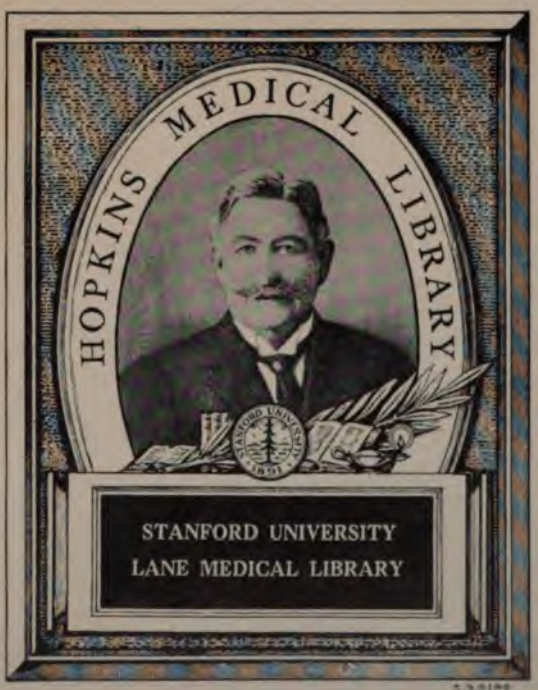
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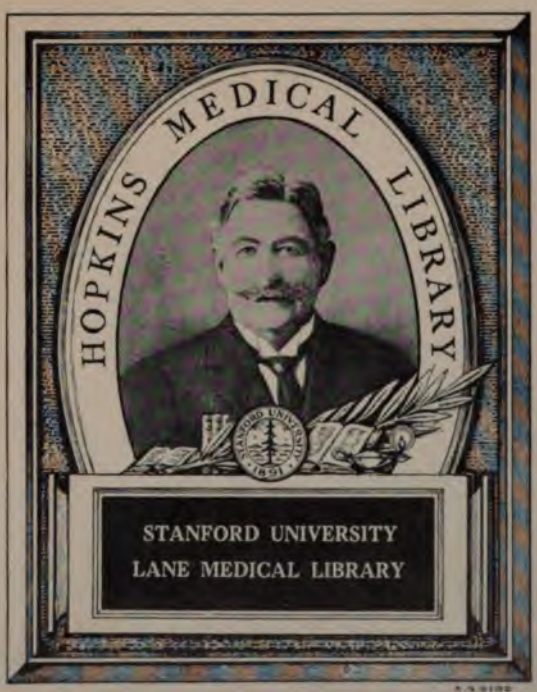
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RECENT ESSAYS

BY VARIOUS AUTHORS

ON

BACTERIA

IN RELATION TO DISEASE.

SELECTED AND EDITED BY

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London:

NEW SYDENHAM SOCIETY.

MDCCCLXXXVI.

PREFATORY NOTE.

About a year ago the Council of the New Sydenham Society decided on the production of a volume which should consist of selected Memoirs on Bacteria in Relation to Disease. It was fortunate enough to secure the services of Dr. Watson Cheyne as Editor. To him the selection both of Papers and Plates has been wholly entrusted, but he has been restricted by the Society's law from taking any by English authors.

EDITOR'S PREFACE.

In selecting papers for translation in the accompanying volume care has been taken to choose only those which are trustworthy, and more or less complete. In one or two instances, such as pneumonia* and diphtheria, where the matter is of great importance, abstracts have been given of the best bacteriological work on the subject, although there may not be sufficient evidence of the causal relation of the bacteria described to the disease in question. With these exceptions the evidence in the other cases is so strong as to leave it scarcely a matter for doubt that the bacteria in question are causally related to the disease.

Although these essays cannot replace the special works on bacteriological methods, nevertheless the reader will find in various parts descriptions of the chief methods of cultivating and staining micro-organisms. In their arrangement it was thought well to begin with Koch's paper on the methods of demonstrating bacteria, to follow with the more important results obtained by the use of these methods, and then to give in pretty full detail, under the articles "Antiseptics and Protective Vaccination," abstracts of what can be done in the way of preventing these diseases.

M. Pasteur's papers on inoculation for rabies have been translated on account of the intrinsic importance of the subject, as well as of the great interest felt in it at the present moment. The uncertainty, however, with regard to the period of incubation in man, the comparatively small number of persons who,

* An excellent paper on "Bacteria in Pneumonia," by Dr. Fraenkel, has just appeared in the *Zeitschrift für Klinische Medicin*, Bd. 5 and 6.

after being bitten by dogs which are undoubtedly rabid, develope the disease, and the absence of any conclusive evidence that all the persons inoculated by M. Pasteur have been bitten by dogs which are really rabid, render it necessary to suspend judgment until all the facts and statistics can be published in full.

For the strict proof that a micro-organism is the cause of a disease three things are necessary: firstly, the same species of micro-organism must be constantly present in the affected parts, at any rate during the early period of the disease; secondly, the organisms must be cultivated apart from the body, and thus separated from all the morbid materials; and thirdly, their re-introduction in a suitable manner into the body of an animal capable of being attacked by the disease must be followed by its production. As our knowledge of this department of science has increased, it has been found that wherever bacteria of the same kind are constantly present in the affected parts, and never found under other circumstances (thus excluding putrefactive bacteria), and where all the tests mentioned in the previous sentence can be carried out, these bacteria are the cause of the disease. Hence the constant presence of a special bacterium in a particular disease and its absence elsewhere, even though no further test is carried out, is now very strong evidence in favour of its causal connection with the disease. There are no trustworthy facts known in support of the opposite hypothesis, viz., that they might be the result of the disease. It is especially in human infective diseases that it is difficult to complete the proof, for several of these diseases do not affect the lower animals, and therefore it is impossible to test the bacteria found. In leprosy, for example, we have an instance of a disease which has not yet been transferred to the lower animals, but in which large numbers of specific bacteria are present. Nevertheless, although complete proof cannot be furnished, and although the conditions of infection are practically unknown, there is the strongest reason for believing, from a study of the affected parts

and from analogy, that the leprosy bacilli are the actual virus of the disease.

The selection of papers in this work has been limited, as far as possible, to those dealing with infective diseases in man, but all the human infective diseases have not been included, partly from want of space and partly because the researches were imperfect. Nevertheless it may be well to allude very shortly to some of those which have been omitted.

In gonorrhœal pus micrococci are constantly present in large numbers, and very frequently in the epithelial cells. The "gonococcus" generally occurs in pairs or groups, and the opposing surfaces of the cocci are flattened. These organisms are found at the very commencement of the disease, and diminish in number as the disease becomes chronic. Bumm states that he has succeeded in cultivating them on sterilized blood serum, on which they form a very thin greyish yellow coating, and that the inoculation of this cultivation into the urethra of a healthy woman was followed by a violent gonorrhœa.

In syphilis (see footnote, p. 427) Lustgarten has described an organism (a bacillus) in various secondary and tertiary lesions which stains in a peculiar manner, and which is probably causally related to the disease. At first it was thought that no other organism stained in the same manner, and that therefore the presence of this organism in the discharge might serve as a means of diagnosing the primary chancre. But lately Alvarez and Favel state that they have found bacilli in the smegma taken from under the healthy prepuce which stain almost in precisely the same manner as Lustgarten's bacillus, and as the tubercle bacillus, though they are somewhat less resistant to acids and alcohol. These results have been confirmed by Klemperer, and none of these authors have been able to find Lustgarten's bacilli in syphilitic products. Weigert, however, has found them in sections of gummata, and points out the extreme difficulty of demonstrating them in the tissues. Hence although the probabilities in favour of Lustgarten's view that

his bacillus is the cause of syphilis remain unaltered, yet the discovery of these smegma bacilli has for the present destroyed the hope that the demonstration of bacilli staining in this peculiar manner in the secretions from sores on the penis can be looked on as diagnostic of syphilis.

In rhinoscleroma very short rods about 1½ times as long as broad are found in large numbers, generally embedded in peculiar large cells (v. Frisch). According to Frisch they grow on nutrient jelly, and on blood serum, but this statement requires confirmation. They have also been found in cases of rhinoscleroma by Cornil in France, and by Payne in this country.

In relapsing fever spiral organisms (spirochætæ) are constantly present in the blood during and just before the febrile attack. They disappear in the interval between the attacks, and are found again in small numbers just before the fresh attack begins. Blood containing these organisms when inoculated into monkeys sets up similar phenomena with the presence of spirochætæ in the blood. The spirochætæ have not yet been cultivated outside the body, but there can be no doubt, when all the evidence is taken into consideration, that they are the cause of the disease.

With regard to ague the statements are contradictory, and by no means conclusive. Some time ago Klebs and Tommasi-Crudeli published a research, in which they stated that they had obtained a bacillus which they looked on as the cause of malaria. The experiments, however, on which this view was founded were by no means free from error, and bacteriologists in general have not accepted their views. A much more careful and trustworthy research has lately been published by Drs. Marchiafava and Celli (*Fortschritte der Medicin*, vol. iii., No. 24, 1885). They have not been able to find these bacilli, but have found that in fresh blood taken during an attack there are present in the red corpuscles bodies which have active amæboid movements, and which they term *plasmodia malariæ*. Some of these plasmodia also contain pigment, and apparently have

cilia. They further found that blood containing these bodies was infective. The plasmodia average in size from the $\frac{1}{10}$th to $\frac{1}{2}$ the size of a red blood corpuscle, and when they die they become round and then appear as granular biconcave bodies in the interior of the blood discs, often apparently partially extruded from them. These plasmodia are constantly present in the blood in fresh cases, they become fewer and eventually disappear on the administration of quinine, but in *perniciosa* they are present in large numbers and contain much pigment. Multiplication of these bodies by fission has also been observed. Should these researches be confirmed, and it be demonstrated that the plasmodia are the cause of the disease, we should have in ague an example of an infective disease not due to bacteria, or indeed to vegetable growths at all, but probably to one of the lowest forms of animal life.

A large number of infective diseases in animals have also been demonstrated to be due to bacteria, and some of these have been described in a former publication by this Society (*Traumatic Infective Diseases*, by Dr. Robert Koch). I may enumerate some of these. In the work referred to Koch described among other diseases anthrax, a septicæmia in mice due to small bacilli, and a septicæmia in rabbits due to small oval organisms. These bacteria have been cultivated outside the body, and the disease produced by the cultivated organisms, so that without doubt they are the virus of the disease. Among the pathogenic bacteria more recently described and not referred to in this work, the following are of interest:—Brieger's bacillus, which was found in fæces, and kills guinea-pigs in a few hours when injected subcutaneously.*—A bacillus causing tetanus in mice was found by Nicolaier in earth. These are fine, thin bacilli, somewhat longer than those of mouse septicæmia. When mice are inoculated subcutaneously at the root of the tail with these organisms they remain well for $1\frac{1}{2}$ to $2\frac{1}{2}$ days, and then a tetanic condition, first

* For a list of bacteria, pathogenic and non-pathogenic, which have been cultivated and studied, see Eisenberg's *Bacteriologische Diagnostik*.

of one and then of the other posterior extremity sets in, along with loss of motion in the anterior extremities. In the further course of the disease reflex tetanic spasms occur, and death takes place in 3 to 5 days. On post-mortem examination pus is found at the seat of inoculation, and there and in the surrounding tissue are numerous bacilli; in some cases also these bacilli have been found in the sheath of the sciatic nerve, and in the spinal cord. Similar effects are produced in rabbits and guinea-pigs. These researches are of interest with reference to tetanus in man, with regard to which the opinion is very general in Germany that it is a bacterial disease. In man, however, no specific bacteria have as yet been demonstrated.—In phthisical sputum largish micrococci, arranged in groups of 4, are often found (*Micrococcus tetragenus*). If these are inoculated into mice or guinea-pigs the animals die in 3 to 10 days, and these groups are found in large numbers in the blood-vessels in all the organs. It almost seems as if these organisms aided in the breaking down of the lung in the phthisical process.

What the conditions are which are necessary for infection in these diseases is still a matter requiring investigation. Much no doubt depends on the state of receptivity of the patient, and on the degree of virulence of the poison, but much also depends on other causes, on the discussion of which I will not enter here, as they are at present the subject of investigation.

How the bacteria act in the production of the disease is also a question on which but little has been done. Their mode of action is possibly different in different instances. Probably the most general mode of action is by the production of poisonous chemical substances, which act locally on the affected part, thus setting up the local appearances, and are also absorbed into the system and cause the fever, the general disturbance of the various organs, and the fatal result. In erysipelas, diphtheria, and cholera, for example, there is every reason to believe that the disease is essentially a local one, and that the general constitutional disturbance is not due to the passage of the living and

multiplying virus into the blood, but to the absorption of the poisonous chemical products of its growth. In other instances, however, such as the exanthematic fevers, there is no doubt that the virus is present in the blood itself, though here also in all probability its effect is due to its chemical action. Evidence of this chemical effect is well seen in the case of certain micrococci, where around the plugs of micro-organisms in the blood-vessels an area of dead tissue is found, into which the organisms have not yet penetrated. And to this action, rather than to deficient blood supply, is also due, I believe, in great measure the caseation of tubercular masses.

Attempts have recently been made by various investigators to isolate these chemical products (ptomaïnes), but partly from the great difficulty of the investigation and the large amount of time required, and partly from the fact that cultivations of mixtures of bacteria, and not pure cultivations of the individual organisms, have been employed, the results arrived at are but few and imperfect. The best work on this subject has been lately done by Brieger (*Die Ptomaïne*), and as he is now carrying on researches on pure cultivations of the pathogenic organisms, we may hope for further information on this matter. From putrefying meat, fish, gelatine, &c., he isolated a number of alkaloid substances, some of which were previously known, some however were new. The known substances, most of which had been synthetically made, were neurin, muscarin, dimethylamin, trimethylamin, and triæthylamin; and the new ones he calls neuridin and gadinin. From human cadavera also a number of alkaloids were obtained, the nature of these bodies depending on the stage of decomposition. Of these the following were new—viz., putrescin, saprin, cadaverin, mydalein, and another poisonous base to which he does not give a name. Several of the alkaloids were poisonous to rabbits or guinea-pigs; among which may be mentioned neurin, muscarin, trimethylamin, and mydalein. Brieger also refers to a few preliminary attempts which he has made to analyse the products of pure cultivations of the pathogenic

bacteria. Friedländer's pneumococcus breaks up carbohydrates into formic acid, acetic acid, and æthylic alcohol. A bacillus isolated by Brieger from human fæces, and which kills guinea-pigs, gives propionic acid as the chief product of its growth in grape sugar. The typhoid bacillus gives considerable quantities of æthylic alcohol, and small quantities of volatile fatty acids; it breaks up grape sugar into lactic acid. From its cultivations in meat infusion small quantities of a ptomaine were obtained, which produced marked effects on guinea-pigs. The animals showed slight salivation and increased frequency of respiration. They lost control over the muscles of their extremities and back, without however any true paralysis; the pupils became dilated, and ceased to react to light; there was also profuse diarrhœa, and the animals died in 24 to 48 hours. Although these results are as yet very imperfect, the advances made in the future will probably depend as much on the chemistry of bacteria as on any other department of this science.

W. Watson Cheyne.

CONTENTS.

ON THE

INVESTIGATION OF PATHOGENIC ORGANISMS.

By Dr. ROBERT KOCH,

(*Mittheilungen aus dem Kaiserlichen Gesundheitsamte*, [illegible])

TRANSLATED BY

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ON THE INVESTIGATION OF PATHOGENIC ORGANISMS.

INTRODUCTION.

Up to the present time hygiene has been able to gain but little advantage from the recent strides in our knowledge of the pathogenic organisms. This circumstance is due to the fact that the greater number of the questions relating to the pathogenic micro-organisms which have to be considered from the point of view of practical hygiene, can only be solved by means of trustworthy methods of separating the different kinds of organisms from one another; for as far as hygiene is concerned, we have not merely to discover whether this or that soil or drinking water contains bacteria in general or other lower organisms, but whether among the micro-organisms present there are any which are specific—in other words, which can produce disease. And if the existence of a distinctly harmful organism, or of one, to say the least, suspicious, be demonstrated, then it must be separated from other kinds, which necessarily complicate and mar the observation; all its peculiarities must be studied, its life history, its development, and every condition that is advantageous or disadvantageous to its growth. Knowledge of this kind can only be gained by means of continued cultivations of the different kinds—so-called pure cultures—for which, however, there exists at present no method which is both practical and certain in every respect. I have laboured hard to fill up this gap, and have finally obtained certain results, which although they are susceptible of and

need great improvement, yet up to the present time have proved of great service in the researches carried out into infective diseases and the subject of disinfection at the Sanitary Institute. These will now be described, partly to make known these practical procedures, which are capable of manifold application, and partly to render such work as is based upon the application of these methods, and published in these pages, more easily intelligible.

ENUNCIATION OF THE PROBLEMS TO BE DISCUSSED.

As a rule the following points must be observed in investigating micro-organisms from the point of view of sanitary science. In the first place, it must be definitely determined whether the organisms in question are pathogenic at all, that is to say, whether they can cause disease. Following on that comes the proof of their inoculability, that is, of the possibility of their transference from one individual to another previously healthy, this transference being attempted both on individuals belonging to the same species as those in whom the disease arose spontaneously or was artificially produced, and on individuals of other species.

Next we have to trace out the mode in which the pathogenic organisms enter the animal body, to follow their behaviour outside the body in the air, water, and soil, and finally to determine what influence reagents may exert upon them either in the way of destroying them or preventing their development. Hygiene is only interested in their existence in animal tissues in so far as an explanation of the mode of infection may thereby be rendered possible, whether, for example, the pathogenic organisms are localized in the alimentary canal, whether they pass into the blood, or whether they form resting spores in the interstices of the tissues.

So long as we do not know the direct exciting cause of any particular contagious disease, such as cholera, the plague, &c., or have to determine roughly the insanitary condition of air, water, or earth, and decide upon the value of certain substances as disinfectants, we shall frequently be in the position of having to study the origin and life history of those

kinds of bacteria and fungi which are most nearly allied to the pathogenic organisms, and in this way we may be able to form some more or less probable opinions as to the nature and behaviour of the as yet unknown but presumable cause of the disease.

DETERMINATION OF PATHOGENIC PROPERTIES.

We turn now to the first of the above problems, to the question of the determination of the pathogenic property and infective virulence of micro-organisms. By some observers it is still maintained that bacteria occur in the blood and tissues of the healthy body; this assertion does not rest upon direct microscopic observation, but partly on theoretical considerations, and partly on experiments upon the decomposition of portions of healthy organs, removed and kept under antiseptic precautions. Against these experiments there exist very weighty objections, the consideration of which would carry us here too far from the present subject. This much is certain, that while with the aid of the microscope and other methods of observation even single bacteria can be recognized with certainty in the organs of an animal, no one has hitherto succeeded in discovering the same in the blood and tissues of a healthy individual so unmistakably that there could be no doubt of their having taken up their abode therein during life. When bacteria—and the same thing holds for other micro-organisms—are found in the interior of organs, whether it be in the blood-vessels or lymphatics, or even in the tissues, in such a position that they can only have got there during life, or when the unmistakable effect wrought by micro-organisms on the tissues they invade can be recognized (such, for instance, as necrosis of cells in definite areas, accumulation of leucocytes in their neighbourhood, penetration of the cells by foreign organisms, &c., &c.), then such micro-organisms must be looked upon as pathogenic, at least they must be regarded as suspicious, and the import of the discovery established by further observation.

It is far more difficult to decide upon the pathogenic power of micro-organisms found on the outer surface of the body and on the mucous membranes. In this case it is only the differences

of form between the probably pathological organisms and those harmless parasitic fungi which have been recognized for a long time as always present on the body, or the preponderating numbers of the former that can afford a criterion of their specific importance. Unfortunately very little attention has been devoted to the harmless parasites, a defect which makes itself peculiarly felt in relation to the various affections of the alimentary canal, and thus great care is necessary before accepting statements as to pathogenic bacteria in that region; all doubt cannot be removed from assertions on this point, since confusion is possible owing to the fact that organisms habitually present in the alimentary canal may under certain circumstances (which even if extraordinary may also be favourable) increase in number and so assume importance from their mere quantity. But the time is certainly not far distant when the harmless parasitic organisms of the healthy body will be so well known that they will be recognized to be such at once; and if the necessity should arise of distinguishing the specific varieties, the former will be separated with certainty from the pathogenic kinds which will have more recently found an entrance.

DEMONSTRATION OF THE PATHOGENIC ORGANISMS.

In proceeding to search for suspected pathogenic organisms (and chiefly for bacteria) in diseased tissues, great, and sometimes almost insurmountable, difficulties will be met with at the outset if the research is carried on by the ordinary methods of microscopical observation without special details of preparation and examination. For while many pathogenic bacteria are so distinct in size, characteristic form, and movement that they cannot be easily overlooked, others possess such a simple outline, and are so small that when they are mixed up with similarly shaped degenerated parts of tissue and corpuscles it may be impossible to distinguish them. Fortunately, however, the bacteria have one peculiarity which renders it possible to overcome these difficulties. This peculiarity is simply the power of taking up and retaining the colours of certain dyes, especially the anilines. The very fluids, however, in which bacteria most commonly occur, such as blood, mucus, lymph, &c., produce a

precipitate when freely treated with the aniline dyes, which precipitate is also coloured, and either imitates the granular and thread-like shape of bacteria, or else when in large amount hides the micro-organisms; hence further modes of preparation of the material are necessary before the bacteria in it can be satisfactorily demonstrated by means of staining solutions. In searching the stained preparation with the microscope, too, it is absolutely necessary to pay special attention to the illumination of the object if the full advantage of the method of staining is to be obtained.

Several years ago I described at length the most suitable methods of demonstrating bacteria in fluids and animal tissues, and these have been partly published in F. Cohn's *Beiträge zur Biologie der Pflanzen*, Bd. 2, Heft 3, and partly in my pamphlet on Traumatic Infective Diseases. For the details of these methods I must, not to be prolix, refer to the above-mentioned papers, and shall now only mention those points in which improvement has lately been made, or about which there has been some misapprehension, rendering further explanation necessary.

MICRO-ORGANISMS IN FLUIDS.

The process of rendering the bacteria in fluids, such as blood, pus, and lymph, visible by means of staining reagents, consists in spreading the fluid containing them in as thin a layer as possible on a cover-glass, drying it, and then subjecting it to the action of the staining solution. If the bacterial fluid contains none or very little albumin, then the staining almost always succeeds in a striking manner. But if it is more or less albuminous, then the dried layer adheres imperfectly to the cover-glass, and the greater part of it may be softened by the staining fluid, separated, and washed off from the glass. And further, since the albumin is not rendered insoluble by the drying process, it mixes with the staining fluid and forms with the dye a precipitate which clings to the cover-glass, thus covering and concealing everything. This disadvantage can be almost completely got rid of if a solution of aniline brown in glycerine is used instead of the watery solutions of fuchsin (magenta), methyl violet, &c., which are commonly employed. In the above-mentioned paper on the investigation of bacteria, the glycerine solution of aniline

brown is strongly recommended, and all the photographs in which bacteria are represented in blood or lymph were taken from preparations stained with this solution. In spite of the above warning many continue to attempt to stain preparations of blood in watery solutions, and yet in a most incomprehensible way the inevitable failures are laid to the charge of the method. Even quite lately M. Wolff* has asserted that the processes advocated by myself do not afford a sure means of diagnosing bacteria. He complains that he endeavoured in vain to obtain his preparations of blood (which he stained with watery solutions) free from "granules and particles."† Besides the well-known fact that in skilful hands a great deal can be accomplished with the above-mentioned method, from among many valuable researches I may quote a paper published by Ogston, who has demonstrated in very numerous cases different kinds of bacteria in fluids taken from the human body, and who has come to the following opinion from his practical observations, viz., that "it is impossible to confound them (micro-organisms) with any such granular bodies as those alluded to by Wolff" (*The British Medical Journal*, 1881, March 12).

