also large numbers of free bacilli. In these cases then, when the resistance of the peritoneum—so perfect against the bacilli of gelatine culture—has broken down and been overcome, presumably by the additional introduction of toxin, we find numbers of phagocytes, whereas in the other case when the resistance of the peritoneum has successfully been maintained—*e.g.*, in the case of using bacilli of gelatine culture—there is no sign of phagocytes.

To the same group of phenomena belongs the occurrence of numerous phagocytes, *i.e.*, leucocytes filled with the microbe, in cases when the injection has produced fatal infection and where there is just the reverse of immunity either of a particular tissue or of the animal as, for instance, in fatal mouse septicæmia, in fatal swine erysipelas, when the presence of leucocytes filled with living bacilli is a conspicuous feature. Add to this the same condition in the leucocytes of the purulent secretion in Koch's Egyptian

ophthalmia, in gonorrhœa, in tubercle, and particularly in the leprosy cells of the leprous tubercles. The presence of the microbes in the interior of cells in these cases means just the reverse of a destruction of the microbes by the cells, it means a destruction of the cells by the microbes, the latter multiplying in the former and thereby producing their (the cells') ultimate destruction.

The occasional local leucocytosis observed in connection with immunity, *i.e.* occurring at the seat of introduction of bacteria, is explained by a remarkable attraction which the introduced microbes seem to exert on the leucocytes.

Pfeffer made the first observations as to the remarkable power possessed by different chemical substances towards bacteria and other micro-organisms, substances which either attract or repel bacteria, these phenomena being spoken of as chemiotaxis, the former as positive, the latter as negative chemiotaxis. Pfeffer (*Unters. a. d. bot. Inst. Tübingen*, 1887, p. 582) found that motile organisms (bacteria, flagellata, and volvocinea) are stimulated by many organic and inorganic substances in solution—positive chemiotaxis. To mention only a few of the substances, the salts of potassium have a great "stimulating" power, likewise peptone, glycerine, morphine. Alcohol, free acids, and free alkalies have a negative chemiotactic action, *i.e.*, repel the microbes. Ali Cohen (*Centr. f. Bakt. und Parasit.*, viii. 6) made systematic observations on this same subject with various kinds of bacteria.

Gabritschevsky, Massart and Bordet (*Annales de l'Institut Pasteur*, 1891, iv. 6), and others tested then the action of bacteria on leucocytes, introducing chemical substances in capillary glass tubes into the living body of animals, and then examining these capillary tubes and seeing whether they attracted leucocytes or not; in this way they found

that chemical substances either attract or do not attract leucocytes. Thus, for instance, Massart and Bordet found the lactic acid acting powerfully—negative chemiotaxis; Buchner found collagen, alkali albumen, gluten casein acting powerfully—positive chemiotaxis. Now, Buchner argues, and I think with justice (*Centralbl. f. Bakt. und Parasit.*, x. 22 and 23), that when in an insusceptible animal leucocytosis does occur at the seat of inoculation this leucocytosis is not an expression of the commencing battle between the microbes and the leucocytes, as is maintained by Metchnikoff and his followers, but is due to a positive chemiotactic action on the part of the bacteria (dead or alive), by which the leucocytes are attracted. Extensive leucocytosis (suppuration) has been shown by Koch to occur after injection of tuberculin containing the products of the tubercle bacilli previously killed; suppuration (miliary abscesses) has been produced by Prudden and Hodenpel in the rabbit after injection into the vascular system of the substance of the tubercle bacilli, previously sterilised; also inserting sterilised tubercle culture by means of capillary glass tubes into the subcutaneous tissue of the rabbit proves positive chemiotactic attractions of the dead bacilli towards leucocytes. This chemiotaxis is brought about by substances—protein—derived from the bacteria themselves, and is dependent on the previous inimical action on the bacteria by the tissue *per se*. Where the tissue *per se* possesses this action the bacteria are either only weakened or destroyed, and only under this condition does their substance—protein—become available to attract the leucocytes; in such cases the weakened and also the killed bacteria are easily taken up by the leucocytes, and these then help to remove them. Under this theory the phagocytosis observed at the seat of the inoculation is therefore dependent on the

preceding alteration of the bacteria. But in the case of a susceptible animal, that is, when the introduction of the bacteria produces general infection and no local leucocytosis at the seat of inoculation, the bacteria, because they remain vigorous and because they withstand the action of the tissue, do not yield the chemiotactic substance—protein—and therefore no leucocytes are attracted to the seat of the inoculation.