It was nevertheless well worth while to improve the methods of staining bacteria in albuminous fluids so that they might give accurate results even in unpractised hands; the most fundamental point to be secured was this, that the film of albumin fixed on the cover-glass should be converted into an insoluble form. If the prepared cover-glasses are kept for some time it will be observed that after a few days, at times only after some weeks, the film is harder, sticks closer to the cover-glass, and produces much less precipitate with the staining fluid.

* Virch., *Archiv. für Pathologische Anatomie*, Bd. lxxxi. Hft. 2 u. 3.

† In criticising my methods of research Wolff has fallen into the error of quoting a few passages from my pamphlet on Traumatic Infective Diseases, relating to the examination of bacteria in sections taken from organs which had been hardened in alcohol, whilst Wolff himself only worked with the dry method, and with that form of it least adapted for his purpose. If Wolff had acquainted himself with the two methods described by me in full detail, then he must have perceived very soon that the staining of the dried film on the cover-glass had a totally different effect to that obtained in sections of tissues by Weigert's nuclear staining method, and therefore having failed in his application of one method, he should not have concluded that the other was useless. I trust that this explanation will suffice to show that I maintain the perfect accuracy of those passages that have been attacked by Wolff, concerning the possibility of diagnosing bacteria in tissues with certainty.

Still better results are obtained, and the dried film can be rendered insoluble more quickly if the cover-glass is placed in solutions which have the power of coagulating albumin, such as solutions of chromic acid, the chromates, alum, tannin, &c. From the favourable action of alcohol in coagulating albuminous compounds when tissues containing bacteria are hardened in it I was led finally to harden in alcohol the albuminous film fixed on the cover-glass, and thus I obtained the result desired. If the preparation be placed in absolute alcohol for some time the film becomes quite insoluble, and takes the staining equally and in a most satisfactory manner. No granules nor other confusing precipitates complicate the diagnosis of micrococci or other kinds of bacteria existing in blood, pus, &c. There is only one uncertainty about the method of hardening in alcohol, and that is the determination of the time during which the preparation should remain in the spirit. At times a few days only are sufficient, but often it is not until several weeks have elapsed that the necessary degree of insolubility of the albuminous film is attained. It is therefore advisable to prepare a considerable number of cover-glasses, and to take one out of the alcohol from time to time and test it by staining.

But in the investigation of infective diseases it is very often desirable to determine at once the existence of bacteria in the organs of an animal, as, for example, in examining the results of an inoculation, and in deciding on further inoculations, &c. In such cases obviously one cannot wait until the hardening in alcohol is complete. It was necessary, therefore, if the method was to be of practical utility under all circumstances, that some means should be devised to provide for this contingency. When Ehrlich* published the remarkable results of his observations on the differentiation of the various granular blood corpuscles which was obtained by heating preparations of blood, it seemed important to investigate the action of heat on preparations of bacteria. Ehrlich subjects a thin film of blood dried on the cover-glass to a temperature of 120°—130° C. for one or several hours. Under the action of such intense heat the film of blood became completely fixed and insoluble, but experiments showed that the bacteria lost their power of taking up the colouring matter. For our object it was,

* *Verhandlungen der physiologischen Gesellschaft zu Berlin*, 1878—79, No. 20. *Zeitschrift für Klinische Medicin*, Bd. i. Hft. 3.

however, only necessary to continue the action of heat until the albuminous substances were rendered insoluble, and this can be attained in a far shorter time. Thus if the cover-glasses remained only a few minutes exposed to a temperature of from 120°—130° C., the film became so hard that it gave no precipitate with the staining fluid, and yet at the same time became perfectly stained. The duration of the action of the heat necessary to obtain a successful result is, however, quite uncertain, for while the preparation was sometimes ready in two minutes, at others it had to be heated for from five to ten minutes. It is most important to notice that some bacteria (for example *Bacillus anthracis*) if heated and stained appear somewhat altered, they appear thinner and more slender than when stained with the glycerine brown process, and, moreover, the peculiar segmentation of the anthrax bacilli is not so distinct.*

On this point I will take the opportunity of remarking that slight differences (similar to those just mentioned) between bacteria will be observed, especially as to their breadth, &c., if the specimens are prepared differently or stained with different staining reagents. Hence for the exact comparison of different kinds absolutely the same process must be employed in preparing the specimens. If, for instance, one wishes to demonstrate most easily the characteristic segmentation of the anthrax bacilli, which of itself serves as an infallible diagnostic point, then, as has already been described, staining with glycerine brown must be carefully performed. Although this particular form of the anthrax bacilli is seen by means of other staining substances it is not then so evident that one could base a diagnosis upon it, and even if by other methods of preparation one cannot find this characteristic of anthrax bacilli, it is certainly not justifiable to conclude, as Zürn† has done, that segmentation of the bacilli does not occur.

In spite of the above-mentioned drawback, the heating process

* A rough rule now given by Dr. Koch is to pass the cover-glass three times through a gas flame about the rate at which one cuts bread.—Ed.

† Separatdruck aus dem I. Bericht der neuen landwirthshaftlichen Institut der Universität Leipzig, 1881.

Zürn relies for support of his assertion on a few photographs of anthrax bacilli which accompany his publication. But these photographs do not satisfy the most modest requirements which may be demanded of micro-photographic illustrations, and if the preparations are no better, then it is perfectly clear how Zürn arrived at his anomalous result.

is a valuable addition to the methods of investigating bacteria. In the researches into infective diseases at the Institute of Public Health it is constantly employed, and indeed has become indispensable. At every post-mortem examination of an animal which has been the subject of an infective disease the blood, the lymph at the point of inoculation, serum from the lungs, spleen, and if necessary from other organs, are in this way examined, and according to the results of these observations (which necessarily are only of a preliminary character, and are supplemented by subsequent careful investigation of the organs hardened in alcohol) the further course of the research is determined upon. As regards the choice of dyes, we have also to thank Ehrlich* for introducing a new aniline colour, methylene blue, which is to be strongly recommended, since it is of especial value in staining heated preparations. In difficult and doubtful cases it is advisable to try other aniline dyes, for many bacteria are quite peculiar in the way in which they take up colouring matters, a fact to which I shall return later on. Wherever it is possible, a few preparations should be stained brown, so as to render it possible to take photographs of the bacteria, for although the remarkably powerful and concentrated tint of the red and blue aniline colours catches the eye far more readily than the somewhat sombre brown colours, still no one has succeeded hitherto in obtaining† good photographs of bacteria which have been stained either blue or red and mounted in Canada balsam, whilst there is not the least difficulty in obtaining photographic pictures from preparations stained yellow or brown.

In preparations in which the film has been rendered insoluble by treatment with alcohol, or by heat in the way described above, and coloured by a suitable staining fluid, there should be no granular precipitate or particles of colouring matter, &c.; therefore only pre-existing organized elements, which are contained in the fluid spread out on the cover-glass, are stained, whilst if the

* *Zeitschrift für Klinische Medicin*, Bd. ii. § 710.

† If preparations stained red or blue are photographed when mounted in a solution of potassium acetate, or some other solution with a low refractive index, the picture on the negative is not due to the action of the blue or red aniline colour to which the layer of collodion is insensitive, but it is due to the difference between the refractive index of the protoplasm of the bacteria and that of the fluid which surrounds them. The picture obtained is, therefore, not really an actinic result such as we are accustomed to see in bacteria mounted in Canada balsam, and it cannot, under any circumstances, be compared with the latter.

staining solution is neither too weak nor too strong, the dried residue of the fluid, *i.e.*, the dried plasma, is indicated by a faint tint. Hence only the cells and their products, whether of natural origin or formed artificially, can supply material which might be confounded with micro-organisms.

With respect to the artificial products just mentioned, every one who has made a few observations on blood, pus, lymph, &c., will soon convince himself that the more dilute the fluid (to be investigated) is, the less will the shape of the cells contained in it be altered when it is spread out on the cover-glass. With blood, for instance, the white blood corpuscles, with rare exceptions, preserve their rounded outline, and appear after drying as circles, in which lie multiform, frequently ribbon-shaped, nuclei. But if the fluid is thick and tenacious, such as is so often the case with the tissue juices of organs such as the spleen or lungs, it is scarcely possible to spread it out in a thin layer without distorting the cell elements more or less, or even tearing and crushing them, in consequence of which comet-shaped figures are often produced, of which the remains of the cell nucleus form the head, while the tail is made up of the rest of the nuclear protoplasm stretched out behind. The explanation of these frequently fantastical appearances is quite evident. Even a few neighbouring fields of the microscope show all transition forms, from the thread-like figures which lie at the borders of the mass where it is thinnest, to the unaltered, *i.e.*, uninjured, cell nuclei at the thicker parts of the preparation. One would therefore think that these crushed cell nuclei, which at the first glance can be recognized as such, could not be mistaken for micro-organisms, and yet this has been the case. Thus Fokker,* in particular, believed from his researches that he had discovered that there were two kinds of splenic fever. In one of these the well-known bacilli were easily found, while in the other kind they were wholly absent, or only present in very small number. In this latter form, however, he found long threads, which as he said were connected with the lymph corpuscles and resembled spermatozoa, the lymph corpuscle forming the head, and the thread-like body the tail. Fokker took these appearances (which he called fungus mycelium [*Pilz-*

* *Centralblatt für Medicinischen Wissenschaften*, 1880, No. 44; 1881, No. 2. *Weekblad van hed Nederlandsch Tydschrift voor Geneeskunde*, 1881, No. 4.

draden]) to be genuine fungi introduced by inoculation, which, when taken up by the lymph corpuscles, grew inside the same, distended them longitudinally, and at last bored their way out at one end. Finally, Fokker found the same appearances in normal spleen, but even that did not teach him the true nature of his putative fungus threads, but he concluded therefrom that fungi normally occurred in the tissues of the body. A drawing which accompanies his publication leaves it beyond doubt that Fokker's fungus threads are drawn out cell nuclei.

To mistake the granules of granular corpuscles for micrococci is much more excusable, especially of those bodies termed by Ehrlich plasma cells (*Mastzellen*). The granules in many of these corpuscles appear to be very loosely held together, the corpuscles are easily destroyed in spreading out the material on the cover-glass, the granules become freed, and to the unpractised eye simulate micrococci either single or arranged in groups. Similar corpuscles of especially large size and regularly developed occur in the blood, and especially in the spleen and lungs, of white rats, less frequently in white mice, and I have seen preparations from the above-mentioned organs in which deeply stained masses of granules from the crushed corpuscles lay distributed in broad streaks in such quantities that the sight would have elicited a cry of joy from an enthusiastic micrococcus hunter. But these preparations were obtained from healthy animals, and by searching the same specimens more carefully I found many uninjured corpuscles in which the granules were arranged around a feebly stained nucleus, and could be easily recognized as forming constituent parts of the granular corpuscles. Moreover, these granules are almost always of unequal size, and can also be often distinguished from micrococci by the peculiar tint they assume with staining reagents. In every case observations of this sort demand the exercise of caution, and if there is any doubt about them, comparison must be made between them and similar preparations taken from normal animals, and sections taken from the hardened tissues which show the doubtful masses of granules in their natural position and relation to surrounding structures. I have never yet met with a case in which it was not possible to distinguish with certainty between micrococci on the one hand, and the elements of the granular corpuscles on the other. I strongly advise any one who is

engaged upon experimental researches into infective diseases to acquire perfect experience in this province of the subject by making himself familiar with the results of Ehrlich's observations upon the granular corpuscles.

Again, Ehrlich's observations are most valuable with respect to the study of infective diseases in another direction: Ehrlich discovered that among the blood corpuscles, which every one supposed to be practically alike in size and everything else, well-marked differences could be demonstrated by means of staining reagents, or, to speak more exactly, by the character of their chemical reaction, which led him to believe that these differences had to do with the origin and physiological function of the corpuscles. Well now, it may be asked, What has the differential staining of the blood corpuscles to do with infective diseases? Simply this, that in one or more groups of infective diseases the contagion might be a body resembling a leucocyte, possibly an amœboid form, and in this case it would be of the greatest value if we possessed unfailing means of differentiation such as Ehrlich's staining process undoubtedly offers. It is undoubtedly a one-sided view, though it is one at the present time very generally adopted, that every unknown contagium must be a bacterium (schizomycetes). Is it not just as likely that other kinds of micro-organisms might live as parasites in animal tissues, although I cannot assert that they would only exist as amœboid forms? There are others which belong to the class of protista,* which are also suspicious. The belief, how-

* This conjecture has received earlier confirmation than I expected. Von Wittich announced recently in the *Centralblatt für Medicinischen Wissenschaften*, 1881, No. 4, that he had found spirilla in the blood of Hamster rats. This led me to immediately investigate the blood of these animals. One of the animals which I had procured for this purpose died spontaneously on the second day of its imprisonment; but it showed on both days the symptoms of severe constitutional disturbance, and did not appear, like the animals observed by V. Wittich, to be perfectly healthy up to death. At the post-mortem examination no changes could be found in the internal organs such as could be considered to be causes of its death. However, I found in the blood innumerable organisms, which in their movements did not resemble spirilla or Spirochætæ in the least, but moved extremely actively, with a serpentine course, among the blood corpuscles. When a drop of the blood was examined on a hollow slide, these parasites arranged themselves in absolute rest at the border of the drop, so that their shape could be accurately determined. They possess a spindle-shaped body of finely granular substance. On the anterior portion of this spindle-shaped swelling there are usually one or two dark granules, behind which the spindle is gradually extended as a long thread, which appeared to me to terminate in several instances in a double flagellum. These parasites have clearly no relationship to spirilla and spirochætæ, and I believe that they belong to the class of flagellate monads, and are most probably identical

ever, that amœboid forms can play the part of parasites is alluded to here simply because a very striking example occurs in the vegetable kingdom. There is a curious disease which attacks cabbage plants, and which remained for a long time a riddle to botanists until Woronin* found out the explanation of it. He showed that an organism in the shape of a colourless finely granular protoplasmic sphere, probably one of the simplest forms of the myxomycetes, penetrated into the roots of the cabbage plant. When lodged in a parenchyma cell of the root this parasite, which was termed by Woronin *Plasmodiophora brassicæ*, fused itself with the protoplasm of the cell so that at first it could not be distinguished from the cell contents, but a little later made its presence obvious by characteristic changes in the cell. The subsequent most interesting course which the development of the plasmodiophora follows does not further interest us here. Only the early period of its residence in the root is for the moment of importance. For, supposing that extremely small masses of colourless protoplasm could find their way in a similar manner into the juices of the animal tissues, and multiply there, would the state of matters be clearer than in the cabbage root, where it is impossible to differentiate the parasites from the protoplasm of the cells? One would certainly be inclined to consider such protoplasmic masses as fragmentary debris of leucocytes if one could not separate them distinctly by delicate staining methods. In studying *Plasmodiophora* Woronin had already been led to very similar considerations. He conjectured that many pathological growths and swellings which occur in the animal body owe their origin and development to a peculiar irritation, &c., set up by small myxamœbæ, which, forcing their way into the animal tissues, develop into plasmodia.

Eidam† also, who confirms Woronin's observations, expresses himself in the same way, and considers it possible that the etiology of many hitherto unexplained infective diseases, in which fruit-

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with the "flagellated organisms" described by Lewis as occurring in the blood of rats (*Quart. Journ. of Micros. Sci.*, xix., 1879). They stain fairly well with Bismark brown. At another time four other Hamster rats fell ill, spontaneously, and died; numerous flagellate monads were also found in the blood of all these animals.

* Pringsheim's *Jahrbücher für Wissenschaftliche Botanik*, 11 Bd., 1878.

† *Der Landwirth Allgemeine landwirthschaftliche Zeitung*, 1880, No. 97.

less search had been made for bacteria, depends upon an invasion by parasites which cannot be distinguished from the tissue elements of the body, and behave in fact just as plasmodiophora.

I have alluded at considerable length to the instance of plasmodiophora because it affords a valuable warning against merely hunting for bacteria, as is so frequently done now when search is made for living contagia, and it shows us that we should also direct our attention to the other organized elements of the infected blood or organs.

MICRO-ORGANISMS IN ANIMAL TISSUES.

To the foregoing account of the investigation of fluids must now be added that of the investigation of animal organs, which will give us information as to the position and distribution of pathogenic organisms in the tissues, their relation to the surrounding cells, &c.

Since we have to do with objects of the smallest dimensions, which are only to be recognized in the very thinnest sections of the tissues under investigation, great advantage will be gained by the preparation of sections by the microtome. As for the further treatment of the sections with staining solutions, dehydration, clearing and mounting in Canada balsam, as well as the advantages and method of use of Abbé's substage condenser, I must refer the reader to the full description which I gave in my pamphlet on the Traumatic Infective Diseases,* to which I have very little to add.

In the first place, I must again repeat that investigation is not to be exclusively directed to bacteria, but attention must also be paid to all other kinds of micro-organisms which may possibly be

* Some time after these lines had been written I became acquainted with Weigert's publication on the Technology of the Microscopic Investigation of Bacteria (Virchow's *Archiv.*, Bd. 84, Heft 2). Weigert claims for himself, and correctly, the merit of having been the first to apply the method of nuclear staining to the investigation of micro-organisms. In my first published paper I laid especial stress upon the fact (and I repeat it again here) that I have to thank Weigert for the knowledge of the application of nuclear staining to the demonstration of bacteria in sections of hardened tissues, and that I simply called attention to the necessity of employing Abbé's condenser for accurate diagnosis of stained bacteria in sections. Finally, I am very glad to find that Weigert, as the result of his extensive experience in regard to the presence of bacteria, which possibly we may not be able to demonstrate with the means of staining at present at our disposal, comes to conclusions similar to those which I have developed further on.

present, and that especial care is to be taken in the preparation of the portions of tissue, and more especially in the hardening processes. Up to the present hardening in alcohol has proved the most valuable procedure in regard to bacteria, but whether it is so for all kinds of micro-parasites is, to say the least of it, doubtful, and it is certainly advisable to employ other hardening reagents as well, such as chromic acid and osmic acid.*

I learnt one fact, which appears to me to be worthy of mention, in experimenting on staining pathogenic bacteria with various dyes, especially in staining with the brown stain to be presently mentioned. It is the very different reaction which different kinds of bacteria show towards certain colouring matters. At the outset it appeared as if all kinds of bacteria would behave alike in this respect, but such is not the case; just as the different kinds of bacteria show many other special points of difference among each other, so also do they differ in their power of taking up certain staining dyes. To quote a most striking example of this kind I will mention that while the spirillum of relapsing fever in a layer of blood dried on a cover-glass is stained intensely by fuchsin, methyl violet, or gentian violet, &c., it is not possible to stain the same organism in tissues by using the same dye and the nuclear staining method. In my experiments the greatest success was obtained with brown aniline dyes, but even this was rather poor, so that the search after spirochætæ in tissues is not one of the easiest of undertakings. Since it is considered by most people impossible to demonstrate spirochætæ in hardened tissues, I publish, as a proof that they can be stained in the same, photographs taken from sections of the brain of a monkey which was inoculated with relapsing fever, and died at the acme of the disease.

The bacilli of leprosy, on the other hand, behave in a diametrically opposite way. They must have been quite recently dried on the cover-glass to take the stain. Even if only a short time elapses after the drying process they will not stain at all. If hardened in alcohol for a very long time, even for some

* The practical value of this suggestion will be endorsed by many workers in bacteriology. It will be found of advantage to harden some tissues in bichromate of ammonia, 2 per cent. solution, for a fortnight or three weeks before placing in alcohol. Specimens hardened originally in alcohol for some time can be improved, as regards the ease with which the micro-organisms can be demonstrated in them, by being placed in a similar solution of bichromate.—V. H.

years, they will stain very easily with fuchsin, gentian, violet, &c., but very badly with aniline brown.

All micrococci stain equally intensely with blue, red, and brown aniline dyes. But among the bacilli there are various differences, many taking all the aniline stains powerfully, while others, as for example the short bacillus first described by Eberth in typhoid fever, stain less intensely though not so feebly as would appear from Eberth's description.

These differences in capability among bacteria to take up colouring matters merit attention, because they partly afford evidence of difference among the various kinds of bacteria as to their chemical properties, and partly also indicate the necessity for caution in estimating the value of negative results, as after consideration of what has been said it is not improbable that there may be some kinds of bacteria which will not take up the colouring matters at present in ordinary use.

This opportunity may be taken of mentioning a small point with regard to staining which under certain circumstances may be of the greatest service. It consists in moderately warming the staining solution, as a result of which the time which the staining takes is greatly shortened, and at the same time the latter is considerably intensified. Indeed it would appear that some pathogenic bacteria can only be sufficiently darkly stained by this method; the heating, however, must not be carried higher than 40°—50° C., for otherwise sections which contain a considerable proportion of connective tissue will begin to shrink up.

PHOTOGRAPHIC REPRESENTATION OF MICRO-ORGANISMS.

Photographs are of the highest importance in investigating micro-organisms, for if ever a pure objective perception, free from every preconceived idea is necessary, it is in these investigations. But hitherto the exact opposite has prevailed, and nowhere have more numerous and highly coloured ideas been conceived, and therefore more confusion of meaning caused, than in the study of the pathogenic micro-organisms. For no one will deny that the difference in the conception of the characteristics of one and the same object almost always depends upon the fact that the object appeared to the one observer under a different

form to that in which it was seen by a second. It must be remembered that here we have to do entirely with microscopic objects, and that two observers with the microscope cannot see the same object simultaneously and form a joint opinion with regard to it, but that first one and then the other looks at the object in question; and as all microscopists know, even the slightest turn of the fine adjustment causes so small an object as a bacterium either to disappear entirely from the field, or to appear with different markings and shadows. Agreement will always be facilitated when the observations are made with the same instrument, that is to say, with the same illumination, the same objective, and the same magnifying power. But if the conditions under which the microscopic object is seen are very different, if, for instance, one observer works with a small, another with a large diaphragm, or one with a high and the other with a low eye-piece, &c., or if the preparation and staining of the object is dissimilar, if moreover it is mounted in fluids of different degrees of refrangibility, how can any one wonder when one microscopist asserts that the object as seen by him is quite different from that described by another, perhaps thicker or thinner, or more or less bright, or that possibly he cannot see it at all, and therefore denies its existence? and how can the error of observation be detected in such a case, on whichever side it may have arisen, among the many possibilities indicated? Was it due to the mode of preparation of the specimen, or to the method of using the microscope, that the observers came to different opinions about the same object? To decide this would be impossible without the aid of some other expedient, for each of the disputants would naturally maintain his own interpretation of the facts, and medical science would not know which to credit.

For these misunderstandings, which have in microscopy already frequently been proved to exercise a most injurious influence on the progress of the science, there is only one remedy, and that is photography, which comes both as a harmonizing mediator and as an instructress. Indeed, under certain circumstances, the photographic picture of a microscopical object is more valuable than the original preparation, for if I give some one a microscopical preparation with the object of his examining some particular spot in it, for instance a lymph vessel containing bacteria, I have no guarantee that

he will find the right place in it, or if he does find it, that he will select the proper focus or the best illumination, &c. Photography, on the contrary, gives the microscopical picture once and for all, and reproduces it without the slightest error, in exactly the same focus, magnification, and illumination as when it was taken. Moreover, nothing is more simple than to come to an agreement as to what a photograph exhibits, for several observers can simultaneously look at the picture which before was accessible only to one individual at a time; one can point out with the finger the object in question, measure it with compasses, compare it with other photographs similarly taken, whether of the same or of other objects; in short, one can do everything which may aid in the comprehension of the disputed point.