In connection with the phenomena of chemiotaxis it ought to be borne in mind that just as certain bacteria exert an attraction to the leucocytes, so also is it imaginable that the cells and tissues exert chemical attraction on certain bacteria, just as in the case of Pfeffer's experiments. This at any rate offers a ready explanation of the conspicuous attraction that one or the other tissue seems to exert towards certain specific microbes. It is well known that in the acute exanthemata the skin is the tissue which preeminently exerts such a positive chemiotaxis on the specific microbes. In anthrax, in typhoid fever, in malaria, in relapsing fever, the spleen has a conspicuous attractiveness for the microbes ; in tuberculosis it is the lymphatic tissues and the spleen. In this disease the lymph-cells seem to be the particular nidus for the growth and multiplication of the bacilli. It is quite possible that the presence of saprophytes in the lymph-cells of the superficial parts of the tonsil, pharynx, and Peyer's glands (Bizzozero, Ribbert, Ruffer) is to be explained in this way, viz., that these cells possess a chemiotactic action, being a more favourable nidus for the growth of the bacteria.

The conclusion which we think justified in making is that the phenomenon of mechanical phagocytosis in Metchnikoff's original sense is in some cases unquestionably a sign of weakening and destruction of the microbes, but it cannot

be the primary and essential part to which the resistance and immunity of the tissue or the animalis are due. This does not deny the possibility that leucocytes do and can take up microbes still in a living and even active state, but, what seems from all that has been said highly improbable, that such phagocytosis is the first phenomenon in the destruction and neutralisation of the microbes introduced into a tissue, or that it is sufficiently extensive or sufficiently early to account for the rapid and complete destruction of the microbes introduced that in some cases is noticeable. This forces us to assume that spontaneous resistance or immunity is primarily and essentially due to an inimical action of the blood and tissue or tissues *per se* on the microbe, a view which as we shall see harmonises well not only with the facts concerning natural immunity, but in a still more marked manner with acquired, active, or artificially produced immunity.

The first definite proof as to the germicidal power of blood was given by Fodor (*Deutsche med. Wochenschrift*, 1887, No. 34), then Nutall (*Zeitschrift f. Hygiene*, 1888, iv. p. 353), Niessen (*Zeitschr. f. Hygiene*, 1889, vi. p. 487), Behring (*Centralbl. f. Klin. Med.* 1888, No. 38), and particularly Buchner (*Centralbl. f. Bakt. u. Parasit.*, 1889, vol. v. p. 25, vol. vi. pp. 14, 21; *Archiv f. Hygiene*, 1890, p. 85), and others have shown that the plasmatic fluids of the body—lymph and blood—have in their fresh and living state the power to destroy and kill bacteria brought into contact with them. The experiments of Buchner, Nutall, and Niessen have shown that the fresh blood plasma used in the test-tube has a remarkable power of doing this, although this power differs considerably as regards different species. Thus, micrococcus aquatilis, cholera spirillum, anthrax bacillus, typhoid bacillus, and the bacillus of Friedländer are easily killed after a few minutes (five to twenty

minutes), while others, *e.g.*, staphylococcus pyogenes aureus and albus, streptococcus erysipelatos, bacillus of fowl cholera and swine fever, and proteus hominis, are only very slightly affected by it; on proteus vulgaris, bacillus fluorescens liquescens, bacillus aquatilis, and bacillus prodigiosus it has no appreciable effect. But also in the cases where the fresh blood exerts its inimical action this only takes place if the relative number of bacteria added is limited, for the killing power of a given quantity of fresh blood is limited, so that if the number of bacteria introduced be too large the killing power of the blood does not extend to all bacteria; and having been consumed and exhausted in killing a certain number of them, others escape, and these, then, are capable of rapidly multiplying, as in any other medium. The power of the blood to kill certain bacteria rests with the plasma, and it is the same power that also kills the leucocytes. There is a remarkable parallelism between blood plasma and leucocytes on the one hand and blood plasma and bacteria on the other, for when the blood plasma kills the leucocytes it also kills bacteria, *e.g.*, fresh blood and blood plasma; but fresh peptonised blood and peptonised plasma, which have not this power on the former, have it not on the latter. When blood is heated to 52° or 58° C. for twenty to thirty minutes (Nutall) it loses the power of killing bacteria, which it otherwise killed; blood mixed with magnesium sulphate loses the killing power; when blood is kept for several hours it also loses this power. Blood to which bacteria had been added and thereby killed coagulates quicker (Grohmann), just as blood which kills the leucocytes coagulates quicker.

Buchner (*Centralbl. f. Bakt. und Parasit.*, vi.) has made very extensive observations on the germicidal power of blood plasma and blood serum; he points out an important

antagonistic action vested in these fluids, on the one hand, as to their power of being *nutritive*, and, on the other, as to being *germicidal*; the first depends on materials no longer living, *e.g.*, dissolved or broken-down blood-corpuscles, the latter on the "living" or "active" condition of albumen. Buchner shows that the circulating blood possesses the germicidal property in a higher degree than blood after removal from the body: evidently the former contains in a much smaller degree the particular nutritive elements than the latter, which of course contains the products of the dead or broken-down blood-corpuscles. Buchner further shows that the germicidal power of the blood is not directly dependent on the leucocytes, and further that it depends on the albumen present in the plasma or serum, as long as this is in combination with salt, or, as he terms it, is in an "active" state. Plasma or serum free of cells acts germicidally; if from it, by dialysis, the salt is removed, it loses its germicidal power; the salts of the plasma or serum themselves possess, however, no germicidal power. Lubarsch (*Fortschritte d. Medizin*, Bd. viii., No. 17) thinks it probable that the germicidal action and inhibitive power of the living tissues may in a large measure depend on the chemical activity of the tissue cells, that is, on chemical substances excreted or produced by the cells; hence the battle against bacteria is essentially of a bio-chemical nature, as has been ably demonstrated by Petruschki in a series of papers.

The substance or substances to which the plasma, serum, or tissue juices owe their germicidal power are called by Buchner *Alexines* (ἀλέξειν, to protect). It must however be clear that, whatever the exact nature of these alexines, they cannot be the same, either in all animals or for the different pathogenic bacteria. The alexines against anthrax in an insusceptible animal, *e.g.*, rat, frog, cannot be the same as the

alexines in glanders in an almost insusceptible animal, as the tame mouse. Nor can the alexines which are present in the tissues, and which act germicidally on saprophytic bacteria, be the same as the alexines protective in insusceptible animals against specific bacteria. Again, the alexines protecting a naturally insusceptible animal against a specific microbe cannot be the same as the substances protecting against a second infection a susceptible animal which has passed through one mild attack ; that is to say, the natural immunity of an individual cannot be due to the same kind of protective substance as the acquired immunity.

Moreover, it has been shown that the inhibitory power possessed by the blood (serum), though it can be greatly increased and rendered specific against a particular species of microbes by previous injections of a particular animal with this microbe (artificial immunisation), may be and sometimes is already naturally present in the normal animal : Roux (*Annales de l'Inst. Pasteur*, September, 1894), for instance, finds the blood-serum of a normal horse possessed of a certain high degree of resisting or inhibitory power against diphtheria, Löffler (*Centralbl. f. Bakteriologie*, February, 1896) finds the blood of a normal dog possessed of inhibitory power against the typhoid bacillus, Cobbett (*Journal of Pathology and Bacteriology*, January, 1896) finds the blood-serum of a normal horse possessed of a certain amount of inhibitory power against diphtheria toxin as also against the living diphtheria bacilli.