Another perhaps more important use of photography is to be found in the powerful control which it enables a microscopist to exercise over his own observations. Drawings of microscopical objects are almost never absolutely true to nature; they are always prettier and appear with sharper lines and stronger shadows than the original, and, as happens not unfrequently, a sharper line or darker shadow in particular places may give the figure quite another signification. The drawing, further, is no criterion of the value of the preparation, for even a badly prepared, and in itself a doubtful specimen may be represented by a beautiful and apparently convincing drawing. This is obviously impossible with photographic illustrations, in which the shadows in the preparation form the picture itself, and in which, as it were, the microscopical object copies itself; it is therefore impossible to introduce the slightest improvement into the details of the picture. It further becomes necessary to make preparations which shall not only satisfy the demands of the observer, but which also by reason of their demonstrable accuracy can meet general criticism. Drawings of microscopic objects can scarcely be criticised, since the drawing is often involuntarily executed according to the mental bias of its author. On the other hand, if an investigator publishes a photograph he frees himself from every subjective influence in representing his preparations, and places to a certain extent the object of his researches before his audience, and admits them to take a direct share in his observation. Moreover, the consciousness that a photograph of the result of his

work will expose him to much wider criticism by the scientific world, compels a microscopist to check the accuracy of his observations, and not to hurry the publication of the result before he is certain of his facts. The general adoption of photography in microscopy would no doubt prevent many immature publications. A few examples will show how especially valuable photography is in the investigation of the various problems of infective disease.

Lewis,* who has been for a long time engaged in studying bacteria, has among other things investigated, as opportunity offered in the epidemics which are so common in India, the spirillum of relapsing fever. He came to the opinion, after examining my photographs in Cohn's *Beiträge*, that the spirillum of Indian relapsing fever was rather thicker than the European organism. Lewis is well known as a conscientious scientific observer, and his observations merit careful attention. The science of the future, however, would have been burdened with two different spirilla, the Indian and the European, and we should also have had to count on the possibility, as Lewis pointed out, of there being two kinds of relapsing fever produced by these different spirilla. Fortunately, however, Lewis published at the time photographs of his Indian spirillum, and so the difference can be at once accounted for. In Lewis's photographs the spirilla and blood corpuscles are surrounded by diffraction lines, an infallible sign that, compared with the amount of light, he had employed too small a diaphragm hole in his investigation, and probably also in measuring the object. Every microscopist knows that the narrower the diaphragm hole the broader and darker does the edge of the object appear, and that if at the same time the light is very intense (and it is probable that it was so in Lewis's specimens), as where sunlight is employed, the edge of the object will appear broad and dark from the number of surrounding diffraction lines. Further, every microscopist who is acquainted with the latest methods of research knows that it is best not to illuminate stained bacteria with a narrow opening, but on the contrary with a very wide one (indeed under certain circumstances no diaphragm is employed at all), or with diffused light, by which means full advantage is taken of the effect of the stain, and a perfectly

* *The Microscopic Organisms found in the Blood of Man and Animals*. Calcutta, 1878.

sharp contour obtained. All my photographs were made by employing this diffuse illumination, and not the slightest trace of a diffraction line can be seen in them. Thus Lewis has compared the true diameter of the spirilla as shown in my photographs with his own, which are broadened by the diffraction band. If he had simply published a drawing possibly the error would never have been detected, because the diffraction bands, which are easily recognizable, would not have been delineated.*

Photography has also cleared up another point. When I found in Letzerich's publications descriptions of plasma corpuscles, plasma balls, swarming of micrococci, &c., I never, even with the best intention, could conceive what Letzerich really meant, or what he had actually seen, until he published photographic illustrations to his work on the morphological differences of the schizomycetes. One glance at these photographs showed immediately that the plasma corpuscles and balls were simply ordinary zooglæa colonies of micrococci which occur in isinglass jelly, in which they remain aggregated for a longer time than when they grow in fluids. Finally the jelly liquefies, and then the nests of micrococci break up (swarm).

I must add some remarks about Zürn's photographs, to which I have already referred.† They suffer from all the errors which can possibly occur in micro-photographs, for they lack all sharpness, for the most part are not properly focussed, have excessively marked diffraction bands, and what is most reprehensible, they are partly touched up. But for all that these imperfect photographs are, to my mind, infinitely more valuable than the finest drawing. Zürn's photographs show at a glance what value is to be attached to his opinions about anthrax and the bacilli occurring in diseases like splenic fever; for in spite of Zürn's belief that the bacillus anthracis has no characteristic shape, even the blurred pictures in his photographs are obviously those of the real anthrax bacilli, and they can be distinguished from other kinds of bacilli. Whoever will take the trouble to compare my photographs (Cohn's *Beiträge*, Bd. 2, Hft. 3, Plate XVI., Nos. 5 and 6)

* I may mention, in addition, that I had the opportunity of photographing, in my own way, some genuine Indian spirilla of relapsing fever, which I received from Dr. Carter, of Bombay, and they can be seen to be identical with the European.

† *l. c.*

with those of Zürn, and especially the bacillus anthracis in Zürn's fig. 4, Plate II., and figs. 2 and 4 on Plate I., which are also supposed to be bacillus anthracis, will recognize that they are the putrefactive bacilli which are figured in my photograph No. 6. At the time when I described this last photograph I pointed out the risk of confounding it with other somewhat similar bacilli, which, however (as can be seen from the accompanying figures), can be distinguished with certainty from them. The instance of Zürn's observations show that this warning was well justified. I can only repeat it, and refer for further details on this point to the researches on splenic fever and septicæmia which follow.*

A further example will show the necessity of employing photography to illustrate publications on infective diseases.

Semmer† has made additions to scientific knowledge by numerous researches on pathogenic bacteria, which he believes he has found in hydrophobia, distemper, septicæmia, cattle plague, glanders, and typhus fever. What opinion should one come to as to Semmer's statements when one looks at the figures of his pathogenic organisms which accompany the papers referred to? I do not wish to assert that Semmer had no bacteria at all before him, although his figures could be taken to represent anything else as well as bacteria; but what kind of bacteria were seen, and whether they could be regarded as really pathogenic, seems to me uncertain, to say the least of it, especially if one compares the organisms of hydrophobia with those of the disease as it is seen in cattle, and the bacillus anthracis (which look almost like the spirochætæ of the tartar of the teeth) with the bacteria of distemper and typhus fever.

After what I have said, and bearing in mind my criticisms of Semmer's drawings and Fokker's observations, to which I could add many like them, no one I think will blame me if I exercise great scepticism about every drawing of micro-organisms, the accuracy of which I cannot control by examining the original preparation; and I cannot too strongly urge every one who is working at these problems to take up the suggestion that they should support their discoveries by the convincing proof of photographic illustrations. I am not saying, of course,

* These are not translated in this volume.—Ed.

† Virchow's *Archiv.*, Bd. 70, S. 371.

that photography is always to supplant drawings; that can and will never be, and moreover, in many instances nothing but drawings are possible. But where photography is applicable (and, as experience shows, this is almost without exception the case with regard to micro-organisms) it must, in the interest of the subject, be made use of to the fullest extent. If any one cannot or does not wish to attempt the undoubtedly much more difficult task of photographing preparations of sections, he can display the micro-organisms simply by cover-glass preparations (since they are easily found in the blood or tissue juice of an organ it is desirable to investigate), and so can readily photograph them himself, or have them photographed. At the same time, however, it must never be forgotten that photography reproduces the object absolutely as it appears according to the individual ability of each microscopist. The mode of illumination must therefore be exactly the same in photographing the object as was found best for examining it with the eye. If the markings on diatoms are best seen with direct sunlight and oblique illumination, then they will be most accurately photographed under the same conditions; and just as no one examines stained organisms in direct sunlight, so they ought not to be photographed thus.

Three conditions must be fulfilled if good photographs are to be obtained of stained objects. Those parts of the preparation which it is desired should stand out markedly in the picture, such, for instance, as bacteria, cell nuclei, &c., must be stained as deeply as possible with a colour which absorbs the blue rays, and therefore acts on the sensitive plate just like black colours, which absorb all the light; for this purpose yellow and brown dyes surpass all others. The best method of determining the suitableness of the stain is to look at the stained preparation in monochromatic blue light, such, for instance, as may be obtained by passing the light through a solution of ammonio-sulphate of copper, under which condition the cell nuclei, bacteria, &c., should appear as more or less black bodies on a blue ground.

Photographs with high powers can only be obtained with the aid of sunlight, nevertheless for the many different reasons already mentioned, the direct projection of sunlight upon the object to be photographed is disadvantageous, and it therefore must be diffused by the interposition of one or more plates of ground glass.

The third requirement is an illuminating condenser of such construction that the dispersed sunlight brightly illuminates the object from all sides and prevents the appearance of the structure picture.* These are essentially the same conditions as are employed to obtain the best optical effects, and only those who have not the slightest knowledge of photography can suspect that therein can lie special modes of manipulation by means of which it is possible to represent by photography more than what actually exists. It should not be forgotten, in the treatment of the negative and development of the prints, that the photographic picture should not be merely an illustration, but that it should occupy the first rank as a proof, to a certain extent a document about the reliability of which there must not be the slightest doubt. Thus the least touching up of the negative or prints wholly destroys their value; this must be so obvious as not to require more than passing notice, but since retouched micro-photographs are published it is necessary to draw attention to the point, and once and for all to protest most energetically against the use of retouched negatives.

The extreme importance with which I regard photography has led me to apply it to my own researches as far as possible, and after I had succeeded in photographing bacteria dried on a cover-glass to make similar pictures of bacteria lying in tissues as seen in sections. As it is not easy to obtain a good differential stain of the nuclei and bacteria with aniline brown, I first directed my efforts to the photography of blue and red stained preparations, by the aid of the dry plate process, and by interposing glasses of suitable tints. After many fruitless experiments this method had to be abandoned, and the other method (already found useful in the case of cover-glass preparations) of staining the object brown had to be adopted. In many cases this gave excellent results, in others again, as compared with the results of staining with blue or red stains, there was much to be desired, and further improvement is necessary. To show, however, what can be accomplished by following the foregoing instructions I publish at the end of this paper a number of specimens from my collection of negatives, which must be admitted to possess an interest attaching to the object they illustrate, as well as to the fact that they are photographs.

* Probably Dr. Koch means diffraction lines.—V. H.

INOCULABILITY OF THE PATHOGENIC MICRO-ORGANISMS.

By the methods described above the existence of micro-organisms in animal tissues may be demonstrated, and if the investigation shows that they are present in large numbers, or that they have caused irritation or gangrene, &c., in the invaded tissues, their pathogenic power is thus rendered certain. A second question which interests us is, whether these micro-organisms, which are known to be pathogenic, are also infectious, that is, whether they can be transferred from one animal to another. The terms pathogenic and infectious must not be confused with one another; it is perfectly possible to conceive the existence of organisms which, since they can penetrate in the tissues of the animal body and set up diseases therein, are truly pathogenic, but still have not the power of infecting, that is, of passing from one animal to another and causing disease in that one also. Granting that intermittent fever is a bacterial disease, a supposition still requiring further proof, it would furnish a striking instance of the existence of a pathogenic but not infectious micro-organism. The terms pathogenic and infectious are not therefore identical, and if a parasite is shown to be pathogenic it must be experimentally established in addition whether it is infectious or not.

In order that our procedures should be rewarded by a positive result, the conditions existing in nature must be adhered to as closely as possible, a precaution which was neglected in the early days of experimental research into infective diseases, and frequently even at the present day. People have experimentally endeavoured in the most primitive way to communicate to dogs, cats, rabbits, guinea-pigs, and the like, diseases which have hitherto only been observed in man. Experience has, however, gradually taught us that it is not a matter of indifference what species of animal is employed for the experiment, and that the method by which the inoculation is performed has the greatest influence on the success of the experiment. To attempt to treat all these points would carry us too far at present, and I can only briefly indicate the most important of them in order to indicate the principles according to which our work should be carried on.

So far as the species of animal (to be experimented upon) goes it is important to choose one of the same species as that from which the contagium was obtained. Other closely allied species are to be employed only when this condition cannot be fulfilled. It is thus especially necessary when engaged with human infective diseases to take the animals nearest to man, viz., monkeys; of the necessity for this we have a striking example in relapsing fever, which, inoculable in no species of the lower animals save the monkey, invariably produces the disease in these creatures. But the experiment should not stop at the point as to whether the infection can be communicated to individuals of the same or closely allied species, but should investigate the reaction to the infective material of as many different kinds of animals as possible. Very peculiar variations in the action of the parasitic organism will thus be met with which are very valuable for the study of the disease under investigation. There are some kinds of animals which react invariably and promptly to inoculation with infective material, to which others may prove more or less refractory. Differences are also observable in the way in which it may extend throughout the body of the animal, for the same bacteria which produce a fatal disease in one species may simply evoke in another a non-fatal local disorder. Most instructive observations have been made in this way on the extraordinary sensitiveness of pathogenic bacteria towards the soil on which they are sown; on some they are able to grow, on others they die out. Within the same genus, *e.g.*, in the rodents, the infection may succeed in some and fail in others. On a former occasion I was able to point out a very remarkable example of this—namely, while it was extremely easy to succeed in infecting a house-mouse with the small bacilli of mouse septicæmia, it was found to be impossible to kill field-mice with the same parasite. Although this sounds quite paradoxical, the facts were confirmed by numerous experiments, and more lately numerous similar observations have been made in other cases. Just to mention a few of these: while mice are so sensitive to the virus of anthrax that they can be employed as a perfectly sure reagent for determining the activity of a specimen of bacillus anthracis, rats, on the other hand, appear to enjoy more or less immunity against this disease. The septicæmia of rabbits kills rabbits and mice with absolute certainty, does not affect

rats and guinea-pigs, but is easily communicable to sparrows and pigeons. In connexion with this subject also the different behaviour of animals of the same species but of different ages towards infection may be mentioned as being very remarkable, and this has been observed several times, especially with regard to infection with anthrax, and has been described by different authors. Thus while very young dogs are pretty easily infected with anthrax, old dogs are not, and the same difference may be observed in rats. The same thing is observable with the septicæmia of mice, which if it is inoculated into quite young rabbits causes a general and fatal disease, just as in mice, while in older animals it only produces a local disturbance. A further inquiry into these most interesting conditions is given in several of the papers on these subjects.* I only wish to point out here how extremely important the proper selection of animals for experiment is, and chiefly in experiments on immunity, which are at present attracting much attention, and concerning which it is sufficient merely to indicate how error can arise when old and young animals are taken at random for such experiments, for the older animals may possibly already be insured against the infection. The peculiar affinity which pathogenic bacteria show for definite species of animals recalls similar facts with regard to the behaviour of parasites generally, since they often confine themselves in the most special way to one species of plant or animal. These facts are so well known about the higher parasites that they are universally accepted as proved. It would occur to no one, for example, to attempt experiments on the breeding of tape-worms in water, because allies of the tape-worms live in water. Is it not practically the same undertaking as the cultivation of the tape-worms in water when, as one constantly hears and reads, some one has made culture experiments with the most delicate micro-parasites in ordinary Cohn's or Pasteur's fluid? Those who wish to commence culture experiments with the pathogenic organisms cannot bear this fact too much in mind.

A scarcely less important consideration deserves notice—namely, the manner in which the introduction of the infective material should be carried out. The method most commonly employed is inoculation (*Impfung*). We are accustomed to

* These papers have not been translated.—ED.

understand by the term inoculation the application of the virus to a slight superficial wound of the dermis, and it is according to this definition quite incorrect to call a wound which penetrates into the subcutaneous tissues an inoculation. Lately, however, this interpretation of the term inoculation has been extended, and it now includes all conceivable methods, this use finding favour with the French experimenters especially, who include under the expression, vaccination, subcutaneous and intravenous injection and other methods of infection. Such an extension of a term would have no importance if it did not at the same time introduce a confusion of meaning, as in the present instance; for these different methods of communicating the virus are by no means similar in their effects. Under certain circumstances an inoculation may have a totally different effect to a subcutaneous injection, although the infective material is exactly the same in the two cases. A good example of this is to be found in one of the following papers on the Bacilli of Malignant Œdema (the so-called *Vibrion-septique*). Far too little weight is also laid on the quantity of infective material employed. It is only by employing very small quantities that we can avoid the action of the soluble chemical products of decomposition which may produce poisoning instead of the particular infection desired. However, it must be admitted there are some pathogenic bacteria which it is absolutely necessary to introduce in considerable quantity in order to obtain their definite action. It is thus very necessary in making experiments on communicability to try all the different methods in use, and, still more, never to omit in describing the experiments to give exact details of the mode of infection, whether simple vaccination, subcutaneous injection, or transplantation, &c., &c.

Whenever in the following researches an inoculation is spoken of, a genuine vaccination is meant, and whenever any other method is employed it will be so fully described as not to leave the slightest doubt as to the mode of communication. There still remain some observations which I should like to make with regard to the methods of infection.

A true inoculation on mice is scarcely practicable. Only in the ear can one make such a small wound that it can be reckoned as a simple cutaneous lesion. A skin incision elsewhere if ever so light passes immediately into the subcutaneous

tissue, and should be correctly described as a subcutaneous inoculation. The method of placing the infective matter in a small pocket-shaped wound of the skin, employed, for example, in investigating the presence of possible infective materials in earth, can under no circumstances be regarded as a simple inoculation, and I would include in the same category infection by means of the introduction of small pieces of thread under the skin, and similar methods which I shall refer to on another occasion.

All instruments employed in infection experiments must of course be subjected to trustworthy disinfection, which according to my experience in this sort of work is only to be obtained by prolonged heating at 150° C. and above. One often reads that disinfection was accomplished by the aid of alcohol, carbolic acid, and the like, but the experiments on the action of various disinfectants on the spores of bacillus anthracis which are described elsewhere show how untrustworthy these substances are. There is nothing for it then but to disinfect things at a high temperature, and there is no difficulty in this respect with many instruments, such as knives, needles, &c., for they are simply heated in the flame. The proper disinfection of syringes which are used for subcutaneous injection is rather more complicated, for the ordinary syringes made of glass and metal become absolutely useless after being exposed for several hours to a temperature of 150° C., and a less degree of heat would not secure adequate disinfection. I cannot conceal my belief that many an experiment has been frustrated by this difficulty, and that many an inexplicable result of subcutaneous injection is to be attributed to incomplete disinfection of the syringe. To meet this objection we employ a specially constructed syringe in our experiments, the metal fittings of which screw directly on to the glass, and are made tight by cork washers, which of course can be changed as required. The piston is made to fit by winding wool or cotton round it. Every time it is used the syringe must be heated in an oven for one or two hours at 150° C., and then the piston is to be moistened with distilled water, which has been sterilized in a digester. By following these precautions it is impossible to mix the infective matter of one experiment with that of a former one.

In all cases in which the local action of infective material is

to be studied, inoculation on the ear or cornea, and transplantation into the anterior chamber of the eye is particularly recommended. I do not think that it is necessary to give a full description of these methods since they have come into general use everywhere.

Although artificial infection by means of inhalation has been frequently practised, no method has yet been discovered which is free from objection. When it is carried on through tracheal fistulæ there is the possibility of infecting the wound in the trachea, and when through the mouth or nose much of the infective material is swallowed, and, in Buchner's method of exposing the whole animal to a spray of infective material, there is the possibility of some getting into a slight wound on the surface of the body. In fact it is most desirable that some satisfactory mode of procedure should be devised for this mode of infection.

There is still one indispensable condition attached to all experiments on infection, viz., that no reliance should be placed on one experiment, and that the requisite control experiments should never be omitted. How often one meets with the statement that some suspected substance or fluid had been inoculated into an animal, or injected subcutaneously; that the animal fell ill, and possibly died, and that this fatal result was clearly a direct result of the inoculation, and that the illness was an example of the affection in question. And yet it is quite evident that a single such experiment is as good as useless. For in the first place it must be shown that this solitary result was neither a mistake nor an accident, and that the inoculation can produce in the animals experimented upon in every case, or in a very large majority of cases, the disease or death, so that every accidental circumstance may be excluded. A further point, and one on which I lay special stress, seeing how often it is omitted, is the necessity of first making certain that one has to do with a really infective material. The fact that a material when injected subcutaneously, or into a vein, or into the abdominal cavity or elsewhere, causes a pathogenic effect does not in the least show that this material possesses infective power. For unorganized soluble substances can also exert a similar action. Only when it can be shown that a disease is successfully communicated from one individual to another by such

a small quantity of the infective material that to have produced the disease it must have multiplied in the body, can such a material be regarded as infective. From this it follows that if any one wishes to know for certain whether he is experimenting with an infective material he cannot possibly remain satisfied with one experiment, but must carry out a more or less extensive and continuous series of inoculations from one animal to a second, from that to a third, and so on, before he can get rid of the just objection that he is only dealing with the symptoms of simple poisoning, and not with those of an infective disease.

PURE CULTIVATIONS.

After the presence of pathogenic organisms in the body has been definitely ascertained, as well as their capability of multiplying in the tissues, and their communicability from one individual to another, there yet remains a most interesting and most important hygienic problem, namely, the demonstration of the conditions of their growth. It has already been shown at the beginning of this paper that this problem is only to be solved by the aid of pure cultivations of the micro-organism, and it is no exaggeration to say that pure cultivations are the crux of all researches into infective diseases.

Since the extreme value of making pure cultivations has been recognized for a very long time, every one who has been engaged on the question of infective diseases has given much trouble and thought to perfecting the methods of making pure cultivations. For all that, the results of the very latest researches show clearly that we have not advanced very far beyond the early incomplete experiments; we have at most learnt how to avoid the most evident mistakes, and even that has not always been done.

The essential principles of pure cultivation as it is practised now may be condensed as follows.

A sterile nutrient fluid is placed in a disinfected glass vessel, which is plugged "fungus tight" with disinfected cotton wool, and then this fluid is inoculated with the material containing the micro-organisms of which a pure culture is to be obtained. If growth occurs in these first flasks, then further inoculation is performed from these into a second series of similarly prepared vessels by means of a disinfected instrument. In fact it is

almost exactly the same process as in transmitting an infective disease from one animal to another.

Obviously various precautions are employed in these methods, the first of which is to see that the cultivation vessel is really disinfected. The discussion between Pasteur and Bastian on spontaneous generation shows how unimportant this disinfection used to be considered from the well-known question of the former to the latter, namely, "Flambez-vous vos vases avant de vous en servir?"* to which Bastian had to give a negative answer.

The second precaution must be to see that the wool plug really fits so closely as to exclude all fungi, for according to Naegeli's investigations the degree of necessary tightness varies in different cases.

Thirdly, the nutrient fluid must be of suitable composition, and thoroughly sterilized. I have already explained what is to be understood by a suitable pabulum, and I have also shown how difficult it is to obtain it in many cases. I shall assume here that a suitable fluid has been found, and only needs sterilizing. Every one who has had occasion to frequently work with hay infusion, meat extract, or malt extract solutions, knows with what difficulties and dangers one has to contend in successfully attaining this object. Although it is easy enough to sterilize with certainty small quantities of the above and similar nutrient fluids in a proper apparatus, it is extremely difficult to free large quantities of the same from active spores of micro-organisms, a fact which is strikingly shown in the accompanying article on disinfection by means of steam.