The essential and primary element in the resistance or immunity of a tissue or of an animal against the growth and multiplication of a microbe is the power of the tissue juices (plasma, serum, or lymph) to injure or destroy the microbe by virtue of its alexines, that then the so altered microbes may be easily taken up by leucocytes (attracted there) and

further broken up and removed—phagocytosis. It is clear that a compromise between the two views: (*a*) Metchnikoff's of phagocytosis and (*b*) Buchner's of alexines, is easily possible; and, Kanthack's and Hardy's researches having indicated such a compromise, it is satisfactory to find that Metchnikoff himself has already placed himself more in harmony with the ascertained fact of acquired immunity by suggesting that the inimical or inhibitory power of the blood (plasma, serum, and lymph) in acquired immunity is due to the presence in the blood of substances secreted or elaborated by the tissue cells. This is in so far a welcome admission as we can easily extend it to natural immunity by saying that in insusceptible tissues or an insusceptible animal the alexines, like other substances, are secretions, or products, or whatever we like to call them, of the living tissue cells, and this would also well harmonise with Kanthack and Hardy's demonstration of a direct process of destruction of anthrax bacilli by the secretions or the products of living cells (eosinophile cells). Phagocytes, *i.e.*, cells which actually are capable of embodying bacteria living, injured, or dead in the process of the destruction and removal of the microbes from the insusceptible tissue or insusceptible body, are in no way opposed to the theory of alexines, since the alexines themselves are substances produced by, and freed from, the living cell protoplasm, and it would make little difference whether the inhibitory or germicidal action by alexines takes place within the cell protoplasm of some cells, or by the alexines originally produced by the cells but now free in the tissue juices.

Acquired or artificial immunity.—The observation that in some infectious diseases one attack protects against a second underlies, as stated on a previous page, the whole theory and practice of protective inoculations, but only within

recent years has it been possible to demonstrate experimentally the intimate nature and causes of the immunity and resistance acquired by a first attack. Salmon and Theobald Smith, Beumer and Peiper, Ehrlich and Fraenkel, Roux and Chamberland, Roux and Yersin, showed that an animal acquires immunity against a particular infection not only by Pasteur's method, *i.e.* by a first infection with mitigated or attenuated microbes—by a mild attack—but that such immunity can be acquired also by previous injection or injections of the ready-made specific toxins. Behring,[1] Behring and Kitasato,[2] Roux,[3] and others followed this up by showing by more exact methods (Diphtheria and Tetanus) that a definite relation exists between the degree of resistance acquired and the amount and virulence of the infecting material (both microbes and specific toxin) used for the antecedent injection or injections; further that it is possible to raise this resistance up to more or less complete immunity by intermittent, repeated, and gradually increasing doses used in these antecedent injections, allowing the animal time to recover completely before the next injection.

Starting with a small dose or a mitigated virus—microbe or toxin—the mitigation being achieved by heat, chemical reagent, or method of cultivation—the first injections produce slight reaction, if the animal is possessed at the outset of a certain spontaneous resistance; the reaction is greater, large tumour in subcutaneous injections, constitutional disturbance in most cases, if the animal is at the outset more susceptible. The less the initial resistance of the animal or the greater or more virulent the first dose, *i.e.* the greater the reaction on the part of the animal, the greater as a rule is the resistance

[1] *Deutsche med. Woch.* No. 49, 1890.
[2] *Ibid.*, No. 50, 1890.
[3] *Annales de l'Institut Pasteur*, September, 1894.

acquired, the sooner the point of acquired immunity is reached, or in other words the greater or more virulent the subsequent dose that the animal can bear. By repeated injections of gradually increasing doses a high degree of resistance is ultimately reached. While by the method of conferring this acquired or active immunity against a specific disease, used by Behring, Behring and Kitasato, Klemperer, Roux, R. Pfeiffer, and others, the animal is allowed to recover from the previous injection before a further injection of the increased dose is administered, Löffler shows (*Centralblatt f. Bakt. und Parasit.*, February, 1896) that as regards the typhoid bacillus, by injections of small doses of virulent bacilli administered in very short intervals of a few hours, immunity can be acquired already in a few days.