Fourthly, the substance to be inoculated must contain only the micro-organism of which a pure cultivation is required and no other, for if there is the slightest contamination of the infective material with a more rapidly growing form of micro-organism than that which one desires to cultivate, one cannot, as Buchner has very strikingly shown, possibly succeed in getting the desired pure cultivation. Buchner employed a peculiar method of obtaining a pure material for starting his researches on bacillus anthracis. He inoculated his nutrient solutions with such an extremely dilute virus that according to a rough estimate only one bacillus was placed in the cultivation flask, and he decided from the character of the macroscopical

* *Bulletin de l'Academie de Méd.*, 1879, p. 1230.

ppearance of the culture which developed whether it was pure or not. Now I shall have to show later on that there are bacilli the macroscopical appearances of the development of which in fluids exactly resembles those of anthrax bacilli, and that consequently if they happened to be mixed with anthrax bacilli they could not be distinguished by Buchner's process. It would be quite impossible to apply this method to other bacteria than those which exhibit a perfectly characteristic form in a nutrient fluid, to kinds, for instance, which, as many do, simply cause a turbidity in the fluid. The difficulty then of obtaining a perfectly pure material at the outset still remains to be got over in the great majority of cases, and this cannot be done with the methods of making pure cultivations at present in use.

Fifthly, precautions must be taken that no spores of extraneous organisms from the air fall into the culture fluid while the first inoculation, and indeed while every subsequent inoculation is being made. This is a risk which no experimenter can ever protect his pure cultures from with absolute certainty. Even if he take out the protecting wool plug only for an extremely short time, even though the early cultivations are successful, the probability increases with each further inoculation that a contamination will occur.

To meet this difficulty as far as possible it is best as a rule to start several samples of the cultivation at the same time, and then only to inoculate further from that one which remains absolutely pure, as may be proved by careful naked eye inspection or by microscopical investigation. Unfortunately one cannot place any dependence on this method, for I have already explained how uncertain macroscopical differentiation of different cultures is, and as for the microscopical it could only tell us whether the small droplet (which is taken as a test and mounted on a slide) is free from contamination, and if there were only a few specimens of other kinds of organisms existing in the drop, it would be impossible to find them with certainty in the midst of the mass of the other organisms. As a matter of fact the first traces of contamination cannot be recognized with any certainty in a fluid either macroscopically or microscopically; and if perchance a further inoculation is made from a culture which, although supposed to be pure, is really impure, and the contaminating organisms surpass the original ones in activity of growth, the possibility of further

pure cultivation is hopelessly lost; even in the next generation the microscope would leave scarcely any doubt of the contamination, a piece of information, however, which comes too late, as it would be quite impossible then to turn out the numerous unbidden guests.

In order to obtain greater accuracy in conducting a long series of pure cultures in nutrient fluids, there is only one method according to my experience, viz., that which I employed in my earlier experiments, and especially in investigating the development of bacillus anthracis. It consists in working with so small a quantity of the culture fluid that the whole of it can be kept under microscopical observation, and consequently its purity ascertained. This is accomplished by making a number of glass cells by means of cover-glasses and hollowed slides, and placing on the under surface of the cover-glass a drop of the nutrient fluid, which must be spread out into a perfectly flat layer in order that it may be surveyed with any magnifying power that one may find necessary in investigating micro-organisms. The "seed" is then sown at the border of the nutrient pabulum, and its further development and the purity of the culture ascertained from time to time by the microscope. If the preparation and inoculation of the cultivation is performed rapidly and dexterously one may reckon with certainty on at least half, and sometimes more, of the cultures remaining perfectly pure and suitable for further transplantation. Unfortunately this method, which is so extremely useful in cultivating the easily recognized bacillus anthracis, leaves us in the lurch when we wish to cultivate very small micro-organisms which scarcely possess a characteristic form. A further defect of the above process is that it only affords a very small supply of air, and that the gaseous products of decomposition, which exert an inhibitory effect on the development of the cultivation, accumulate in the small space. For these reasons this method is only applicable in a few cases.

On the whole it is truly depressing to attempt pure cultivations; and any one who has undertaken cultivations of micro-organisms in the usual way, but who has not taken care to avoid all the sources of error which I have indicated—which, by the way, I am convinced is practically impossible—has only himself to blame if the results of his investigations are not held by scientific men to have been obtained by sufficiently exact methods, and are not

therefore accepted as convincing. This remark is especially applicable to the researches (carried on with really remarkable, if blind, zeal) now issued in quantities from Pasteur's laboratory, and which describe incredible facts with regard to pure cultivations of the organisms of hydrophobia, sheep-pox, pleuro-pneumonia, &c.*

I have already frequently stated that pure cultures are absolutely indispensable for the further development of our knowledge of pathogenic organisms and of everything connected with them, and that somehow or other we must find means of obtaining an easily applicable and exact method. To me there seems no prospect of adequately improving the method at present in vogue. Inoculations and reinoculations have been performed under shelter of an antiseptic spray. It is not impossible that some bacteria present in the air and capable of multiplication may thereby be destroyed in the air, but my experiments on the disinfection of the spores of bacillus anthracis and other bacteria show how feeble are the ordinary antiseptic means in their effect on spores. From my experiments it is perfectly clear that a spray of carbolic or salicylic acid, or of Condy's fluid, &c., can have no action at all on spores by reason of the short time that the reagent is in contact with them, and that therefore in spite of the spray a whole crowd of spores suspended in the air may infect the culture fluid at each experiment. Klebs introduced an improvement, which consisted in making the upper part of the plug which closes the culture flask of heated asbestos. Just before taking a small quantity of material for investigation or further cultivation the asbestos is reheated, and so any spores entangled in its meshes destroyed, then a hole is bored through the plug with a heated needle and a portion of the material taken out by a heated capillary tube. I consider this, however, to be only another addition to the complications with which the procedure of pure cultivations is already loaded, without affording a corresponding amount of protection against possible contaminations. For though only a very small opening is made in the plug the possible entrance of spores is thereby merely diminished and never altogether prevented, and in conveying the material from one flask to the other the glass tube

* In my opinion Dr. Koch rather over-rates the difficulty of carrying on pure cultivations in fluids.—Ed.

employed must pass through the air and so possibly become contaminated.

It being perfectly clear that efforts in this direction are in vain, I have abandoned the principles on which pure cultures have hitherto been conducted, and have struck out a new path, to which I was led by a simple observation, which any one can repeat. If a boiled potato is divided, and the cut surface exposed to the air for a few hours, and then placed in a moist chamber (as, for instance, on a plate under a bell-jar lined with wet filter-paper) so as to prevent drying, there will be found by the second or third day (according to the temperature of the room) on the surface of the potato numerous and very varied droplets, almost all of which appear to differ from each other. A few of these droplets are white and porcellanous, while others are yellow, brown, grey, or reddish; and while some appear like a flattened out drop of water, others are hemispherical or warty. All grow more or less rapidly, and between them appears the mycelium of the higher fungi; later the solitary droplets become fused together, and soon marked decomposition of the potato occurs. If a specimen is taken from each of these droplets so long as they remain distinctly isolated from each other, and is examined by drying and staining a layer of it on a cover-glass, it will be seen that each is composed of a perfectly definite kind of micro-organism. One, for example, will show enormous micrococci, another very minute ones, a third may show micrococci arranged in chains, while other colonies, especially those which are spread out flat like a membrane, are composed of bacilli of various size and arrangement. Many consist of torulæ, and amongst them may be seen here and there the mycelium of one of the higher fungi sprouting from a spore. The source of all these different organisms will not long remain a matter of doubt if another potato is taken, and peeled with a previously heated knife, so that none of the skin may remain which might contain, at least in the earth clinging to it, spores of bacilli which had not been killed by the brief boiling. It must not be exposed to the air, but kept in a disinfected vessel which is plugged with wool, under which circumstances it will be found that no droplets appear, no organisms settle on it, and it consequently remains unaltered, until after several weeks it becomes dried up. That the organisms which developed as small drop-like colonies on the

first potato fell on it from the air is obvious, and indeed one often finds a small particle of dust or thread in the centre of the small colony, which has clearly served as a carrier of the organisms, whether as dry but still living bacteria, as spores, or as yeast-cells. But that there should be no confusion on this point, I must add that a few of the colonies spreading from the margin may start from spores which are contained on the skin, or rather in the earth sticking to it.

The question now arises, What do we learn from this observation of these colonies growing on potatoes? We learn this most striking fact, that with a few exceptions every droplet or colony is a pure culture, and remains so until by growth it pushes into the territory of a neighbour, and the individuals of each colony mingle; the exceptions being those cases in which possibly two spores fell quite close to one another, so that the colonies they produce soon of necessity merge into one another, or where possibly a particle of dust is covered with several different spores, and these develope simultaneously. If an equally broad surface of a nutrient fluid, instead of the potato, had been exposed to the influence of the air, numerous organisms would without doubt have fallen into it, indeed approximately as many kinds as in the case of the potato, but their development would have proceeded very differently. The motile bacteria would have dispersed themselves rapidly throughout the fluid, and would have become mixed with the immobile ones, which at first develope to some extent in small floating colonies, and would have pushed these about by their active movements, and distributed them throughout the fluid. Again, some organisms would swim about at the bottom of the liquid, and others in the upper layers; and many which found a place on the potato, where they could multiply undisturbed, would never develop in the fluid at all, being choked by the over-growth of the more rapidly multiplying forms. To sum up: the whole fluid would from the commencement afford on microscopical investigation a picture of a confused mixture of different forms, and never even in the remotest sense of the word could be called a pure cultivation. In what lies this marked difference between the soil which is offered by the potato and by the fluid respectively? It only consists in this, that the former is a solid soil, and prevents the different kinds of organisms (even those which are motile) from

mixing with one another, whilst with regard to the latter, the fluid substratum, there is no possibility of the different species remaining separate from one another.

It became important to utilize further the advantages which a solid cultivation soil offers for pure cultivations. For this purpose some of the colonies above described as spontaneously growing on boiled potato were spread out as much as possible on the recently cut surface of another potato, which was then placed in a moist chamber. There soon appeared (even on the following day, or the next but one) a plentiful growth of the micro-organism thus sown, which preserved exactly the same characteristics exhibited by the original colony. If it was yellow, and consisted of small micrococci, then the potato infected with it appeared covered with a broad yellow layer which consisted of the same minute micrococci. Other kinds of micrococci, the different kinds of bacilli, torulæ, and fungi, &c., all behaved in just the same way. All could be obtained in large numbers and in completely pure cultivation pretty quickly after a few successive inoculations on potatoes from the originally very small colony. A very careful exclusion of the air from these cultures is not necessary, for if here and there the germs of other organisms fall on to the potato they can only develop where they fall, and spread slowly, but never endanger the whole culture. Besides, the characteristic appearance of their colonies show them to be so obviously foreigners among the organisms under cultivation that these accidental contaminations can easily be avoided in future cultivations. The culture is only endangered when it is kept such a long time that the extraneous organism is enabled to attain a considerable development, and experience soon showed the proper moment for further cultivation in order to have always an absolutely pure culture. In this way it was quite possible to obtain faultlessly pure cultures in an extremely simple way, at least of all those organisms which find a suitable soil in boiled potato, and their number is by no means small. As I have just indicated, many different kinds of micrococci and bacilli will grow luxuriantly on potatoes, and it was important to transplant on to this kind of soil other well-known bacteria of great practical interest. If hay bacilli were placed on boiled potato, a plentiful growth was obtained, which formed a white creamy layer over the

cut surface of the potato, and could be distinguished at the first glance from the colonies of other bacilli which grow spontaneously on potatoes, more especially from the most common kind of bacilli, which form small moist spots at the edge of the potato, spread as a veil-like wrinkled membrane over the surface, and produce a viscous ropy mucus. After this experiment had proved successful another potato was infected with bacillus anthracis; this also grew most luxuriantly, as I shall have occasion to mention more fully in another place. But attempts at cultivating on potatoes other bacteria, which are known to be pathogenic from experiments on animals, gave a negative result.

However, the principle being thus established it only remained to adapt it to all cases. Nothing would be gained by my describing all the experiments I made in attempting to discover a soil suitable for all pathogenic organisms, and at the same time resembling the boiled potato in being solid, and therefore I will state the result of these experiments, which in their present form provide a thoroughly satisfactory procedure for pure cultivation in the large majority of cases, and in time when further perfected will doubtless meet all wants.

When I saw that it was scarcely possible to construct a kind of universal nutrient fluid which should be equally suitable for all kinds of micro-organisms, I devoted my attention to discover how well-known and new nutrient solutions could be converted from the fluid into the solid condition, and I found the best way of accomplishing this was to mix gelatine with the nutrient liquid. Isinglass and other gelatinizing substances are not nearly so useful. The mixture of nutrient fluid and gelatine, which I shall call "nutrient jelly" for the sake of brevity, is prepared in the following way: gelatine is soaked in distilled water and then dissolved by heat; to it is added the nutrient fluid in such quantity that the mixture shall contain in definite proportions the necessary quantities of gelatine and nutritive material. I have found the most convenient amount of gelatine in the nutrient gelatine mixture to be about 2½ to 3 per cent. But if the nutrient fluid and gelatine are to be mixed in equal parts, then in order that the nutrient jelly may be brought up to 2½ per cent. of gelatine,* the gelatine solution must be made 5 per cent. to begin with, and of course the nutrient solution

* 10 per cent. of gelatine is now generally employed.—Ed.

must have double the quantity of nutritive material in it; for example, for a nutrient jelly containing 1 per cent. extract of meat there must be prepared a 2 per cent. watery solution of extract of meat. The gelatine may also be soaked and dissolved at once in the nutrient fluid. Usually gelatine has a slightly acid reaction, and therefore it is necessary, at least if the nutrient jelly is to be used for cultivating bacteria, to neutralize it with carbonate of potash, carbonate of soda, or the basic phosphate of soda; the nutrient jelly is then boiled again and filtered, because precipitates often form in the mixing and neutralizing, and moreover the gelatine is often dirty. Meanwhile a flask plugged with wool is disinfected by heating it for a long time at 150° C., and into it is poured the nutrient jelly. It is then plugged again and heated to the boiling point, and this boiling ought only to be carried on for a short time, since it is simply done to kill the adult and easily destroyed micro-organisms in the jelly. The spores in it would only be destroyed by prolonged boiling, but that is out of the question here, because under those circumstances the gelatine would lose its power of gelatinizing, and for the same reason the nutrient gelatine cannot be sterilized at a high temperature in a digester. The nutrient gelatine is still not perfectly sterilized by the preceding manœuvres, but that is of no consequence. If the nutrient solution were fluid the germinating spores contained in it would grow into bacteria, and multiplying rapidly, spread through the whole of the fluid, giving the first warning of their presence by turbidity about the second or third day. It would then be impossible to save such a fluid, since its original composition would be altered, and it would probably be again laden with innumerable newly formed spores. But the case is quite otherwise with the nutrient jelly, which shows at once the great advantage of the solidity of the nutrient substance in determining the presence of bacteria in it. Thus on the next day, or somewhat later, the perfectly clear gelatine is found to show, when held up to the light, a varying number of whitish spots pretty equally distributed through it. If the gelatine is left to its fate these small points soon grow into little balls, and increase more and more in size till they form a space which is shown by movement of the vessel to be filled with liquid gelatine, and finally the whole mass of nutrient jelly melts

into a turbid fluid. The smallest colonies which grow out of the white points consist of bacilli, as can easily be determined by examining them with the microscope. When one knows this, and wishes to sterilize the jelly, one would naturally not wait until these colonies had reached a considerable size, but would kill them by boiling as soon as they could be recognized with the naked eye. Herein, as already stated, there lies this great advantage of using nutrient gelatine, namely, that the very earliest development of bacteria cannot be overlooked, for bacteria growing from a single spore must lie packed together for some time, and so cannot escape observation even while their number is relatively small, and at the same time are seen so early that they have not yet formed spores or materially altered the composition of the nutrient solution. A further superiority of the nutrient jelly over nutrient fluids is, that one can estimate from the number of colonies which develop the number of germs which were originally present; and not only so, but also the mode in which the organisms entered the vessel containing the nutrient jelly can be seen at the first glance. For all the germs which survived the boiling of the gelatine are diffused pretty uniformly throughout the fluid mass, and appear later as colonies springing up in the substance of the consolidated jelly; while, on the other hand, all the organisms which come into contact with the jelly after it has solidified having fallen, for instance, from pieces of imperfectly disinfected wool covered with organisms, from particles of dust which have come from the air and got through an inefficient plug, or from germs adhering to the walls of the flask, particularly to its neck, all these subsequent contaminations must be deposited on the surface of the jelly, where alone they can grow into colonies. Thus one is always in a position to control the suitability of the nutrient material for pure cultivations, and to recognize at once all the mistakes which may occur during its preparation, and to correct them speedily. It is unnecessary to say anything further as to the value of this constant guard against the possible commission of errors, nor how an investigator can soon acquire practice and great security in the use of this method. It takes very little time to ascertain whether gelatine prepared with this or that nutrient solution is easy or difficult to sterilize.

In many instances sterilization is very easily accomplished, for example, with alkaline urine or Pasteur's fluid when in the shape of nutritive jelly, usually one boiling suffices, but it is much more tedious with other kinds, such as meat extract or hay infusion jelly, which have to be raised to the boiling point once a day for several days in succession, because it cannot be imagined that all the spores will germinate at the same time. Single colonies sometimes appear some days after the last boiling, and show by their position in the middle of the jelly that they were there from the commencement and did not enter subsequently. But as I have just said, if this does happen it can be perceived in time by frequently looking at the jelly, a thing which ought not to be neglected during the first week, and then it can be arrested by another boiling.

As for the further application and treatment of this nutrient jelly for pure cultures, it is above all advisable that a number of test tubes plugged with cotton wool should be filled with a convenient quantity of the jelly (both test tubes and wool having been thoroughly disinfected with heat), so that an ample supply of the jelly may be always ready without having to melt up the whole mass every time it is wanted, and further run the risk of contamination. It will be found most convenient not to put more than a small quantity, say 10 to 15 c.cm., in each tube.

Since the method of pure culture with potato is so convenient and certain in execution I have contrived to employ the nutrient jelly in a similar way. It is poured into flat watch-glasses, small glass capsules, &c.; but according to my experience the most convenient way, especially when the cultures have to be examined microscopically, is to spread out the jelly in broad streaks on glass slides, which can then easily be placed under the microscope. This can be done with a pipette first disinfected, and of course the slides must be perfectly clean and exposed to a temperature of 150° C. before being used. The streaks should be about two mm. thick, and when the jelly solidifies, which it does in a few minutes, the slides are laid on small slips of glass which are broad enough to carry two or three slides lying side by side, and finally several (five or six) layers of such slips and slides may be placed one above another, and the whole then kept in a moist chamber. I usually employ

for the latter purpose glass vessels which are covered with a bell-jar with a flat top, and the inside of which is lined with damp filter paper. In such vessels the jelly can remain two or three weeks without drying up. The sowing of the organisms to be cultivated is accomplished thus: as small a quantity as possible of the fluid or substance containing the organisms is taken by means of a previously heated needle or platinum wire, and then drawn in several cross lines (usually five or six) rapidly over the surface of the jelly. The needle is used in much the same way as the lancet in vaccinating by incision, and it is better to make the cuts as shallow as possible, just as in inoculating; in fact this process might very well be called an inoculation. The inoculation of several slides is performed in the same way, so that without extra trouble or appreciable loss of time twelve or fifteen separate cultures can be started, for each inoculated tract represents a perfectly independent culture. Indeed the number is really much greater, for each section of a line can be considered by itself, and will afford material for further cultivation.

Once covered with a more or less perfectly fitting glass bell-jar, the cultures do not need any further protection from the constantly imminent risk of contamination. It is impossible to avoid the entrance of extraneous organisms either at the time of making the inoculation or examining them with the microscope; but any which may then fall upon the jelly can only develop at the spot where it fell, and it will very rarely happen that a foreign colony pitches upon the inoculation line or its immediate neighbourhood. Besides it is inconceivable that the whole number of cultures should be so covered in a short time by such crowds of spores as to render further cultivation impracticable, and as a matter of fact this does not occur, at any rate where the bell-jar is not lifted too frequently. Usually pure cultures grow so rapidly as to arrive at their maximum development in a few days, when further inoculation should be performed, more particularly when, as is the case with many kinds of bacteria, the jelly becomes fluid by their rapid growth; and further, when spore formation has already occurred there is no object in leaving the culture for a longer time, and it should be transplanted as soon as possible. If cultures are to be preserved from contamination for a considerable time they must of course be guarded by wool, under which circumstance also the nutritive

gelatine proves itself a very useful material, because the purity of the growth of the colonies which started from the point sown can be ascertained at once with practice by means of their well-marked forms and general characteristics without the necessity of microscopical examination, while other contaminations lying close to the point of inoculation can be immediately recognized as such.

Cultures develop very slowly at low temperatures, and most organisms require a considerable degree of heat to be able to thrive. The cultures on jelly grow best at 20° to 25° C., and hitherto I have not found any organisms that could be cultivated which would not grow at this temperature. But if it is necessary to use temperatures above 30° C., at which point gelatine melts, this method must either be abandoned, or in order to obtain some of its advantages the solid jelly kept in vessels protected by wool must be inoculated; and then if no foreign colonies are visible in it after it has been kept at 25° C. for about twenty-four hours (thus affording the greatest probability of success in getting an inoculation free from contamination), the culture may be placed at the temperature of the incubator.

A very important requirement necessary for carrying out pure cultivations, the difficulty of obtaining which, as I have already shown, must have vitiated the majority of pure culture experiments hitherto carried on, is the selection of a perfectly pure material for the first inoculation, and this can easily be got with the aid of the nutrient jelly. If, for example, it be required to make a pure culture of the septicæmic organisms which are found in the blood of a septicæmic animal there is no necessity to take extraordinary preparations with sprays, heated capillary tubes, &c. (which after all are not satisfactory); it is quite sufficient, avoiding of course all gross contamination, which can be done without any difficulty, to take some of the blood on a previously heated needle from the freshly opened heart or a blood-vessel which has been laid bare, and inoculate in a considerable number of lines the surface of the nutrient jelly. A few fungi and colonies of micrococci which can scarcely be excluded from a post-mortem examination of an animal may grow in a few of the lines, but besides these there will be a greater or less number of perfectly pure colonies of septicæmic organisms, easily recognizable under a low power of

the microscope by their peculiar dull polish and extremely fine granular appearance, and of these there will be a sufficient number which are suitable for further inoculation. To detect these a dissecting microscope may be used if necessary. Success may be looked for equally certainly, if less easily obtained, even if only a few of the organisms under investigation were present. It would only then be requisite that the bacteric mixture should be well diluted and inoculation performed in a great many places. Under these circumstances it is often advantageous to inoculate the jelly while still fluid, and thus to scatter the different organisms over a larger area, or an extremely small quantity of the substance can be well mixed with the fluid jelly, which can then be poured out on slides, and the colonies of the particular species of organisms under investigation sought out with the microscope.

I have already drawn attention to the fact that different nutrient soils are required for different kinds of micro-organisms. To mention only one of the most marked examples, fungi and bacteria do not grow with equal facility on the same soil, for the former thrive best on acid soils, while the latter prefer neutral or slightly alkaline ones. Hence it is necessary to work with different kinds of nutrient jelly corresponding as far as possible to the requirements of the different groups of micro-organisms, and even to the individual species of the same group.