The fact that a specific immunity can be thus acquired by injections of specific toxin, that is to say, by the repeated injections of the pure toxin elaborated by a particular microbe, and separated from the latter by filtration, *e.g.* diphtheria toxin, tetanus toxin, typhoid toxin, &c., proves conclusively that the condition of this immunity cannot be due to a phagocytic action of the leucocytes; no microbes being used for the injections, there are no microbes to be swallowed up and destroyed.

Now, the most striking fact that was first demonstrated by Behring and his co-workers is this: the blood or blood-serum of an animal actively immunised, or, generally speaking, of the animal body which has acquired immunity in one way or another against a particular infectious disease, possesses a definite and measurable power to confer immunity, *passive immunity*, against that particular disease if injected into a normal animal; more than that: it is capable of modifying or even completely neutralising—curing—the

effect of an already-established infection in a normal (unprepared) animal.

This immunising and curative power of the blood-serum of the actively immunised animal body is commensurate with the degree of immunisation, so that the blood-serum of an animal in which the active immunisation has been carried to a high degree has itself measurably greater immunising and curative power than the blood-serum of an animal not immunised to the same degree (Diphtheria, Tetanus).

Behring uses as the standard for measuring (in diphtheria) this potency of the blood-serum by taking as *unit* the amount of serum required to completely neutralise a dose of pure toxin that would produce death in ten guinea-pigs each of about 300 grams weight in thirty to thirty-six hours. As was mentioned in the chapter on Diphtheria, Roux and Yersin, who first separated the diphtheria toxin elaborated by the diphtheria bacilli in broth cultures, showed that the injection into the subcutaneous tissue of the guinea-pig of a fatal dose of this pure toxin produces the same tumour at the seat of injection and the same subsequent symptoms and death of the animal as does the injection of the active and living diphtheria bacilli. Behring's unit of potency of diphtheria serum is the amount of serum required to inject—antecedently or simultaneously, or shortly after—in order to neutralise, *i.e.* prevent from producing tumour, disease, and death, a tenfold fatal dose of toxin in a guinea-pig of 300 grams weight. This measure of the serum is then its *antitoxic* potency. Behring has carried the active immunisation against diphtheria of animals: sheep, goat, to such a high degree that the (diphtheria) antitoxic potency of the serum of these animals reaches the high

figure of about 90, 130, and even 200 units, or, more accurately stated, 7·5 cc. of serum possess 600, 1,000, or 1,500 antitoxic units respectively. Roux submits horses—as a rule not possessed of great susceptibility for diphtheria at the outset—to repeated injections with pure and powerful diphtheria toxin, starting by injecting subcutaneously small doses of attenuated toxin, then gradually increasing the dose of the pure toxin, till after many injections the intravascular injection of enormous doses—250 cc.—of the most powerful toxin do not produce more than a transitory result. After three months' immunisation he obtains an antitoxic serum which is possessed of great potency: one cubic centimetre being capable of neutralising a fatal dose of pure toxin for 10,000, 20,000, 50,000, 100,000 and even 200,000 grams guinea-pig, or, put differently, $\frac{1}{50}$, $\frac{1}{100}$, $\frac{1}{250}$, $\frac{1}{500}$, and even $\frac{1}{1000}$ of a cubic centimetre of the antitoxic serum can completely neutralise the effect of a fatal dose of toxin injected into a guinea-pig of 200 grams weight. But while this serum possesses the high antitoxic power, *i.e.* the neutralising power of toxin, both when injected into the unprepared animal shortly before or simultaneously with, or some hours—six or even twelve hours—after the toxin, its germicidal potency, *i.e.* its action against the living microbes (Diphtheria bacilli), is considerably less, though it is for a time at least considerable. Thus, for instance, of Behring's diphtheria antitoxic serum, marked 600 units, $\frac{1}{10}$ of a cubic centimetre is required to completely neutralise for a guinea-pig of 500 grams weight a fatal dose of living culture of the diphtheria bacilli, $\frac{1}{20}$ of a cc. does not prevent the formation of a tumour, although the animal does not die, but recovers after some days, $\frac{1}{100}$ of a cc. neither prevents the formation of a tumour nor the fatal issue.

I have succeeded in obtaining serum of considerable anti-

toxic *and* immunising power by subjecting the horse to repeated injections with large doses of living diphtheria bacilli; in one horse already after three weeks, in another after four weeks, the serum had the same immunising power as in horses done after Roux's method with pure toxin for a considerably longer period.

The immunising power of antitoxic diphtheria serum, *i.e.* the power of the serum when injected into a normal guinea-pig to protect the animal against subsequent infection with the diphtheria bacilli, is only of a temporary character, being of short duration, generally from a few days to a few weeks, and depends on the amount of serum injected. The immunising action of the injection into a guinea-pig of a subfatal dose of living culture of bacillus diphtheriæ is of considerably longer duration, but it must be added that in that of the bacillus of diphtheria, unlike with some other microbes—anthrax, cholera, typhoid, colon, &c.—the resistance of the guinea-pig against new and further infection is comparatively limited.

It is clear from the facts above recorded that during the process of "*active immunisation*," as first practised by Behring and Kitasato, Behring, Roux, and others, the blood (and blood-serum) of the immunised animal contains substances, *antitoxins* or *antibodies*, as a result of the antecedent injections of toxin. Of what nature are these bodies? Are they a result of the activity of the cells and tissues, a reactive secretion of new substances (ferment) by the cells in consequence of successful and effective stimulation by the toxin (Roux), or are they the original toxin modified and chemically altered by the tissue cells (Buchner)? Are they of the nature of albumins like the toxalbumins, or are they bodies more resembling ferments?

As regards the diphtheria antitoxins Aronson's mode of

separating them and obtaining them in a concentrated form from the serum of immunised animals suggests that they are of the nature of ferments like the diphtheria toxin itself of Roux and Yersin, the diphtheria antitoxin also in other ways comporting itself like a ferment, *e.g.* the diminution and final destruction of its potency by heat of 65 to 70° C.

One of the most remarkable results of immunisation against particular toxins was achieved by Ehrlich[1] with Ricin and Abrin. By feeding animals with one or the other of these poisons he was able to gradually immunise them, and just as in Behring's experiments was able to achieve a high degree of immunity; moreover the blood of these animals possessed antitoxic (antiricin or antiabrin respectively) potency (immunising and curative) commensurate with and proportionate to the amounts of the antecedent toxins used for the immunisation.

And last but not least Sewall,[2] having shown that immunity against rattlesnake poison can be conferred on an animal by antecedent subfatal doses of this poison, Calmette[3] was able to produce antitoxic serum (in the rabbit) by immunising with repeated injections of at first small subfatal and gradually increasing doses of snake venom so much so that the serum of highly immunised animals is capable of conferring protection or passive immunity and even exert curative action against snake venom in normal unprepared animals. Fraser[4] has confirmed these observations.

We may take it then as a general law that an animal can

[1] *Deutsche med. Woch.*, 1891, Nos. 32 and 44.
[2] *Journal of Physiology*, 1887, p. 203.
[3] *Annales de l'Institut Pasteur*, May 1894, April 1895.
[4] *Royal Society of Edinburgh*, June 3 and July 15, 1895.

be immunised against a particular toxin, and that its blood and blood-serum thereby acquire a proportionate *specific antitoxic potency*.