We have employed several kinds of nutrient jelly in our experiments, some being found useful for one class of cases and others for another. Those meriting special notice are hay infusion jelly, which is an excellent material for many kinds of bacilli; wheat infusion jelly; jelly prepared with aqueous humour; jelly made with meat extract and peptone; another kind prepared with meat infusion and peptone is especially valuable for many pathogenic bacteria;* but unquestionably the best nutrient material for pathogenic bacteria is a nutrient jelly made out of blood serum and gelatine, with regard to which I must make a few remarks on account of its great value. This nutrient jelly cannot be sterilized by boiling because the serum albumin would coagulate; great care must therefore be taken to obtain the blood serum in

* The jelly now mostly employed is composed of 1 lb. meat, 100 grammes gelatine, 10 to 30 grammes peptone, 1 gramme chloride of sodium, and 1 litre of water, neutralized with carbonate of soda.—Ed.

the first instance as free as possible from contamination in the following way. The blood must be received into a perfectly pure vessel and allowed to stand until a firm clot has formed; the upper layer of the clot is then carefully separated from the wall of the vessel, which is then covered and set in a cold place for one or two days until a sufficient quantity of clear and only slightly coloured serum has collected. This is then drawn off with a previously heated pipette and mixed with an equal part of fluid and sterilized 5 per cent. gelatine, and finally the mixture is poured into disinfected test tubes which are plugged with sterilized wool. This serum jelly is sterilized by placing the tubes several times into a water-bath which is kept at a temperature of 52° C., at first for half an hour to one hour daily, and then less frequently. In this way I have always succeeded in obtaining perfectly sterilized blood serum jelly.

For cultivating the moulds decoctions of plums or of horse dung were used, since these afford an extremely favourable soil for their growth.*

Pure cultures accomplished with nutrient jelly have this especial advantage besides those just described, namely, that they can readily be controlled by the microscope without spoiling them or in any way checking their further growth. And although, of course, only low powers can be employed for this purpose, still these are amply sufficient for watching the cultures and looking for a good place from which to transplant. Thus, for example, it is perfectly easy to place slides covered with nutrient jelly under the microscope without any further preparation, and to examine them with Hartnack's objective 4 and ocular 3, or Zeiss' objective AA, with a high ocular and a small diaphragm. With this magnification one can often recognize the individual bacteria in the colonies, at any rate at the edge of the mass the individual large bacilli, sarcinæ and torulæ can be easily seen. If a more powerful objective has to be employed to settle doubtful cases then one of the inoculation streaks must be sacrificed and covered with a cover-glass, after which examination of the colonies can without any trouble be made even with immersion lenses. As a rule, however, this is unnecessary, because after one has observed under a low power a considerable number of colonies of bacteria, fungi, &c., whether sown naturally or artificially on nutrient jelly,

* A paste made of bread crumbs is now generally used.—Ed.

one will very soon see that the colonies of each kind as they grow on the gelatine possess perfectly characteristic peculiarities of form, colour, and mode of growth, which render them very easily distinguishable one from the other. There is really nothing extraordinary in this, when we remember that similar conditions occur in every province of natural science, especially in those cases where a number of individuals of the same kind is collected together. If the group is so far away that the individuals cannot be recognized, or if they are individually so small as not to be visible with the unaided eye, nevertheless, from the characteristics of the whole mass taken together, in other words of the colony, the species to which they belong can be recognzied with more or less accuracy, since the characters of the whole group are simply the sum of the characters of single individuals.

Take for example colours. We may find that it is impossible to distinguish the colour of single specimens of animals or plants if they are very small and widely scattered, but when a large number of individuals of the same species are closely aggregated the summated tint of them all produces an effect which is very readily recognized. And it is just the same with motion. A small object barely or not at all visible to the naked eye cannot be identified when it is single; thus, for example, a bird flying in the extreme distance may be so indistinctly seen as to render it impossible to determine its species. But this state of things is quite altered when a covey of birds is seen moving even at the same distance. It is not merely the larger number which attracts notice, but a practised eye can recognize by the grouping and the character of their movements the species of birds which compose the covey. From other characteristic features of aggregated masses may be deduced the characters of the constituent parts. The circumstances are precisely the same in the present instance with regard to the masses and colonies formed by micro-organisms, only that in most of these cases the characteristic peculiarities of each mass cannot be properly made out without the help of a moderate magnification. By means of the microscope, however, it is so easy to recognize the characteristic differences of colour, size, form, &c., of the individual colonies, that the colonies formed by various species can readily be differentiated from each other; for example, bacillus anthracis cannot be confounded with the hay bacillus (bacillus subtilis)

when these are growing side by side on gelatine. For while the anthrax bacilli are non-motile, and always form woolly masses composed of long undulating threads, often curling round each other, the hay bacilli only grow as long threads in the youngest colonies, and as soon as they develop further and liquefy the gelatine, as is always the case, they are seen in the interior of the colony to take the form of actively moving rods, which bore into the solid gelatine at the margin in a direction at right angles to the original mass, so that the colony appears to be surrounded by a crown of rays. In fact it presents so absolutely different a picture from that of the anthrax colony, as well as from other kinds, that by the diagnostic signs just described it can be picked out from among all other varieties. Other forms are shown by other bacilli, of which the majority of movable kinds grow in a wreath-like form, somewhat similar to the hay bacillus but differing from it in the outline and breadth of the rays; others again present a spreading and most complicated root-like appearance; in some disinfection experiments I obtained a broad bacillus which had remarkable powers of resistance to heat, and which grew in flat colonies on gelatine, in which the bacilli were arranged side by side like a mosaic, and neither moved nor formed any threads. It would take me too long to describe all the different kinds of bacilli which I have thus examined, and there no doubt are many more forms. The various kinds of micrococci are still more numerous, and present every possible shape, from the simple colourless granular colonies to the coarse knob-like masses, which are often of a brown, red, yellow, or white colour, and while often heaped up spirally are sometimes spread out in flat, leafy masses.

The little heaps which sarcinæ form are easily recognizable, and there are here also different kinds. The varieties of torulæ behave in a similar way. Many fungi grow luxuriantly on gelatine, and can be differentiated from one another by their fructification. When colonies of bacteria lie in the interior of the gelatine, their special peculiarities do not appear so obviously as when they are allowed to develop freely on the surface of the gelatine exposed to the air. For this reason it is best only to compare the colonies growing on the surface with one another, and to inoculate a free surface with any doubtful kind that is buried deeply, and there allow it to develop freely.

I have performed very numerous and extensive series of pure cultures with pathogenic and non-pathogenic micro-organisms on boiled potatoes and nutrient jelly, without once meeting with any obvious alteration in the characteristics of the organisms. Whenever observed, even if cultivated (pure) for months, they always preserve their external appearances, as well as their physiological peculiarities, remaining perfectly constant from the beginning to the end of the investigation; even when the nutritive pabulum was at various times altered, when the interval of time between the various transplantations was at one time as long as possible, and at another as short as possible, or when in one series spore formation was allowed to occur, while in another further inoculations were made before spores appeared, there was still no change whatever in the specific characters of the organisms. Of course contaminations of the most various kinds occurred, and that these were accidental contaminations, and not the transformation of one organism into another, was evident. If, for example, twelve out of fifteen inoculation streaks of anthrax bacilli on jelly develop pure cultures, while in two of the remainder brown masses of micrococci are mixed with the anthrax bacilli, and in the third hay bacilli appear at one point only of the long streak, and further, if a few more colonies of micrococci, hay bacilli, and moulds develop on the jelly away from the inoculation streaks, no one would assert that in one such streak, and at one place only, the anthrax bacilli became converted at once without intermediate forms into genuine hay bacilli, &c. Such an assertion would necessitate the conclusion that, since all the above streaks were under the same conditions, in the two contaminated with micrococci the anthrax bacilli must have become changed into micrococci, and that the colonies of micrococci, hay bacilli, and moulds scattered between the uncontaminated streaks must have arisen by *generatio equivoca*. No one would willingly come to such a conclusion as this last, but would rather say that the independent colonies sprang from spores in the air which had fallen on the jelly. If this is admitted, then there is nothing against the view that micrococci happened to fall on two of the streaks, while hay bacilli fell on one spot in the third.

This illustration is not by any means taken from very unusual circumstances. On the contrary, exactly similar admixtures with extraneous organisms occur in making pure cultivations. Hence

the objection which might be brought against the immutability of the species in my method of cultivation does not hold good, namely, that by only employing the best and purest parts for inoculation no opportunity is offered for the conversion of one kind into another. For in my method only those forms whose characteristics were markedly different from those of the kind cultivated were avoided; if slight transition forms between one species and another had been present, then, as the differences could only in the first instance have been very slight, they could not have been recognized, and hence, without doubt, these slightly altered organisms would have been carried over in the inoculation, and thus, finally, it would have been impossible to avoid obtaining the morphologically altered species. Besides, my methods do not offer the least hindrance to the evolution of a physiologically different variety, since the selection of material for further cultivation is not guided by physiological but by morphological criteria. But I repeat again, that in all my experiments I have never met with either a morphological or physiological metamorphosis of species.

The general principle has always been followed in botany and zoology, that every organism hitherto unknown, and described for the first time, should be named and registered provisionally as a distinct species. It has indeed happened that individual forms, considered to be distinct species, have been shown to be merely developmental stages of a species already well known. It has, however, been far more frequently necessary, after the employment of better methods and instruments in fresh investigations, to break up what had hitherto been supposed to be a single species into several kinds. This valuable and generally applicable principle of regarding all new forms, whose characteristics markedly differ from each other, as different so long as their connexion has not been indisputably proved, has been departed from to a most extraordinary extent by some in regard to micro-organisms, and especially to bacteria. From the commencement of investigations into bacteria, from Hallier to Nägeli and Buchner, we meet with attempts to group all bacteria together (although, as cannot be denied, they differ very much in their characteristics), and to make only one, or at most two species. If the attempt to transform one well-known species of bacteria into another by inoculation or cultivation should ever prove

successful, it would always be possible to group in one class the forms thus proved to be connected with one another. But as yet such a proof is wanting, and hence there is not the slightest reason for departing in bacteriology from the principles of the other natural sciences. If at the beginning a few species too many are adopted, this can in nowise injure the science; but if from the first the desirability and necessity of investigating the different forms of bacteria, and of thus adding to our knowledge, were censured, all further advance in our knowledge of this subject would be barred, and this would, of course, be the severest imaginable injury to this young and most promising branch of science. Truth and knowledge must undoubtedly force their way to the front in this as in all other branches of science, and all untenable hypotheses must go to the wall.

But how often an accurate and painstaking inquiry, advancing, as it always does, with slow and laborious effort, is thrust into the background for a time by highly promising theories, which apparently resolve even the most difficult problems with the utmost ease. And even if no permanent injury results to science thereby, the false direction given to it exerts at any rate a temporary evil influence on the most important questions of sanitation, and also colours the teaching and practice of the same.

It seems to me, therefore, that the only proper line to take (and this is not at all a crotchety view) is to make a careful separation between all the various micro-organisms, more particularly bacteria, which we meet with, and more especially in regard to the latter, to adhere strictly to the principle *that all those bacteria which retain their characteristics, by which they differ from one another, unaltered on the same soil and after several transplantations (so-called generations), should be regarded as different one from another, whether or not they be spoken of as species, varieties, &c.*

Before I conclude these remarks on pure cultivations, I must guard myself against an objection which will certainly be urged against me. It will be said that my method of making pure cultivations is not at all new, and that the idea of cultivating bacteria on potatoes and in gelatine is also well known. That is quite true. It has certainly been known for a long time that some bacteria grow very well on boiled potatoes, and they have

also been cultivated in gelatine and isinglass, but the advantages offered by a firm nutritious pabulum were not recognized, for the isinglass and gelatine were added in such small quantities to cultivation fluids that they did not gelatinize and form solid nutrient soils, or when enough isinglass was used to make the pabulum solid the cultivation was begun with impure material; and besides, the cultures were kept at the body temperature in an incubator, and thus the jelly must have liquefied. Wernich's researches on micrococcus prodigiosus show that the cultivations up till now made on potatoes cannot be regarded as pure cultures, and with regard to this I would refer to Gaffky's work in this volume, in which will be found a full criticism of these investigations.

The peculiarity of my method is that it supplies a firm, and, where possible, a transparent pabulum; that its composition can be varied to any extent, and suited to the organism under observation; that all precautions against the possibility of after contamination are rendered superfluous; that subsequent cultivation can be carried out by a larger number of single cultures, of which of course only those cultures which remain pure are employed for further cultivation; and that, finally, a constant control over the state of the culture can be obtained by the use of the microscope. In almost all those points my method differs from those hitherto employed, and especially also from the former attempts at cultivation with potatoes and isinglass referred to above.

After the above facts were made out, it seemed very important to utilize the excellent qualities of the nutrient jelly for other allied investigations, more especially where it was necessary to learn the number and kinds of micro-organisms present under various circumstances; for example, in the air, in water, in soil, in articles of commerce, in food, &c.

METHOD OF INVESTIGATING AIR.

Every one who has made, or indeed seen, even a few cultures on jelly, will be convinced of the ease with which the particulate constituents of air can be collected in that way, and of the convenience and accuracy with which the number and species of the

different organisms can be noted. It would only be necessary, in order to get comparative results, to bring the desired quantity of air in contact with a known surface-area of nutrient jelly in such a way that all spores, &c., contained in it must be deposited on the nutritive soil.

It is, however, practically very difficult to fulfil these requirements, although it seems at first sight very easy. The earliest attempts in this direction were made by drawing air through sterilized wool by an aspirator, and then distributing the wool, loaded with the dust from the air, in liquefied nutrient jelly, all possibility of further entrance of spores from the air being prevented by air-tight or dust-tight plugs. This method was in so far successful that the colonies of bacteria and moulds developed readily, but in consequence of their growing in the interior of the jelly, and being hidden here and there by the fibres of cotton wool, we do not obtain nearly so striking and beautiful a result as when the colonies spring from spores, &c., deposited on the surface of the jelly. For this reason this method has been for the present given up. Although inapplicable for the general investigation of air, still this method could be employed in certain cases, as, for example, where known quantities of air have to be examined quickly. The next attempt was to aspirate the air (in the manner employed in other well-known methods of examining air) against a drop of glycerine, or against a glass-plate covered with glycerine jelly, and then the drop of glycerine or the glycerine jelly was mixed with enough nutrient jelly to neutralize any injurious effect which the glycerine might exert on the pabulum. Experimental researches into the action of glycerine on micro-organisms carried on at the same time showed, however, that glycerine has no injurious action on the spores of bacilli, moulds, or torulæ, but that it kills in a comparatively short time bacteria which are not in the spore stage, and which otherwise are still capable of development when but recently dried; hence the result is that only colonies of bacilli, yeast, and moulds are formed in the gelatine, and, consequently, this method makes it impossible to form an accurate idea how many active organisms the air may contain, because many forms are destroyed owing to the conditions under which they are collected. Besides, I got the impression that the force of the current of air, which for this purpose must be pretty consider-

able, carries many particles of dust and spores, past the glycerine or glycerine jelly, and these, consequently, are not deposited. When an equal quantity of air was taken from the same place and at the same time, and filtered through wool, it was found that, quite apart from the possibly destructive action of glycerine on micrococci, many more moulds and bacilli developed than when glycerine was employed. The next attempt was to drive the air against the jelly. When a narrow tube was employed for this purpose, it was found that the jelly close to the orifice quickly dried on its upper surface, so that the particles of dust would not stick to it; and, on the other hand, when a wide opening was employed, control experiments showed that very few spores, &c., were deposited on the jelly at all, and so this method was also discarded.

The numerous drawbacks which are entailed by employing a more or less rapidly moving current of air led me to try the simple deposition of the solid constituents of the air from a quiescent or very gently moving column of air. If the column of air employed is of sufficient height, it is possible to calculate to a certain extent on obtaining similar quantities of air, which will deposit their solid particles on the nutrient jelly within the same period of time. It was further necessary to arrange the vessel in which the jelly is put so that the colonies upon it may be examined under the microscope without disturbance, as I have described above with regard to cultivations on microscopic slides.

An apparatus was therefore constructed on these principles, and it appears to me to afford a simple and adequate means for ordinary investigation. At the bottom of a glass cylinder, 6 cm. diameter and 18 cm. high, is placed a flat glass capsule to hold the jelly, 5·5 cm. diameter and 1 cm. high (minus the thickness of the bottom); the glass capsule is easily lifted out of the cylinder by means of a piece of tin bent at right angles. At least twenty of such vessels should be prepared for a series of investigations on air. The cylinder, capsule, and strip of tin are then to be cleansed, the cylinder plugged with cotton wool, and the whole heated for 1 to 2 hours at 150° C. When the vessel has cooled the cotton plug is removed for a moment, the capsule lifted to the margin of the cylinder half filled with nutrient jelly, replaced, and the cylinder immediately

replugged with the wool. If in doing this a few spores from the air of the laboratory should fall into the jelly, they sink and develop in its interior and not on the surface. As soon as the jelly has solidified the apparatus may be used as follows: the wool plug is removed and kept meanwhile safe from contamination* at the place where the air is to be examined, and the vessel is left with the gelatine exposed to the air for a definite period of time, say, for instance, 5, 10, 12, or 24 hours. It is then plugged again with the wool so as to prevent any further entrance of organisms; and the development of the colonies is encouraged by keeping it at a temperature of from 20° to 25° C. The earliest minute colonies will appear as droplets or round white spots in from 24 to 30 hours. By the second day the growth is usually so far advanced that microscopical investigation may be made, and the examination of the colonies performed with the aid of a lens. This must not be delayed longer than two days, otherwise the colonies will be too large and may have run into one another. The composition of the jelly is naturally of especial importance in order to obtain successful cultivations of the very varied kinds of organisms which are found in the air, because it must afford as good soil both for the growth of bacteria and for the growth of the torulæ and higher fungi. As the result of a series of experiments in which I compared the values of different nutrient jellies, it seemed to me that a jelly prepared with wheat infusion might be most suitable for investigating air, for the different kinds of micro-organisms seem to develop equally well in it. Still I would strongly recommend that, supposing an observer has plenty of time and material at his disposal, he should expose as many different pabula as possible to the air, as, for example, boiled potatoes, plum infusion jelly, blood-serum jelly, wheat infusion jelly, &c., in different glasses, and all for the same space of time. If it is only a question as to the pathogenic organisms present (as may be the case in investigating the air of sick-rooms), it is best to employ peptonized meat infusion jelly, and especially blood-serum jelly.

In the course of last winter I carried on for a few weeks a fairly regular series of observations on the organisms in the air, using wheat infusion jelly, to test for myself the practicability

* The simplest way is to place it in a sterilized vessel.

of the method, and to obtain information to some extent as to the existence of germs in the air. The method of investigating air by filtering through wool or aspirating against glycerine drops, as used hitherto, gives a fairly accurate idea of the amount of dust present in the air, and is sufficient for correct enumeration of large spores such as those of moulds, but no method up to this time provided for the accurate estimation of the actual number of living organisms, spores, &c., in the air. Up to a certain point my method undoubtedly supplies this want. It is, of course, impossible to say in what volume of air the organisms which are deposited in the jelly were contained, but on the average in every experiment it must be a comparatively and equally large quantity of air from which the dust is deposited. Even when the apparatus is placed in the open, in more or less freely moving air, the height of the cylinder is such that the air in the lower end of it is practically at rest. From the experiments referred to above, which, as I have stated, are only of value as giving a general idea, I have found that the air of my laboratory contains much fewer bacteria but more mould spores than does the air of the garden of the veterinary school. In a museum, which, at the time the experiments were made, was seldom entered, there were markedly fewer bacteria and fungi than in the laboratory. Very few moulds and bacteria developed in a glass which was exposed for three days in a cupboard the door of which was not firmly closed; on the other hand, nearly as many bacteria and fungi grew on the jelly which was placed near the cages of the experimental animals as when it was put out in the open air. Even in winter the open air contains so many active spores, &c., of different micro-organisms, that after an exposure of the glass for 24 hours frequently over a hundred single colonies grow on the gelatine, which then, after development occurred, appeared to be thickly studded with droplets and small spots. Indeed when it was exposed for only 12 hours the number of colonies varied between 40 and 80, too many for a rapid review, and for further observation of the individual colonies. Hence it seems to me best to expose the glasses for only 4 to 6 hours, and even for a shorter time if the air is very impure.

A systematic investigation must always be completed by

further cultivation of the organisms found in the atmosphere, and by the testing of them as to their pathogenic or other special properties. I had not then time to do this, and so am compelled to postpone further observations in this direction to a future occasion.*

* The above method of testing air is only a qualitative one, and can hardly be reckoned to give quantitative results. Dr. Hesse (*Ueber quantitative Bestimmung der in der Luft enthaltenen Micro-organismen: Mittheilungen aus dem Gesundheitsamte*, vol. ii.) has devised an excellent method for quantitative analysis. He describes his apparatus as follows:—

"The method devised by me, and employed in my investigations, consists essentially in drawing air through long tubes, the walls of which are covered with a layer of solidified nutrient jelly. The current of air is regulated and measured by means of an aspirator. From the number of colonies which develop on the jelly, and from the quantity of air employed, an accurate estimate is obtained of the number of germs in the air. This, however, only gives the number of germs which come in contact with the nutrient jelly, and which can grow under the conditions under which they are placed, such as at the temperature and on the nutrient soil employed.

"APPARATUS.—For these experiments one requires glass tubing, nutrient jelly, and an aspirator.

"GLASS-TUBING.—The most convenient tubes to employ are about 70 cm. long and 3·5 cm. broad, having a capacity of about 670 c.cm. The tubes, the edges of which at each end are somewhat thickened or bent outwards, are now prepared for the reception of the jelly; over one end a closely fitting caoutchouc cap is fastened, having a central hole of about 1 cm. in diameter, and over this a second entire cap is placed; in this way the tube is completely closed at this end (fig. 1). If one only applies a single cap over the end of the tube, as I did in my earlier experiments, there is a danger of detaching the jelly from the glass when the cap is removed, and therefore I used to cut out a small central hole in the cap before the commencement of the experiment; but by the use of two caps there is no danger of disturbance of the jelly by the removal of the outer cap.

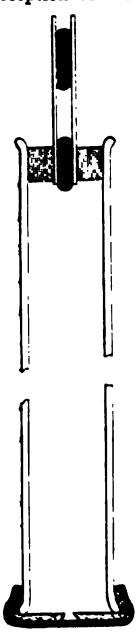

Fig. 1.

"Into these tubes 50 c.cm. of fluid nutrient jelly are introduced by means of a pipette. This quantity is sufficient to cover the inner wall of the tube completely." (The nutrient jelly which Hesse found to be best was the peptonized meat jelly of the formula given in the footnote on p. 46.)

"Into the other end of the tube now containing the jelly a tightly fitting caoutchouc cork about 2 c.cm. in diameter is introduced. The central part of this cork is perforated with a hole about 1 cm. in diameter, and through this passes a piece of glass tubing about 10 cm. long and 1 cm. wide, and containing two plugs of cotton wool. The plug nearest the large tube projects a little beyond the end of the small glass tube. (See fig. 1.) This small piece of tubing is for the purpose of easily connecting the large tube with the aspirator, of equalizing the atmospheric pressure inside and outside the tube, and to act as a filter of the air. The use of the two wool plugs is in order to meet requirements to be referred to later, which make it desirable to remove a part of the contents or to alter the position.

"The tubes, &c., are by no means free from micro-organisms even though the greatest care and cleanliness be observed. Although these accidental

INVESTIGATION OF EARTH.