Antitoxic diphtheritic blood-serum does not act anti-microbically or germicidally *in vitro*, for, as Wright has shown, antitoxic blood-serum of a diphtheria-immunised horse forms a good artificial medium for the growth of the diphtheria bacilli.

This production of acquired or active immunity by toxin, is apparently not the same as is created in the animal body under natural conditions, that is when the animal body acquires immunity against a particular infectious disease by a previous attack of the disease. In the natural condition when the animal body is subject to an attack the specific microbe, having found entrance, lives and multiplies within the infected body and causes the particular disease, and after the body recovers and the microbes again disappear it is found some time afterwards that it has acquired the power to resist a new infection with the microbe, or if this be injected in an otherwise sufficient dose the animal and its tissues resist it, the microbes cannot now live in such a tissue or such an animal body, they degenerate and die and produce no disease. Evidently during the first attack something was formed in the animal which after the disease has passed off is present in the blood and tissues and which acts inimically, germicidally against the same microbe. This germicidal substance does not appear immediately on recovery (in Fraenkel's experiments on diphtheria in the guinea-pig it requires two to three weeks for its appearance); the same holds good for pneumonia, for cholera, typhoid, and others; further this germicidal or immunising action of the blood and tissues does not in all cases last for an

indefinite time, in some, *e.g.* variola, scarlet fever, and the acute exanthemata in general, it seems as a rule to persist for lifetime, in others, *e.g.*, erysipelas, diphtheria, it is of a more limited duration. We said above that this naturally acquired immunity is apparently not of the same character as that producible by repeated toxin injections, but in reality it may be the same, since also in the naturally acquired immunity against a particular infectious disease by a previous attack this attack is caused by the toxin elaborated by the microbe in the infected body, so that after all the difference in the two methods is merely this, that in the one, the Behring's method, the toxins are prepared outside the animal body in artificial cultures, while in the other, *i.e.* the immunity acquired under natural conditions or by Pasteur's method of protective inoculations, the toxins are elaborated by the microbe within the infected animal body.

But is there really no difference between the immunity acquired in the two methods? We have already indicated that the antitoxic power of diphtheria serum prepared after Behring or Roux by toxin injections is incomparably greater than the immunising, or germicidal, or antimicrobic power, and it can be further shown that while an animal can by repeated injections of dead bacterial bodies be well immunised and protected against an otherwise fatal dose of the same bacterial bodies in a living state it is not protected against an otherwise fatal dose of the specific toxin. A guinea-pig is repeatedly intraperitoneally or subcutaneously injected with dead cholera vibrios or dead vibrios of Finkler, bacillus coli or bacillus of typhoid, bacillus prodigiosus or proteus (scraped from the slanting surface of an active Agar culture, then distributed in sterile bouillon and finally thoroughly

sterilised by being heated to 60–70° C. for ten or twelve minutes). The dose for subcutaneous must be larger than for intraperitoneal injection; the dose is at first subfatal, but sufficient to produce distinct illness, then after a week or ten days a second injection is made with a larger dose, then a third, a fourth, a fifth, and a sixth injection, till no reaction at all follows. If ultimately, a fortnight after the last injection, such a prepared animal be tested with a dose of living microbes more than sufficient to kill an unprepared control guinea-pig, it will be found that the prepared animal shows no reaction whatever, and that the living microbe very soon after its injection degenerates and disappears.

From these and similar experiments as also from experiments such as immunisation of guinea-pigs by intraperitoneal repeated injections with diphtheria bacilli, it seems feasible to assume that the immunising or germicidal or antimicrobic potency of the blood-serum in naturally acquired immunity as also in immunity produced by injection of living or dead microbes owes its origin principally or in part to substances derived from the bacterial bodies.

Now, in the case of the vibrio choleræ or of vibrio Finkler, by cultivating them in solidified blood-serum, which is liquefied by the growth, it will be found that after some weeks' growth at 37° C. a powerful toxin is produced in these cultures, which when used free of the living bacilli (or after sterilisation by heat) affects and kills guinea-pigs previously immunised by dead vibrios against living cultures in the same way and to the same degree as unprepared animals.