The examination of earth for the organisms contained therein is a much simpler matter, and does not require any special apparatus. The portion to be tested is sprinkled on jelly spread on microscopic slides, care being taken that the particles of earth do not lie too closely together. If subsequent contamination from aerial spores, &c., is to be perfectly excluded,

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impurities could be recognized from the position in which the colonies appear (mostly in the substance of the gelatine), nevertheless in consequence of their further development the result of the experiment would not be satisfactory; and further, the innermost wool plug, which, as will be later seen, is employed to control the accuracy of the experiment, would on account of its contamination be useless for that purpose. Hence it is necessary to destroy these organisms. This is most conveniently done by exposing the tube with the gelatine, &c., for one to two hours to a current of steam at or near 100° C. For this purpose I employ an apparatus similar to that described by Koch, Gaffky, and Löffler (vol. i. of these *Mittheilungen*, p. 332). (See fig. 2.) By means of it six tubes can be sterilized at one time. It consists of a cylindrical tin vessel about 1 m. high, 13 cm. wide, covered with a tin cap and surrounded by felt; this cylinder is fixed on the top of a tin vessel filled with water, and 20 cm. high and 13·5 cm. wide.

"After the tube has been removed from the steaming apparatus, and while the jelly is still fluid, it is moved in various directions under a cold water tap till the jelly solidifies. (Thus an even coating over the whole tube is obtained, but in later experiments it was not found necessary to have the whole wall of the tube covered, as the organisms fell to the side on which the tube lay.) The tube is now washed externally in a 1 per cent. corrosive sublimate solution, and fixed horizontally on a stand. (This stand is somewhat similar to that used in photography. See fig. 3.)"

By means of an elastic tube attached to the narrow glass tubing the aspirator is now connected with this tube. (Hesse generally uses two 10 litre flasks.) The one nearest the apparatus is filled with water, the other is empty. By setting a syphon arrangement into action the water flows from the upper to the lower flask, and the air which takes its place must pass through the apparatus. The rapidity of flow is easily regulated by a stopcock. When everything is ready, the outer caoutchouc cap over

Fig. 2

an arrangement must be adopted like that used for inves
air. As a rule it is impossible to confuse the spore
are contained in the earth with those which may fall
jelly from the air, for the first-mentioned organisms st
the particles of sand and earth. For this class of researc
prepared with wheat infusion or peptonized meat infu
be found to be particularly suitable. Some experi
made on the organisms contained in the earth, altho
in number, still afforded fairly constant results, and p

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the end of the large tube is removed, and the water is made to flow from
to the lower flask at a moderate rate. It was found experimentally th
dust was deposited in the large tube unless the flow was very rapid.

Fig. 3.

sufficient quantity of air has been drawn through, the aspirator is detache
tube laid horizontally at a suitable temperature (20° to 25° C.). In tw
days colonies are evident which may be counted and investigated.—

the general conclusion that the upper layers of the soil are particularly rich in spores of micro-organisms (the great majority being bacilli). In perfectly fresh earth micrococci are usually found, but almost always in small numbers. In testing very impure places, such as soils impregnated with manure, the number of micrococci exceeds that of the bacilli, and moulds were also present, but this is of course simply a local peculiarity. Bacilli, on the other hand, occur constantly, and always in large numbers, in the superficial layers of the ground round dwelling houses, and where gardening and agriculture is carried on. I have found them in the earth of the garden of the Veterinary School in Berlin in as large numbers as in the earth of a disused cemetery, and in earth taken from gardens and ploughed fields at a long distance from any thickly populated parts. If the portions of earth are first dried for a few weeks micrococci do not appear in the cultures, while the bacilli are as numerous as before. Since it has been known for some time that micrococci do not form spores, and consequently can survive the dried condition but a very short time, it is obvious that while the micrococci are killed by the drying, the bacilli must be present in the earth in the form of spores. This view is confirmed by the fact that the germs of the bacilli in the earth can withstand degrees of heat which are fatal to everything except spores. This we have found to be the case in experiments on disinfection. Since only spores and very rarely bacilli are found in the earth, it seems to me very probable that these spores are not developed at the spot where they are found, but are brought to the soil in manure and putrefying material; it is also very possible that they may be swept off by a current of air from the place where they are developed, carried a long way, and deposited on the earth, and hence become mixed up in its upper layers. The chief bacilli found in earth are hay bacilli and the bacilli which form the root-like colonies already described; but besides these there are often a few more, usually 6 or 8, very well marked species of bacilli.

I observed one very striking fact, which, however, I do not assert to be invariably true as it is only based on a few observations. It consists in the steady but rapid decrease in the number of micro-organisms in the earth strata according to

the depth, so that at the depth of one metre the undisturbed soil is always free from bacteria. I have proved this to be the case even in the midst of Berlin, in soil freshly excavated for buildings, cultivation on gelatine of the soil from the depth of one metre showing no bacilli, and only a few solitary colonies of very small micrococci. In one case the earth was taken at 2 metres depth from the foundations of a new house close to the tank *Panke* in the Philippstrasse, at the level of and scarcely 2 metres distant from the water, and yet these specimens of soil proved to be extraordinarily poor in micro-organisms. It is well to remember, however, that my observations were only made in winter, and it is possible that in summer the facts are different. Nevertheless if, according to the universally accepted view, there is a luxuriant development of micro-organisms in the ground, water, and the earth in its neighbourhood, the spores of these organisms must be left behind, and ought to be found in winter in the lower layers of the soil as easily as they can be demonstrated in the upper surface. But as this is not the case it appears to me very doubtful whether any considerable number of micro-organisms exist in the deeper layers.

INVESTIGATION OF WATER.

The study of water by the help of nutrient jelly likewise offers no difficulties. A known quantity of the water in question is mixed with a large quantity of liquefied jelly, and the vessel is then plugged with cotton wool and the colonies allowed to grow in the jelly until they can easily be recognized with the microscope, and specimens taken from them for future cultivation. With regard to the last point it is best to pour out the mixture into flat vessels, and to allow it to solidify so that the colonies may be scattered as far as possible and be readily reached with the needle. It is also of advantage to use a very clear and colourless jelly, such as wheat infusion jelly, because in this method of research the colonies develop in the substance of the jelly. As regards the quantity of water to be used, I may say that in some instances in which 1 cc. of water was mixed with 10 cc. of jelly very few bacterial colonies appeared, while in another instance the micro-organisms (among which were colonies

formed of very short rods, and therefore belonging to the subclass bacteria, and also numerous moulds) were present in such numbers as to make a complete examination impossible, so that the water had to be diluted 10 to 20 fold before it could be used for cultivations.

INVESTIGATION OF DUST.

Dust is a very interesting substance for cultivation on jelly, and I believed at first that such cultures would afford examples of every imaginable organism infesting the air, but I very soon found that this belief was not warranted by the facts. For while a fair number of moulds and a few bacilli grow from freshly deposited dust only a few micrococci develop, as compared with the numbers which are found when the jelly is exposed to the dust floating in the air. Old dust, furthermore, such as may be collected from the interior of furniture or from out-of-the-way corners, only produces moulds and a considerable number of bacilli when sown on jelly; so that we have here another confirmation of the fact that the majority of organisms in the air die very soon when subjected to the drying process, and that only the spores of moulds and bacilli (especially the latter) preserve their vitality.

INVESTIGATION OF MISCELLANEOUS OBJECTS.

Little explanation is necessary to show that the most diverse objects can be examined in exactly the same way as air, water, earth, and dust, and tested as to the presence of micro-organisms capable of development. The field of research which the above-mentioned novel method opens to us is so enormous that it is most important that it should be attacked by many different workers. Besides the investigation of air, water, and earth, with special reference also to the ground and rain water in very many different places, and at various periods of the year, it is also most important that special investigations should be made into the air of rooms which are inhabited or not, namely, school-rooms, sick-rooms, mortuaries, and manufactories, especially in those which are overcrowded, and where perishable or readily decomposing substances are employed. In addition to

these, everything which can possibly act as a carrier of pathogeni organisms, or affords them shelter in any way, must also b thoroughly examined, such, for instance, as brickwork, wood work, carpets, clothes, articles of commerce, such as money, &c and, most important of all, articles of food, such as mill sausages, &c. In connexion with the subject of sausages w may draw attention to the frequency with which cases of sausag poisoning have been published lately.

THE

ETIOLOGY OF TUBERCULOSIS.

By Dr. R. KOCH,

(*Mittheilungen aus dem Gesundheitsamte*, Vol. ii., 1884.)

TRANSLATED BY

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culosis of the thoracic duct, and Weigert's detection of tubercle in the walls of veins in miliary tuberculosis. These facts, however, bear only upon the diffusion of the tubercular virus within the body; we cannot conclude from them that the virus is communicable from one individual to another, or, in other words, that the disease is truly infectious.

With the latter question experimental pathology has dealt in the most exact way. The course of experimental research into the infective nature of tuberculosis has lately been fully considered on several occasions (S. Johne, *Die Geschichte der Tuberkulose*, Leipzig, 1883), so that I shall limit myself to a few remarks on the points of most importance.

Isolated, imperfect attempts at the artificial production of tuberculosis made at the end of the last century gave only negative results. The credit of the first successful experiments belongs to Klencke, who in the year 1843 succeeded in inducing an extensive tuberculosis of the lungs and liver in rabbits, by inoculation with portions of miliary and infiltrating tubercles from Man; and he did this by the introduction of these masses into the veins of the neck. He did not continue his researches, and they were consequently soon forgotten. In the meantime Villemin undertook an experimental investigation into the nature of tuberculosis, working in a methodical and thorough manner. He inoculated not only with tubercular material from human beings, but also from cases of bovine tuberculosis, and proved experimentally the identity of the latter disease with human tuberculosis. Villemin's researches, from the number of his experiments, the careful manner in which they were carried out and the employment of suitable control experiments, appeared to have decided the question in favour of the infective theory. The numerous workers, however, who repeated Villemin's experiments after the same or a modified method, arrived at very contradictory results. The partisans of the infective theory, amongst whom Klebs must be specially noticed, sought to improve the details of the experimental method and to free it from the imperfections surrounding it; its opponents strove, on the contrary, to prove that tubercular material contained no specific virus, and that true tuberculosis could be induced by inoculation with *non-tubercular* material. To the decision of this question Cohnheim and Salomonsen

contributed largely by selecting for inoculation, in a moment of inspiration, the anterior chamber of a rabbit's eye. By this means it is possible to separate the cases in which successful inoculation with tubercular material has been accomplished from those in which some other infective material has been introduced with the tubercular virus. Subcutaneous inoculation with such material often causes a more or less widely diffused cheesy infiltration, not unlike that of tubercle. But in the eye these substances give rise only to a general inflammation of short duration, which cannot in any case be mistaken for the slow and characteristic development of tuberculosis resulting from inoculation. The course of a successful tubercular inoculation can be watched throughout by the experimenter. After a fairly long incubation period, single grey nodules, barely visible to the naked eye, appear in the iris, starting from the piece of material introduced. The number of nodules gradually increases, they enlarge, become yellowish in the centre, caseate, and show macroscopically as well as microscopically all the typical characters of the true tubercular nodule. The tubercular infection, however, does not remain limited to the eye, but invades later the whole organism, spreading to the neighbouring lymphatic glands, the lungs, spleen, liver and kidneys. Cohnheim and Salomonsen, as also the observers who repeated their experiments, unanimously state that in no case did tuberculosis of the iris follow an inoculation with non-tubercular material. A further point is that spontaneous tuberculosis of the iris has never been observed in rabbits. This method of infection is, therefore, preferable to all others, in so far as it completely excludes both a vitiation of the experiment by unnoticed errors of manipulation, such as may easily occur in cases of subcutaneous inoculation or introduction of materials into the peritoneal cavity, and also the possibility of mistaking spontaneous tuberculosis for an artificially induced form of the disease. In contradistinction to the earlier experiments on the infective nature of tubercle, the researches of Cohnheim and Salomonsen must, therefore, be regarded as quite free from objection, and they established the fact that tubercular materials, apparently differing widely from each other, are characterized by one and the same specific contagium. But it was impossible at the time to decide upon the nature of this contagium—

whether it consisted of independent organisms, endowed with constant properties, which invaded the body as parasites and rendered it tubercular, or whether it was composed of particles of an organized or even unorganized nature, arising only under certain abnormal conditions within the body and indeed from its own elements.

Judging from the results which had been recently attained concerning the etiology of many infective diseases, it seemed not unlikely that the cause of tuberculosis might also be found in some micro-organism. To arrive at some conclusion on this point, it was obviously necessary to utilize all those methods which had proved of value in the investigation of other infective diseases, and to follow that order of research which had on former occasions proved itself best adapted to the end in view. Consequently, the following plan of inquiry was decided upon. First, to determine whether formed elements, which could neither belong to the constituents of the body, nor have sprung from them, were present in the diseased parts. Were the presence of such foreign elements demonstrated, it would next be necessary to ascertain whether they were organized, and whether they exhibited any signs of possessing independent life, the chief of these being spontaneous movement, so often confounded with molecular motion, the power of growth, increase, and reproduction. Further, the relations of these forms to their surroundings, the behaviour of the neighbouring tissue-elements, the distribution of these forms through the body, their presence in different stages of the morbid process, and similar points, would have to be worked out; these all having a bearing of more or less importance on the causal relation of these forms to the disease under consideration. It seemed possible that the facts thus brought to light might furnish such decisive proof, that only the most extreme sceptic would still maintain that the micro-organisms discovered were concomitants, and not the cause, of the disease. Often, however, there may be grounds for this objection; complete proof of the causal relationship demands, not merely a demonstration of the coincidence of the parasites with the disease, but, beyond this, it must be shown that the parasites directly produce the disease. To obtain this proof, it is necessary to isolate the parasites completely from the diseased organism, and from all the products of the disease to which any

pathogenic influence could be ascribed; then to excite anew the disease with all its special characteristics by the introduction of the parasites alone into a healthy organism.

An example will render this clearer. If the blood of an animal dead of splenic fever is examined, numerous rod-like, colourless, motionless forms are invariably found. One could not at once regard these rods as of vegetable nature, and, as a matter of fact, they were at first held by many to be lifeless crystalline bodies. It was only when they were observed to grow and form spores, which again developed into rods of a similar character, that it could with certainty be said that they were living, and that they belonged to the lowest forms of plant life. Now if an animal be inoculated with the minutest quantity of blood containing these rods from another animal dead of splenic fever, the former will, without fail, die also of splenic fever, and in its blood rods, the so-called anthrax bacilli, will likewise be found. This is, however, not sufficient to prove that the disease was communicated by inoculation with the rods, for other formed and unformed elements of the blood were introduced with them. To prove that the bacilli, and no other constituents of the blood, produce splenic fever, the bacilli must be isolated from the blood and introduced alone. The isolation of the bacilli is best effected by successive pure cultivations. With this object, a small quantity of blood containing the bacilli is placed upon a solid cultivating material suitable for the growth of the bacillus, *e.g.*, prepared nutrient jelly or boiled potato. There they soon begin to multiply, while the remaining elements of the blood, the red and white corpuscles and the serum, remain unchanged. After two or three days, when the bacilli have formed a close mass of spore-bearing threads, the least possible quantity of this mass, no longer blood-red but whitish in colour, is taken and again transferred to gelatine or boiled potato. The bacilli increase now in exactly the same way as in the first cultivation, and form again a thick white covering over the potato; the most careful microscopic examination will now detect hardly any trace of the other elements of the blood. The cultivations are continued on the same plan. After the third or fourth the bacilli may be regarded as isolated from the other constituents of the blood originally transferred with them. If the cultivations are carried

out to the number of twenty or fifty, or even more, it may be accepted as practically certain that not the smallest portion o the morbid products from the body adheres to the bacilli, no can they contain anything of the kind in their interior, for thos first transplanted are no longer present, and their remot descendants have obtained the materials for their developmen from the soil, the potato, upon which they grew. The bacil thus obtained by pure cultivations have consequently no kind connection with the diseased animal from whose blood the fir cultivation was made, nor with the morbid products resultin from the chemical changes occurring in the animal. Yet th give rise to splenic fever of a fatal character in a healthy anim inoculated with them. The animal experimented on becom ill just as quickly and with the same symptoms as if it had be inoculated with fresh anthracic blood, or as if it were sufferi from the spontaneous form of the disease; and in its blood found, as in the natural disease, countless bacilli, possessing same characteristics as the well-known anthrax bacilli. In face of these facts, it is impossible to come to any other conclus than that the splenic fever bacillus is *the cause* of the disea and not merely an accompaniment of it. Now splenic fever d not always present the same clinical spectacle, it appears un different forms in different animals; in Man it may run course of a general infective disease without any striking lo changes; or it may remain purely local, being limited to spot on the skin, the intestine or larynx. Nevertheless, in case where the specific bacilli are found at the seat of dis we must regard them as the cause of the morbid condition, their pathogenic properties are well-known, and it is in ceivable that in the tissues of the same subject they should present at one time as harmless parasites, at another tim pathogenic organisms. These conclusions are so unanswer that no one now opposes them, and science universally acc the bacillus anthracis as the cause both of the common typ splenic fever we are familiar with in our domestic animals, also of the clinically different forms of the disease which o in man.

In my investigations into the etiology of tubercle I l followed the method by which the parasitic nature of spl fever was so effectually established. I first turned my atten

towards proving the presence of a pathogenic organism, thence passing on to isolation and inoculation experiments. I will now proceed to describe the separate steps of the inquiry.

I.—THE DEMONSTRATION OF PATHOGENIC ORGANISMS IN TUBERCULAR ORGANS AND IN THEIR SECRETIONS.

There is no great difficulty attending the examination of pathogenic organisms of the size of the anthrax bacilli, and, like them, present in large numbers in the blood, or of such, like the spirochætæ of relapsing fever, as readily strike the eye by their peculiar motion; the ordinary optical apparatus will be found sufficient. But it is otherwise where we have to do with small pathogenic bacteria sparsely distributed in the substance of the tissues, more especially when proliferation and disintegration of cells have begun to take place at the seat of investigation, as is usually the case. It then becomes necessary to make use of all the more delicate methods of microscopy, such as special modes of preparation and differential staining, and to make the examination with the best optical apparatus, such as oil-immersion lenses and Abbe's condenser.

With regard to tuberculosis, it was to be expected that the discovery of pathogenic organisms might be attended with unusual difficulty, since many attempts had been made to demonstrate them without producing satisfactory results. I began my investigations with material in which the infective virus might confidently be expected to exist, for example, recently developed grey tubercle from the lungs of animals killed three or four weeks after inoculation. Sections of such organs hardened in alcohol were prepared and examined for bacteria after the most trustworthy methods. Grey tubercles were also crushed, spread on cover-glasses, dried and then examined for the presence of micro-organisms. Every attempt to discover bacteria or other micro-organisms in these preparations was unsuccessful. Earlier observations having shown that in certain cases the deepest staining and clearest differentiation of bacteria from surrounding tissues were yielded by the use of stains which were of alkaline reaction, advantage was taken of this fact. Of the common aniline dyes, methylene blue bears the freest addition of alkalies, therefore this staining material was chosen;

and to a watery solution of it caustic potash was added, so long as no precipitate formed, and so long as the fluid remained clear. For this purpose 1 c.cm. of a concentrated alcoholic solution of methylene blue was mixed with 200 c.cm. of distilled water and well shaken; to this 2 c.cm. of a 10 per cent. solution of caustic potash was added, the mixture being repeatedly shaken. When cover-glass preparations were exposed to this staining fluid for twenty-four hours, very fine rod-like forms became apparent in the tubercular mass for the first time, having, as further observation showed, the power of multiplication and of spore formation, and hence belonging to the same group of organisms as the anthrax bacilli. It was incomparably more difficult to recognize these bacilli in sections, amongst the heaped up nuclei and masses of detritus, and an attempt was consequently made to render the tubercle-bacilli more evident by contrast-staining, according to the method by which Weigert succeeded in colouring splenic fever bacilli with one tint and the surrounding tissues with another. This object was attained by using a concentrated watery solution of vesuvin, with which the blue-stained cover-glass preparations and sections were treated until they appeared perfectly brown to the naked eye. Microscopic examination then showed that only the previously blue-stained cell-nuclei and detritus had become brown, while the tubercle-bacilli remained of a beautiful blue, and stood out clearly from their surroundings, so that they were easily recognized even amongst dense masses of nuclei. In the above process methylene blue does not colour the bacilli very deeply, and some practice is needed to demonstrate them successfully in all tubercular organs.

Another method, by which the bacilli are very deeply stained, we owe to Ehrlich. I use it now exclusively, and recommend it highly to all who are beginning to work at the bacilli of tubercle. Ehrlich's method has of late undergone many modifications, and in some respects improvements, amongst which I would place Weigert's plan of mixing the solutions in definite proportions, and Rindfleisch's recommendation to hasten the staining process by warming the staining fluid. In describing minutely my own way of carrying out Ehrlich's process, I would by no means imply that it is necessarily the best, and that other modifications may not give quite as good results. As, however, the staining of tubercle-bacilli seems to present difficulties to many investigators,

it will not be superfluous to give as exact a description as possible of the process.

For the preparation of the staining fluid, aniline-water and a saturated alcoholic solution of methyl violet (N.B., not methylene blue) or fuchsin are necessary. The aniline-water is made by adding about 5 c.cm. of pure aniline—an oily liquid, colourless at first but turning brown later—to 100 c.cm. of distilled water, and shaking the two thoroughly together. From 3 to 4 per cent. of aniline is taken up by the water, and the remainder adheres to the bottom of the vessel in the form of thick drops. The saturated watery solution of aniline so obtained, which is generally ready in about half an hour, is run through a previously moistened filter, to remove the undissolved aniline. The filtrate should be transparent as water and colourless, holding no small drops of aniline in suspension ; if any are found to be present, the liquid must be refiltered. Aniline-water will not keep, and must be freshly prepared each time it is required.

The second ingredient of the staining fluid, the saturated alcoholic solution of methyl violet, is obtained by pouring 100 to 150 c.cm. of absolute alcohol on to a sufficient quantity (20 grm.) of dry methyl violet in a well-stoppered glass vessel, and shaking frequently. After standing for several days some undissolved methyl violet will still remain at the bottom of the flask, which can be gradually brought into solution and utilized by the further addition of alcohol. If fuchsin is used in place of methyl violet (and it seems to have certain advantages for permanent preparations), exactly the same process is followed.

The next step is to mix 11 c.cm. of the alcoholic solution of methyl violet with 100 c.cm. of aniline-water, according to the recommendations of Weigert. I generally add 10 c.cm. of absolute alcohol to this mixture, as I have found that the staining fluid will then keep for about ten days without requiring to be filtered each time before use.

The preparations to be examined for tubercle-bacilli must be made in the following way.

The cover-glasses must be rinsed in nitric acid and cleansed with alcohol, to free them from any adherent particles of grease or dirt which might prevent the substance to be examined from sticking to the glass. The material must then be spread on a cover-glass in the thinnest possible layer. This is most easily managed when we

have to deal with soft cheesy masses, which can be evenly and thinly spread by means of a needle or scalpel. More solid, crumbling, cheesy masses must be carefully crushed with the scalpel and distributed over the cover-glass by repeated manipulations. The preparation of a hard tubercular nodule is still more difficult. It must be completely broken up and torn asunder on the cover-glass. Specimens of sputum, also, need a special method of preparation. It will not do to take any chance streak of mucus from the sputum, which consists not only of secretion from the diseased parts of the lungs, but also of that from the bronchial tubes, of saliva and mucus from the mouth and nose. We want to examine the secretion furnished by the diseased lung, and must therefore confine our attention to the ball-like yellowish masses, often to be found floating singly in the frothy viscous liquid, often, however, forming the greater part of the sputum. One of these yellowish tenacious masses must be brought to the edge of the fluid, and a small piece separated from it with a scalpel must be drawn up to the upper part of the inner surface of the glass. It can now be easily broken up and transferred in particles of any size to the cover-glass, where it must be spread out with the scalpel evenly and in a thin layer, any superfluous material being brought to the edge and removed by means of blotting paper.

The layer must now be left till quite dry, when the cover-glass is exposed to a transient heat, in order to render the layer upon it insoluble in the watery fluids with which it is next to be brought in contact. The heating can be effected by placing the cover-glass for twenty minutes in a dry chamber at a temperature of 110° C., or by holding the cover-glass in forceps and passing it several times, not too quickly, through the flame of a spirit-lamp or gas, care being taken to keep the side on which the specimen lies uppermost, out of direct contact with the flame. It can easily be proved by the following experiment that careful heating such as this has no effect whatever on the bacteria, cells, &c., contained in the specimen. Taking a series of prepared cover-glasses, the first is not heated at all, the second is passed once through the flame, the third twice, and so on. They are then treated with staining fluids, and on examination no difference is seen between the colouring of the bacteria and cell-nuclei in the unheated specimen, and

in those heated once, twice, thrice or four times. The forms also remain unchanged. But if the heating is carried further, and the cover-glasses passed oftener through the flame, the bacteria by degrees lose the property of taking up the colouring matter, whilst the cell-nuclei retain this power even after intense heating. In preparations which have not been heated at all, the dry layer of material is often more or less, perhaps completely, detached, and the albuminoids form with the colouring matters a precipitate upon the cover-glass which obscures the specimen and renders the discovery of minute bacteria very difficult or even impossible. Glasses passed once or twice through a flame give better results, but three times appears to be best. In the latter case the layer adheres evenly to the glass, the albuminoids have become totally or so nearly insoluble that no precipitate forms, the bacteria and cell-nuclei are alike deeply stained, while the ground substance remains colourless or almost so. My plan therefore is, when the specimens are quite dry, which will generally be within a few minutes of spreading them out, to pass them three times moderately quickly through the flame of a Bunsen burner.

After heating, the cover-glass must be floated face downwards in a watch-glass or flat vessel containing the staining fluid, care being taken that no air-bubbles lie beneath it, which would protect the specimen from exposure to the staining fluid at this spot. If the fluid is now heated over a flame till bubbles form, and after once boiling, is left in contact with the cover-glass for about ten minutes, a sufficiently deep colouration will be produced. Better results are obtained by leaving the preparation in the unheated staining fluid for several hours. In all difficult cases, where one has only a few bacilli to deal with, it is best to leave the cover-glass for twelve hours or longer in the staining solution.

If sections are to be examined for tubercle-bacilli, small pieces of the organs required must be thoroughly hardened in absolute alcohol; other methods of hardening interfere with or entirely prevent the staining of the bacilli. The sections need not be very thin, as by double staining it is possible to detect easily single bacilli in fairly thick sections. It is desirable, however, to secure large sections, the distribution of the bacilli being often very unequal, so that in a small section none may

be met with; a microtome is therefore almost indispensable. The sections are placed at once in the staining fluid and left there for at least twelve hours. An immersion of several days will do them no harm.

Both the sections and cover-glass preparations, on being taken out of the staining fluid after the necessary time has elapsed, appear dark blue, almost black. All the tissues are stained about equally, and it is hardly possible to recognize the coarser structures. To render the preparation suitable for microscopic examination, much of the colour must be removed. This can be done in various ways. In my earlier attempts at staining with an alkaline solution of methylene blue, I found that the blue colour could be discharged from the tissue-elements by treatment with a solution of vesuvin. The same can be done with preparations stained after Ehrlich's method. They must be washed in water, placed in a concentrated watery solution of vesuvin and moved about frequently in it, and finally transferred thence to alcohol, when they will be found to have lost the dark blue colour almost completely. Preparations made by Ehrlich's method can, however, be more quickly and effectually decolourized by treatment with dilute nitric acid. I have mentioned that this effect may be produced by other aniline dyes, such as the above-mentioned vesuvin, because by many the action of nitric acid is erroneously regarded as specific; this, however, is not the case, for other acids have a similar power.

Nitric acid diluted with two parts of water is generally used for decolourizing preparations. But there is no need to use it in such a concentrated form, and I have lately diluted the pure acid with three to four parts of water. It may be possible to carry the dilution still further. Care must be taken that no nitrous acid is present in the nitric acid.

In speaking of the decolourization of specimens by nitric acid, I have followed the description given by Ehrlich. This description applies also to the treatment of cover-glass preparations with nitric acid, when they are lightly stained, but a deeper tint, which certainly gives better and more trustworthy results, is not entirely discharged by a few minutes exposure to the action of nitric acid, and sections which, as has been already said, must be left for some time in the staining fluid and deeply coloured, retain a tolerably dark tint after treatment with nitric acid.

Hence the expression "decolourize" is not to be understood literally. Failure in staining bacilli appears often to have been due to the fact that workers have expected the preparations to be quite colourless after treatment with nitric acid, and to attain this result have either stained them too lightly or left them too long in the acid.

Sections that are transferred to nitric acid after lying for twelve hours in the staining fluid lose their blue-black colour in a few seconds and become greenish-blue. If they are now placed in distilled water, the tint immediately changes to blue with a violet shade and becomes darker. Nitric acid, therefore, leaves some colouring matter in the specimen which is insoluble in water, and which, in combination with water, assumes a darker tint. It is evident that this remaining colouring matter is very sparingly soluble also in nitric acid, for when the preparations are again immersed in the acid, the greenish-blue colour returns, but no fainter than on its first appearance, and if they are again washed with water they regain their former darker hue. Hence I conclude that no further decolourization can be obtained by leaving the preparations longer in the acid than a few seconds, at most half a minute. I find, however, that the colouring matter remaining after treatment with nitric acid is soluble in alcohol of the strength of 60 to 70 per cent., if the specimens are transferred direct to alcohol from the acid. A longer immersion of the preparations in water seems at last to make the colouring matter insoluble also in alcohol, and on this account it is necessary to transfer them straight to alcohol from the nitric acid without washing them first in water.

My method of decolourization then is as follows: the preparations are lifted out of the staining fluid with a platinum wire fused into a glass rod and placed in nitric acid diluted with three to four parts of water; in this they are freely moved about for some seconds until they become of a greenish-blue colour, and are then transferred to a vessel containing 60 per cent. of alcohol. They are left in the alcohol for about ten or fifteen minutes, and are then ready for the next staining process.

In preparations treated with nitric acid and alcohol, the tissue-elements are quite colourless or of a very light blue tint, whilst the tubercle-bacilli present an intense blue colour. It is almost impossible to determine the position of the

bacilli in relation to their surroundings in specimens so prepared. It is also very difficult to discover single bacilli in tissues, the structure of which is much obscured by the peculiar mode of illumination presently to be considered, and it is therefore necessary to stain the nuclei of the tissue subsequently. In order to get the strongest possible contrast between the staining of the bacilli and cell-nuclei, a yellow or light brown stain is chosen when the bacilli are blue, and when they are red, green or blue is preferred for the tissue; in the first case vesuvin is best suited, in the second methylene blue. Both of these dyes must be used in weak solution, and the time of their action limited, the object being to obtain a sufficiently distinct colouration of the nuclei, without obscuring single bacilli by masses of deeply stained nuclei. For the contrast stain I am in the habit of using a watery solution of vesuvin freshly filtered, which remains transparent in a layer two centimeters deep. The cover-glass preparations are floated on the liquid, face downwards. Sections must be left in it for some minutes. It is not necessary that the sections should be perfectly colourless when they are transferred from the alcohol to the vesuvin solution, as they have to be treated with alcohol again to remove water, and any blue colouring matter remaining is then dissolved out.

From the vesuvin solution the preparations are again transferred to 60 per cent. alcohol, and from this again to absolute alcohol. The further steps of the process need not be repeated, but I would recommend that oil of turpentine, or, still better, cedar oil, which does not dissolve the aniline dyes out of the specimens, should be used for clearing instead of oil of cloves. With regard to mounting, Canada balsam diluted with oil of turpentine appears to answer best. Very thick balsam, which has to be used warm, cannot be employed, as the heating would quickly decolourize the tubercle-bacilli.

Cover-glass specimens can be examined as soon as the vesuvin solution has been washed off in water, or they may be allowed to dry, and then mounted in Canada balsam. Contrast staining is as a rule not required in examining sputum for tubercle-bacilli, so that preparations of sputum may be placed under the microscope immediately after treatment with nitric acid and alcohol.

To sum up the whole process:

Cover-glass preparations dried in the thinnest possible layer, after drying are heated thrice in a flame.

Sections of objects, thoroughly hardened in alcohol.

Stain with a solution consisting of 100 c.cm. of aniline water, 11 c.cm. of an alcoholic solution of methyl violet (or fuchsin), 10 c.cm. of absolute alcohol.

Let the preparations remain in the staining fluid for at least 12 hours. (The staining of cover-glasses can be hastened by warming the solution.)

Immerse the preparations in dilute (1 to 3) nitric acid for some seconds.

Rinse in 60 per cent. alcohol for some minutes. (It is sufficient to move cover-glass specimens to and fro in the alcohol a few times.)

Place in a dilute solution of vesuvin (or methylene blue) for some minutes.

Wash again in 60 per cent. alcohol, dehydrate in absolute alcohol, clear with cedar oil.

Examine the preparations microscopically.

Mount in Canada balsam if permanent specimens are required.

As to the microscopic examination of the specimens so prepared, my remarks elsewhere* upon the use of the microscope for stained objects hold good.

In the present case, again, we have not to determine relations by means of the different refractive powers of the various tissue-elements, but simply to see as clearly and sharply as possible the colour-relations of the microscopic objects, in other words, the absorption-pictures. The structure-picture is only confusing, and must, therefore, be got rid of; and this is effected most completely, as I have shown, by means of Abbe's well-known substage condenser. As every glass will not bear the peculiar illumination furnished by this apparatus used without a diaphragm, specially constructed objectives become necessary. The larger the angle of aperture of an objective the better suited it is for examining absorption-images with the aid of an Abbe's condenser. Oil-immersion lenses are for this reason the most useful for the examination of coloured specimens.

* *Untersuchungen über die Aetiologie der Wundinfectionskrankheiten.* Leipzig, 1878, p. 31 *et seq.*

Cover-glass specimens when rightly prepared ought to be so thin that the structure-picture is formed by a single layer of objects only, and attracts but little attention. These preparations can therefore be examined simply in water, and a water-immersion objective will, in the absence of a more perfect glass, meet all requirements if the field is sufficiently lighted by means of a condenser. In sections, on the contrary, it is impossible to set aside the structure-picture of several superimposed layers of tissue unless the preparation is placed in a highly refracting liquid to do away with the differences in refraction of the tissue-elements, and unless the full illuminating power of an Abbe's condenser is utilized by means of the wide angular aperture of an oil-immersion lens. It is easy to convince oneself how indispensable the optical aids just mentioned are, by examining a section stained according to the foregoing directions, first in water with a dry or water-immersion objective and a relatively small diaphragm. Fine differences of colour, and consequently small stained bacteria, are hardly to be recognized under these circumstances in tissues containing many nuclei. Sections mounted in glycerine are hardly any better, for the differences in refractive power of the tissue-elements are insufficiently and much too slowly equalized by the glycerine. A great improvement is effected when highly refracting liquids, such as oil of cloves, cedar oil, &c., are used for clearing the sections, for clarification really means the more or less complete obliteration of refractive differences, and therefore of the structure-picture. Even this is not enough to show the colour-images in all their clearness and sharpness; this can be accomplished only by means of the intense illumination from all sides furnished by an Abbe's condenser in conjunction with an oil-immersion lens. So if we wish to examine only cover-glass specimens, and do not require absolute certainty in the result, a microscope with water-immersion objectives and without any illuminating apparatus will suffice. Dry objectives are not on the whole suitable for bacterial investigations. But if it is desired to conduct a trustworthy examination for the smaller bacteria, or to form an independent opinion on the more recent results of bacterial research, recourse must be had to the best optical aids, oil-immersion lenses and an Abbe's condenser. As to the magnifying power necessary in examining for tubercle-bacilli I may

say that 500 to 700 diameters is the most useful, and can be best obtained by the use of $\frac{1}{12}$ inch oil-immersion objective and the corresponding eye-pieces.

In the staining process described above, almost all the tissue-elements of the body behave differently from the tubercle-bacilli. While the latter retain, in spite of treatment with nitric acid, alcohol and vesuvin, the dark blue colour they assumed originally, the animal tissues lose, as before mentioned, the blue tint. After the second staining the cell-nuclei and products of cell-disintegration, as well as the granules of the plasma-cells, become brown.

The horny tissues, hair and epidermis, form an exception to this rule, and remain more or less blue; but in these we shall scarcely have to look for tubercle-bacilli. The discovery of these organisms in tissues is therefore very greatly facilitated by their characteristic behaviour with aniline dyes. Even in the thickest masses of nuclei and amongst disintegrated cells which often assume all manner of shapes, from the tiniest points and micrococcus-like forms to long rod-like bodies, thus resembling collections of bacteria, the individual bacilli can be recognized with absolute certainty by their dark blue colour, standing out as nearly black rods on the brown ground. This remarkable difference in staining is obtained, however, only by carrying out exactly the method described above. Quick and thorough hardening of the organs in alcohol is a necessary condition; any other mode of preparation appears to alter the conditions essential to the result. For though the granules of the plasma-cells generally behave like the cell nuclei and assume a different colour from the tubercle-bacilli, I lately saw a preparation made by Dr. Benda, assistant in the Pathological Institution at Göttingen, in which the tubercle-bacilli could not be distinguished, whilst the granules in the plasma-cells, on the contrary, appeared blue. The organ from which the section was taken had probably been placed in chromic acid, or not hardened rapidly enough in alcohol.

Another point of importance for the demonstration of tubercle-bacilli is that all other bacteria that I am so far acquainted with and have examined give the same colour-reaction by Ehrlich's method as the tissue-elements, and form a decided contrast to the tubercle-bacilli, the only exception being the bacilli of

leprosy, which will be mentioned later. In phthisical sputa there are nearly always numbers of bacteria derived from the buccal cavity. I have never found any of these various bacterial forms give the same colour-reaction as the tubercle-bacilli. This observation has been confirmed by various trustworthy workers, and may be accepted as an established fact. The same holds good where tubercle-bacilli are present in the intestinal contents in tubercular ulceration. If the evacuations from such a case are spread out on a cover-glass and stained according to the foregoing directions, they will appear to consist almost wholly of bacteria, such enormous numbers are present. But the latter, especially the smaller sorts, which would be the most likely to be mistaken for them, stain differently from the tubercle-bacilli. One large variety of bacillus which forms rather large oval terminal spores, behaves very curiously, itself becoming brown, while its spores often retain a distinct or even deep blue tint. These spores apparently stain in this way only for a short time after their formation, later they remain colourless. Such spores are represented in fig. 7 on Plate II. at *a*. Those stained dark blue are probably the young spores. Amongst the various spores in the intestinal contents belonging to other forms of bacilli none were found for some time which stained like the tubercle-bacilli. Dr. Gaffky examined at my request the spores of the anthrax bacillus, hay-bacillus and others, with a view to deciding this point, and found that they remained uncoloured. But he discovered that the spores of moulds, on the contrary, became deep blue; and a certain kind of yeast also seems to stain in the same way. But as it is impossible to mistake tubercle-bacilli for the spores and yeasts just mentioned, these will not interfere with the diagnosis of the bacilli, although their colour-reactions are similar.

I have lately applied Ehrlich's method of staining to many substances containing bacteria—as putrid meat infusion, decomposing urine, decomposing blood, milk, putrefying vegetable infusions, mud from marshes, Berlin ditch-water, &c.—but I have never found bacteria which give the same colour-reaction as tubercle-bacilli. Those who maintain that bacteria staining like the tubercle-bacillus can be found in sputum, putrid liquids, the intestinal contents of healthy subjects and slime from marshes, are in my opinion mistaken, and have founded their conclusions on an erroneous employment of the methods of staining. I feel

justified in making this assertion, because I see almost every day what difficulties the carrying out of this somewhat complicated staining process presents to most workers.

As I indicated in my first communication, only one form of bacillus has up till now been discovered which stains like the tubercle-bacillus, namely, the bacillus of leprosy. This fact is all the more worthy of note, because not only are the parasites of tuberculosis and leprosy evidently nearly related in many ways, but the two diseases themselves resemble each other anatomically as well as etiologically. The staining properties of the two kinds of bacilli are not, however, exactly similar. For although the bacillus of leprosy can be stained by the same method as the tubercle-bacillus, the contrary does not hold good. The former, as Neisser has shown, stains by Weigert's plan for colouring nuclei, but not the latter. So that, although the two kinds of bacilli resemble each other in form, size, &c., it is always possible to distinguish them by trying Weigert's colour-reaction.

The instance of the bacillus of leprosy shows that the tubercle-bacillus holds no strictly exceptional position in its behaviour towards colouring materials, and it is, therefore, not improbable that in time other bacteria may be discovered which have the same staining properties as the tubercle-bacillus. Even if this does prove to be the case, it will have no effect on the conception of the etiological significance of the tubercle-bacillus, for the peculiar colour-reaction is not the only specific property of this organism; it has, we shall see, several biological characteristics which furnish much more weighty reasons for regarding it as specifically distinct from other known forms of bacteria.

In considering this question it is useful to compare corresponding points in the history of splenic fever. We shall then see that the splenic fever bacillus has no specific staining properties, and yet it is universally acknowledged to be a distinct kind of bacterium, and the true cause of splenic fever. Just the same might be granted for the tubercle-bacillus, even if it did not chance to be distinguished by its colour-reaction from other bacteria. Not that the evidence thus afforded has not its diagnostic value, but it would be a great error to think that the etiological significance of the tubercle-bacillus stands or falls with its specific colour-reaction.

It seems to me also not unlikely that other ways of staining

the tubercle-bacilli will shortly be discovered. Ehrlich's method has already undergone various modifications, of which the most important, from a theoretical point of view, is that brought forward by Ziehl, viz., the replacement of the aniline by other substances, such as phenol, resorcin, &c. The statements of some authors that tubercle-bacilli can be stained also with pure fuchsin seem, likewise, to point to the existence of other means by which they may be successfully stained. The diagnostic importance of Ehrlich's process is in no way affected by the discovery of other methods not having an exclusive character, should such come to light. For the fact remains firmly established, in spite of everything, that when Ehrlich's method is strictly followed out, tubercle-bacilli behave in a manner peculiar to themselves, and can thereby be distinguished from all hitherto known bacteria. The method is consequently of the same value as a chemical reaction which renders it possible to distinguish between substances recognizable with difficulty, but it is only of value on the condition that certain definite rules are attended to. It would be particularly interesting to be able to stain the bacilli brown or yellow, as useful photographs of the tubercle-bacilli can only thus be obtained. I have recently succeeded in colouring the tubercle-bacilli of a fairly intense brown, by subjecting the preparations to preliminary treatment with very weak liquor potassæ ($\frac{1}{10}$ per 1,000); but these preparations do not yet fulfil the requirements of photography. Let us hope that this difficulty may be overcome; but in the meantime I have been obliged to give up the idea of a photographic representation, although I wished greatly to be able in this way to compare with certainty the tubercle-bacilli with other similar bacilli as to shape and size.

A further deficiency in the present method of staining tubercle-bacilli is the instability of the preparations. In specimens mounted in Canada balsam, the bacilli begin to lose their deep colour after a longer or shorter time, becoming by degrees more indistinct until at last they completely disappear. Preparations stained with methyl violet and gentian violet fade most quickly; in some cases the colour of the bacilli has disappeared in two days. Specimens stained with fuchsin or an alkaline solution of methylene blue last much longer. Why it is that the colour fades so quickly, when in the case of other bacteria staining with the same dyes will last for years, I cannot explain. But from

the circumstance that in a large series of preparations some have been found to retain their colour for nearly a year, I conclude that there are conditions, yet to be discovered, by the fulfilment of which permanent preparations may be procured.

The faded specimens do not quite lose their value, however, because they can be stained again without much trouble. The Canada balsam is liquefied by heat, the section is taken out carefully with a brush and transferred to oil of turpentine. After twenty-four hours it is placed in absolute alcohol, and, after the lapse of a second twenty-four hours, in the staining fluid, to undergo the whole staining process over again. The tubercle-bacilli take on just as intense a blue colour as the first time, but the tissue does not stain so clearly and well.

It seems to me impossible to find a satisfactory explanation of the different way in which the tubercle-bacilli behave with regard to staining reagents as compared with other bacteria until we know something more of the minute structure and chemical constitution of bacteria. Many circumstances render it probable that tubercle-bacilli, as is known to be the case with other bacteria, are surrounded by a membrane which has a different relation to staining reagents than have the contents. For instance, bacilli coloured with methylene blue appear thinner than those stained with methyl violet or fuchsin. In cultivations also in which the bacilli are closely packed those stained with methyl violet seem to be in contact, whilst between those stained with methylene blue, and apparently thinner than the former, are perceptible intervals. Further, the colour of bacilli deeply stained by methyl violet does not fade uniformly; first an outer layer loses its colour, so that the bacillus is still to be seen as a thinner deeply stained thread, of about the same thickness as a bacillus coloured originally with methylene blue. Finally, the close adhesion to each other of bacilli in cultivations points to the existence of an enveloping cementing substance. So that it is conceivable that a substance of peculiar nature surrounds the bacillus and permits the passage of colouring matter in conjunction with alkalies, aniline, and such like, but is impervious to acids. In the present state of our knowledge we cannot go further than conjecture on this point.

I now pass to the description of the tubercle-bacillus itself. Having described the staining fluids by the use of which it

was first rendered visible, it seems natural now to portray the characteristics of the bacillus in the living state before it has been acted on by any reagents. Preparations for this purpose must be made from tubercular material containing considerable numbers of bacilli, because isolated bacilli cannot be distinguished with certainty in masses of detritus without the help of colour-reaction. I generally use recent tubercular nodules from the lungs of guinea-pigs, after convincing myself that plenty of tubercle-bacilli are present by examining stained specimens. The nodule is crushed in a drop of blood-serum free from bacteria, and the material distributed as finely as possible in the liquid; a sufficient quantity for microscopic examination is then spread out evenly on the under surface of a cover-glass, and fastened by means of vaseline over a glass cell in order to prevent disturbing currents in the liquid and too rapid evaporation.

The ordinary microscopic examination, the illumination being suitably regulated by diaphragms, will reveal in a specimen so prepared lighter places amongst the opaque masses of undefined granules where the formed elements are less closely packed, and here numerous colourless, very fine, short rods can be made out. They occur mostly in small groups; in the isolated rods no movement except the ordinary molecular motion is to be seen. The length of the rods is from a quarter to half the diameter of a red blood-corpuscle. No joints are perceptible, and by this method of examination it is impossible to determine their relation to the surrounding cells, so that if the investigation were carried no further they might be looked upon as lifeless particles rather than as bacteria.