Acquired or artificial immunity against a specific toxin or a specific microbe may be limited to a single tissue or it may involve the whole body, thus Cobbett and Melsome

show (*Journal of Path. and Bact.*, November, 1894) that only a local immunity against the streptococcus of erysipelas or its toxin is produced in the rabbit in one ear previously the seat of erysipelas, and further that also in the process of immunisation of horses against diphtheria the region of a former inoculation acquires resistance against the new dose, whereas a new region of the skin is more suitable for the purpose (Cobbett, *Journal of Path. and Bact.*, January, 1896).

Again, the peritoneum of a guinea-pig may be immunised against the living or dead cholera vibrio by repeated previous intraperitoneal injections of cholera vibrios, without its alimentary canal being immunised against the growth and multiplication of the cholera vibrio (R. Pfeiffer and Wassermann, Klein). Koch and Gaffky showed that sheep successfully vaccinated after Pasteur's method of subcutaneous protective inoculation are still subject to anthrax by ingestion of spores.

R. Pfeiffer in a series of publications (already referred to in the chapter on Cholera) has demonstrated that by repeated intraperitoneal injections of guinea-pigs with living cholera vibrios, at first in small non-fatal, then gradually rising doses, the blood and blood-serum of the animal, as the immunity becomes greater and greater, possesses higher and higher *germicidal* or *immunising potency* against cholera vibrio: the higher the degree of immunisation the greater the germicidal power of the blood-serum. When a definite quantity of this "cholera serum" is mixed with a definite otherwise fatal quantity of living cholera vibrios and injected into an unprepared animal no result follows, the animal survives and remains well; already after a short time, in twenty minutes or so, after injection the vibrios degenerate

and break up into globules and granules. This germicidal action of the cholera serum *in corpore* has been already spoken of as *Pfeiffer's test*, it does not take place *in vitro*. The same law holds good more or less for other microbes : erysipelas, typhoid, Finkler, colon, prodigiosus, so that it seems to possess general application, but it must be added that it is by no means so absolute as is represented by Pfeiffer.

Bordet and Durham (*loc. cit*) show that a "potent serum" acts specifically (*specialised*, Durham) on its particular microbe or races of microbes also *in vitro*, inasmuch as in definite quantity and definite time a potent serum causes a more or less perfect separation, aggregation, and precipitation and loss of motility of the microbe contained in a suspension, without however destroying the microbe, for even long after the microbes have separated active cultures can still be produced with them.

The germicidal action of the serum as shown by Pfeiffer's test is on the whole but not without exception specific, that is to say it is only exerted against the microbe with which the animal had been actively immunised. As is now well known an animal, say a guinea-pig, can be protected intraperitoneally against a fatal dose of the living microbe, *e.g.* vibrio of cholera, by previous repeated intraperitoneal injections of the living or of the dead microbes (Klein), but this immunisation is of á comparatively temporary nature, and does not yield specific germicidal serum unless often repeated and with considerable doses.

A certain resistance, non-specific in nature, of the tissues against microbic action has been produced in various ways : thus Wooldridge showed that the injection of thymus extract

may protect rabbits against anthrax; Kossel, Vaughan, McClintock, produced a refractory condition to microbe infection by the administration of nuclein and nucleinic acid.

A very transitory local immunity of the peritoneum of the guinea-pig has been produced by Pfeiffer and Issaeff (*Archiv f. Hygiene*, vol. xvi. part 2) by intraperitoneal injection of normal serum, saline solution, nucleo-albumin and other substances.

INDEX

A

B

Q

R

S

T

P. 196. The Granular organism.

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Flagella (m. H.S.)

"micrococcus" (really bacillus) Melitensis 1-4

Plague 1-3.

Found cholera 1.

Found Enteritis 1.2

Found disease 4.

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pp. 410-466 Cholera