If the cover-glass is now lifted off the hollow slide, and the liquid containing the bacilli allowed to dry on it and double-stained after the manner described above, the numerous granules and broken-down cells appear brown, while the rods are an intense blue, and are consequently clearly discernible from all recognized elements of the animal tissues among which they occur. The true number also of the bacilli is now seen; and they are found everywhere amongst close heaps of cells as well as in the thinner parts of the preparation. It is to be noticed that the rods appear thinner after staining, which is due to the fact that the unstained specimen had to be examined by light which had passed through a small opening, when inter-

ference lines at the margin of the object caused the diameter to appear greater than natural, whilst for the examination of the stained bacilli full illumination from all sides is used, and all interference spectra are thus excluded.

We can examine objects of the most different character for tubercle-bacilli in the same way, by spreading a thin layer on a cover-glass and staining it. But we do not learn much more by it than that bacilli are present in a certain tissue or liquid, and to what number. We cannot determine by this means their situation and relation to the surrounding tissues. Although examination of a cover-glass preparation suffices for fluids, it can be regarded only as a first step in studying tissues, and must, in order to obtain accurate information concerning the presence and distribution of the tubercle-bacilli in them, be supplemented by examination of sections of the hardened tubercular organs.

As I wished to discover whether bacilli were uniformly present in cases of tuberculosis, I worked with material of the most varying character possible. I obtained it principally from Dr. Friedländer, who readily placed the rich stores of material from the hospital at Friedrichshain at my disposal, and from Dr. Guttmann, the Director of the hospital at Moabit, who also allowed me to examine a number of cases of tuberculosis. It is my pleasant duty to take this opportunity of expressing my thanks to these two gentlemen for the assistance they so kindly gave me.

In the following account of the results furnished by these observations I shall group the cases according to the usual anatomical classification. But before coming to this I have still a few general remarks to make.

When a tubercular nodule is examined by unstained sections without the diffuse illumination of an Abbe's condenser, it appears to consist of closely packed cell forms, and to be very slightly transparent in consequence. When the nodule is caseating in the centre the cells become transformed into an almost opaque finely granular mass, in which fine details cannot be made out. But a very different picture of tubercle will be presented if the sections are mounted in a highly refracting medium and examined by diffuse illumination after nuclear staining. Here, again, the most recent tubercular nodules are seen to consist of masses of stained nuclei, the nuclei, however,

not being so closely packed but that a section of ordinary thickness is sufficiently transparent to allow even the smallest formed elements in the intervals between the nuclei to be clearly distinguished. But the cheesy centres of the tubercular nodules in the specimens have quite an altered appearance; they are almost colourless and perfectly transparent, owing to the fact that the cells in those parts are dead and no longer stain; but here and there the remains of disintegrating nuclei are seen as groups of coloured granules, which are certainly sometimes closely packed, but even then allow of the recognition of all the formed elements. The conditions are similar in the larger cheesy foci. The cheesy substance itself becomes quite transparent by the process of preparation, and shows merely a light greyish-yellow colour interrupted by isolated brown granules or groups of granules. Each tubercle-bacillus can be easily distinguished in it. The ordinary ideas of the microscopic appearances of tubercle and tuberculous tissue must therefore be modified in accordance with the conditions just mentioned, when specimens prepared by the nuclear method of staining and diffusely illuminated are examined or drawn.

Amongst the characteristics of tubercle-bacilli in general, as seen in stained preparations, must be mentioned the following. They invariably appear in the form of small rods of the length (as before mentioned in the description of unstained specimens) of a quarter to half the diameter of a red blood-corpuscle (about 0,0015—0,0035 mm.). Although their length varies their breadth is pretty constant, provided that the same method of staining is used. They appear thinner by my earlier method of staining with alkaline methylene blue than they do by Ehrlich's method. It is difficult to determine accurately the relative size of such small objects without the help of photography. In looking through a large number of my photographs of bacteria for bacilli which correspond to the tubercle-bacilli most closely as to size, I find in F. Cohn's *Beiträge zur Biologie der Pflanzen*, vol. ii., part 3, amongst the photographs on Plate XV., No. 1, one of club-shaped bacilli with terminal spores, among which are very thin small bacilli which would be of about the same size as the tubercle-bacilli if the photograph were magnified 700 instead of 500 times. Some of these bacilli contain spores, and give a fairly good idea of the appearance of the spore-bearing

tubercle-bacilli which we shall mention later. The bacilli of septicæmia in the mouse, of which photographs are given in Struck's *Mittheilungen*, vol. i., Plate VII., fig. 41, are of about the same thickness as the tubercle-bacilli, but shorter on an average.*

The tubercle-bacilli are not, as a rule, quite straight rods, they generally show slight breaks or bends, and often a gentle curve which may increase in the longest forms to such an extent as to reach the first stage of corkscrew structure. This departure from the straight linear form is a point in which the tubercle-bacilli differ from other bacteria which, like those shown in the photograph, resemble them very closely in size.

The distribution of the bacilli in tubercular organs varies much. Sometimes they are heaped together in thick masses, which can be recognized under a low power as containing bacilli by their deep blue colour. Very often they occur only in small numbers. We are most sure of finding them in places where the tubercular process is just beginning, or where it is spreading rapidly. Here they are seen first in moderate numbers among the nuclei of the accumulated cells, which generally take on an epithelioid character at an early stage. On closer examination it appears that a bacillus lies almost always near a nucleus, and in the interior of the cell to which the nucleus belongs. A cell often contains two or even three bacilli. Where the process is farther advanced the number of the bacilli is usually much increased. They are then frequently grouped in small closely packed heaps in which the bacilli lie parallel to each other, and so near that it is often difficult to make out that each group is formed of separate bacilli. In this arrangement the tubercle-bacilli resemble closely the bacilli of leprosy, which are usually thus grouped. The relation of the tubercle-bacilli to the cells can no longer be made out at this stage, because the cells have already undergone considerable change and are on the point of

* In my first communication on the bacilli of tubercle, I could only say that it seemed improbable that Aufrecht, who had previously reported the discovery of rod-like bodies in tubercle, had seen the real tubercle-bacilli. At the Medical Congress at Wiesbaden I had the opportunity of examining a specimen brought forward by Aufrecht, stained with fuchsin, and exhibiting the forms described by him as tubercle-bacilli. These closely resembled anthrax bacilli in length and thickness. If we compare the above-mentioned photograms with those of anthrax bacilli (*e.g.*, fig. 41, Plate VII. of Struck's *Mittheilungen*, vol. i., with fig. 29, Plate V.) there can be no doubt that Aufrecht did not see the tubercle-bacilli.

death. The nuclei are beginning to disintegrate and be transformed into irregularly-shaped particles of very varying size. These become by degrees less numerous, until at last a homogeneous mass is left which will not stain, and in which all the cells originally present are dead. This mass forms the cheesy centre formerly regarded as the essential part of tubercle in which the infective material resided. But this cheesy substance contains as a rule very few bacilli. Only when the death of the cells and their transformation into the cheesy mass destitute of nuclei take place very quickly, do the bacilli remain visible for a time in groups of any size. They evidently retain the power of fixing colouring matters longer than the cells whose destruction they cause. But soon other changes take place in the bacilli; they also die, or pass on to the spore-forming stage, losing gradually their capability of staining. When this happens nothing is left of them in the cheesy substance but their spores, and as, up to the present, no means has been found of staining the spores of the tubercle-bacillus, their presence is revealed after the disappearance of the bacilli only by the infective properties of the cheesy substance in which they are embedded. Owing to the importance which used to be and quite lately has been erroneously attached to the cheesy products of the tubercular process, it may not be superfluous to again lay some stress upon the fact, that in all tubercular affections the tubercle-bacilli are the primary phenomena and are immediately inclosed by collections of cells, whilst the death of the cells with the resulting cheesy transformation is a secondary process. The opinion still held by many that the relation between the tubercle-bacilli and caseation is the reverse of this, that caseation is the first step of the process and prepares a suitable soil for the tubercle-bacilli, is therefore altogether incorrect. For the anatomical comprehension of the tissue changes occurring as a result of the tuberculosis the process of caseation is of importance, but it has not the slightest etiological significance. The reproach has lately* been cast upon me that in my explanation of the etiology of tuberculosis I attached too little importance to the process of caseation; it is not, however, well founded, and implies a misconception of the position I have taken up, which

* Baumgarten, *Ueber die Wege der tuberkulösen Infection*, *Zeitschr. f. Klin. Med.*, vol. vi., Part I.

is to work out the etiological relations of tuberculosis, leaving the anatomical details to pathological anatomists, particularly when they have so little connection with the etiology of the disease as caseation has.

Of greater importance for the question under discussion are the relations of the tubercle-bacilli to the giant-cells occurring so frequently in tubercular tissues.

These peculiar bodies are so numerous in tubercular tissues, that for a long time it was thought that they might be regarded as characteristic of tubercle. Since the giant-cells occur so constantly in the centre of the tubercular nodule, the theory has often been advanced that the tubercular virus has its seat in their interior, it was even said to have been seen there in the form of very small granules. It has now, certainly, been made clear that giant-cells are present in other pathological processes, and are not a specific product of tuberculosis. Nevertheless, the conviction that the infective material must be contained in the giant-cells has proved well founded. For as soon as giant-cells make their appearance in a tubercle they are seen to contain tubercular bacilli. The relation of the bacilli to the giant-cells is a varying one. In all slowly progressing tubercular processes, *i.e.*, scrofula, fungous arthritis, &c., where the bacilli occur only in small numbers, they are found almost exclusively in the giant-cells, and then only one or two at a time (*cfr.* Plate I., fig. 2). But when, corresponding with the greater or less rapidity of the process, the bacilli appear in greater numbers, the giant-cells present contain more, as many as fifty and upwards being inclosed in one cell (*cfr.* Plate II., fig. 5). A solitary bacillus is sometimes not very easily discovered in the interior of a giant-cell, for it may naturally often happen that the rod does not lie horizontally in the specimen but has an oblique or vertical position, so that it appears under the microscope as a point instead of a blue line. Under these circumstances the rod-like form can be recognized only by the fact that the point can be followed for some distance by focussing. As the interior of the giant-cell is a more or less deep brown, the characteristic blue colour of the rod is exchanged for black, owing to the fact that the aniline brown absorbs the blue part of the spectrum so that a blue object seen on a brown ground must appear black. I may take this opportunity of remarking that bacilli seen against

a brown background, as for example when they lie amongst nuclei stained brown, no longer appear blue, but more or less black.

If the discovery of a single bacillus in a giant-cell presents occasionally some difficulties, bacilli occurring in large numbers in a giant-cell, on the other hand, offer a striking picture which cannot be passed over even under a low power. The giant-cells look in this case like little blue circles, surrounded by a brown wall composed of the nuclei of the cell.

The arrangement of the bacilli within the giant-cells is often characteristic. When the nuclei of the giant-cell form a closed circle, and, for example, only one bacillus is present in the cell, it lies generally almost in the middle or only slightly to one side. But we often find the nuclei of a giant-cell, especially one of an oval or elongated form, collected together at one end, a unipolar arrangement in fact. In this case the bacillus is generally in the part of the cell free from nuclei; often in an exactly opposite position to the nuclei, at the furthest point of the unoccupied pole. The idea involuntarily rises in the mind on seeing such giant-cells that there must be a kind of antagonism between the nuclei of the giant-cell and the parasites it contains, which causes them to remain as far apart as possible. This opposition between nuclei and bacilli is most striking when the nuclei of a giant-cell are grouped equatorially, and a bacillus takes up its position at each unoccupied pole, or where there is a bipolar arrangement of the nuclei and each group appears to hold a bacillus in check.

When the number of the bacilli is increased this antagonistic grouping of the nuclei and bacilli may be apparent still (Plate I., fig. 2), but more often a different arrangement comes in. It seems as if, in proportion to their increase in number, the behaviour of the bacilli towards the nuclei became more actively hostile. They crowd more and more towards the periphery of the cell, push between the nuclei, and finally break through the wall formed by them (fig. 5). A further point to be noticed in their behaviour is that they place themselves at this stage constantly with their long axes at right angles to the surface of the giant-cell, so that when either the upper or under arched surface of the cell is viewed under the microscope the bacilli appear as dots; if the cell is focussed, the bacilli take the form of a

circle of blue rods. A considerable increase like this in the number of the bacilli seems always to be followed by degeneration of the giant-cell; for, often, in the neighbourhood of giant-cells filled with many radially-placed bacilli, and particularly towards the centre of the tubercular focus, groups of similarly arranged bacilli are found, but no longer surrounded by brown-coloured nuclei (*cfr.* Plate II., fig. 6). As, besides these, many transition forms are seen, there is no doubt that these groups of radiating bacilli indicate places where giant-cells existed earlier, the nuclei of which have disappeared, leaving only the bacilli.

Without danger of getting involved in hazardous conjectures, the following conclusions as to the relations existing between the bacilli and cellular elements of tubercle may be drawn from the microscopic appearances just described. The occurrence of one or two bacilli in the interior of cells of an epithelioid character must be regarded as the first stage in the formation of tubercle. How the bacilli come there can scarcely be explained otherwise than by supposing that, having no power of locomotion of their own, they are taken up from pre-existing tubercular foci by wandering cells present in the blood or lymph stream or in the tissue itself, and so carried to other parts. This is the only way to account for the extraordinary fact that, frequently, single bacilli or small groups of bacilli occur at equal and relatively great distances, as, for example, in scrofulous, fungous, and lupoid tissues, and generally in all chronic tubercular affections. For a wandering cell which has taken up a bacillus has no such harmless burden as if it had devoured a particle of vermilion or carbon, or other indifferent material. With such a load it might travel a long distance; but under the deleterious influence of the bacillus, changes take place in the white corpuscle which soon bring it to a standstill. Whether it now disintegrates, leaving the bacilli to be taken up by other cells present on the spot, which then assume an epithelioid form, or whether, as it seems to me more probable, the wandering cell bearing the bacillus is itself transformed into an epithelioid, and later into a giant-cell, must be decided by special observations.

The view that the bacilli are borne off originally by the wandering cells and their distribution in the tissues thus regulated, is supported by the following considerations. First, I would refer to an analogous process in which also rod-shaped bacteria

are taken up by the white blood-corpuscles. This occurs in the septicæmia of the mouse, described by me in my *Untersuchungen über die Aetiologie der Wundinfectionskrankheiten* (*cfr. op. cit.* figs. 2 and 8). In this disease the bacilli of septicæmia, which closely resemble the tubercle-bacilli, appear likewise in the interior of the white blood-corpuscles, and in some few cases close to the nucleus; they then multiply very quickly in the cell, destroy the nucleus and burst the cell, and having become free are soon again taken up by other cells, only to work the same ruin, so that within a short time the majority of the white blood-corpuscles are found occupied by bacilli. As we shall see later, the tubercle-bacilli grow much more slowly than the bacilli of septicæmia, and the cells containing them maintain their vitality much longer; hence the great difference in the further course of the two diseases, although the early stage of the invasion by bacteria in each is so very similar.

Direct observation also seems to show that in the first place the tubercle-bacilli are seized upon and carried away by wandering cells. This can be best seen in cases where large numbers of bacilli are introduced immediately into the circulating blood, *e.g.*, by injection into the auricular vein of a rabbit. If the animal be killed soon after infection, the blood is already found to contain numbers of white blood-corpuscles, inclosing one or more of the tubercle-bacilli, and further, here and there in the tissue of the lungs, liver and spleen, true round cells with single or divided nucleus are seen; they contain tubercle-bacilli, and not having as yet taken on the epithelioid form, they exactly resemble white blood-corpuscles. It is hardly possible to find any other explanation of these cells than that they are wandering cells which have seized upon the bacilli in the circulating blood, and carried them away to the neighbouring tissue. The same phenomena are observed in guinea-pigs dying in the first week after the injection of large numbers of tubercle-bacilli into their peritoneal cavities.

A third justification of the above view seems to me contained in the fact that in perfectly dead tissues, and consequently in places where the influence of the living cells cannot reach the bacilli at all, the latter arrange themselves, when growing rapidly, in quite typical groups of the same peculiar form as that assumed by the colonies of bacteria in pure cultures on blood

serum (*cfr.* Plate III., fig. 10). We must therefore consider that these are the forms adopted by the tubercle-bacilli when they develop undisturbed, and when their mode of grouping is determined simply by the displacement and change of situation resulting from growth. Any other order must be regarded as resulting from some disturbing influence, *i.e.*, currents, or the direct action of tissue elements capable of locomotion. In the same way, the peculiar distribution of the bacilli in the giant-cells—namely, their position opposite the nuclei, and their radiating arrangement—is not due to any power of locomotion possessed by the bacilli, but to currents in the protoplasm of the cell; for after the death of the cell the bacilli do not depart from the radiating form, once they have so arranged themselves. When the wandering cell carrying the bacillus has changed into an epithelioid cell and lost its power of motion, the pathogenic influence of the bacillus appears to be extended to the neighbouring cells within a certain radius, whether they have entered the area as wandering cells or have developed on the spot from fixed tissue elements as a result of the stimulus which the bacillus, by means of its products diffused round about, exercises upon the tissues. All cells within a certain area are similarly transformed into epithelioid cells. But the cell containing the bacillus undergoes further changes. It increases more and more in size by continuous multiplication of its nuclei, and at length attains the form and size of the well-known giant-cell. That the development of giant-cells really takes place in this manner can be seen in proper specimens, which show all the intermediate stages between simple epithelioid cells containing only one bacillus, and fully formed multi-nucleated giant-cells with many bacilli. The development of giant-cells can be best studied in tubercular tissues from the ox or horse, in which they are particularly numerous; in them I have many times seen the above-mentioned transition forms between epithelioid cells and giant-cells. The further fate of the perfectly formed giant-cell differs according as the course of the disease is rapid or slow. In the latter case the number of bacilli inclosed in a giant-cell remains small. There are generally only one or two. It is hardly to be supposed that the bacillus seen in a large giant-cell is the same as that which caused its development. In a giant-cell we not infrequently meet with a bacillus which no longer stains so deeply as the

bacilli in neighbouring giant-cells; I have also noticed cases where the giant-cell contains one deeply stained bacillus, and near it a second very faint one which might be overlooked without close attention; further, I have sometimes met with spore-bearing bacilli in the interior of a giant-cell; from all which I conclude that the giant-cell is a tolerably lasting structure, while the bacilli on the contrary have, as a rule, a short life, and are able to maintain their position for any length of time in the giant-cells only by the appearance of a new generation to fill the places of the dying. Now and then they form spores in the interior of a giant-cell, and thus leave behind them the germs of a future race. But, often enough, the bacterial growth appears to die out of the cell, and the empty giant-cell then remains as a sign of the former presence of the bacilli. If, as is often the case, a considerable number of giant-cells occurs in a tubercular tissue, comparatively few of which contain bacilli, it is reasonable to suppose that many of the apparently empty giant-cells contain spores of the tubercle-bacillus, while others, on the contrary, represent the seat of former bacterial growth. One is tempted to institute a comparison with a volcanic region, in which, along with scattered, still active volcanoes, there is a large number of temporarily quiescent, or permanently extinct craters, the latter of which possess in their characteristic form infallible evidence of their former activity.

We have already spoken of the fate of the giant-cells when the bacilli in them multiply rapidly. Then the result is exactly the opposite of that just described; the giant-cell succumbs, ruptured by the bacilli pushing through the wall of nuclei. Its nuclei fall to pieces, break up into small particles, and the cell is destroyed.

I will not now enter upon the question why it is that at one time the bacilli perish or remain for a long period limited to certain spots, passing away their existence without any extensive growth, while at another they multiply greatly, and all the cell elements in their neighbourhood are rapidly destroyed. Only hypotheses can as yet be brought forward, and I shall return to the subject later.

The further changes which take place in tubercular tissues after the development of epithelioid and giant-cells are all of a retrogressive nature. They mostly belong to the sphere of the coagulation necroses (to use Weigert's term), and lead to the

death of the tubercular tissue and to the production of the so-called "cheesy masses" which so often form the centres of tubercular foci. The tubercle-bacilli generally disappear very rapidly in the cheesy masses, so that they can be found only in the more recent nodules, and are almost always wanting in older ones. In other cases, after the disappearance of the bacterial growth there is simple contraction and conversion of the tuberculous tissue into firm connective tissue.

A very important property of the tubercle-bacillus has yet to be mentioned. I refer to its power of spore-formation. F. Cohn, as is well known, was the first to observe in the so-called hay-bacillus the appearance of small highly refracting bodies, which remained behind after the disappearance of the bacilli, and gave rise later to a new generation, and which were therefore regarded as the embryonic forms of the bacilli and called by Cohn spores. The appearance presented by bacilli during spore-formation when stained with aniline dyes and viewed under the microscope is well shown in Photograph No. 76, Plate XIII., in the first volume of these *Mittheilungen*. The bacilli there consist of short segments, generally two in number. Some of these are uniformly deeply stained and resemble exactly the bacilli without spores in Photograph No. 75. In many of the segments, however, may be noticed the appearance of a small clear spot which gradually becomes larger, whilst the stained contents of the rod withdraw further and further towards the two ends, and the sides come to be bounded only by fine lines indicating the contour of the rod. The clear space within the segment is the spore, which is here distinguishable by its want of colour, rather than by its brilliancy, because the specimen is mounted in a highly refracting substance. With a few exceptions, the spores of bacilli have no affinity for aniline dyes. The divisions between the segments are not always so sharply defined as in the bacilli here represented. With many kinds of bacilli, as, for example, the anthrax bacillus, the segments remain close together and form a connected filament which contains the unstained spores at regular intervals. It is the same with the tubercle-bacillus during the stage of spore-formation. The bacillus remains in one piece, and does not divide up into separate joints, but in each joint a bright body appears, so that, after staining, the bacillus looks like a dark filament interrupted by clear oval

spaces. Under the highest powers it can be made out that the tubercle-bacillus when containing spores presents exactly the same appearance as the anthrax bacillus at the same stage, only on a much smaller scale. The spores are oval, bounded by a fine coloured line, from two to six being usually present in a single bacillus. As each spore occupies a segment, we can judge from the number of spores how many segments, or distinct elements, go to form the bacillus. If a substance containing spore-bearing tubercle-bacilli is examined in an unstained condition in a less highly refracting medium, the bacilli seem to be beset with small highly refracting bodies; these must therefore be true spores and not vacuoles or simple gaps in the protoplasm of the bacillus.

After these remarks on the general features of the tubercle-bacilli, I will turn to the description of their behaviour in the different tuberculous processes.

A.—TUBERCULOSIS IN MAN.

1. *Miliary Tuberculosis.*

Nineteen cases were examined in which tubercle, in the form of miliary or submiliary grey nodules, usually with white or slightly yellow centres, affected several organs—lungs, brain, liver, spleen and kidneys. In no case were bacilli absent from the nodules. The smaller and more recent the granulation, the more abundant were the bacilli, particularly in the centre. As soon as the middle of the nodule refuses to stain, showing caseation to have begun, the number of bacilli decreases. In the larger nodules, where extensive caseation had already taken place in the centre, few bacilli were to be seen, and those only between the nuclei of the epithelioid cells in the periphery of the nodule. Occasionally single bacilli or groups of bacilli could be made out in the giant-cells occurring at the margin of the cheesy focus (*cfr.* Plate I., fig. 2). A remarkable phenomenon noticed frequently in chronic processes in the lungs, is the occurrence in most of the giant-cells of black pigment granules near which bacilli can often still be clearly made out (*cfr.* fig. 2). I have not observed

these pigmented giant-cells in other organs; they seem to be limited to the lungs. From the analogy with other appearances to be referred to later, in the lungs of swine and other animals, I assume that these giant-cells developed originally in the interior of an alveolus, and absorbed the pigment of perishing cells which were present in the alveolus.* As the nodule increased, these cells, which at first developed in the immediately adjacent alveoli, became included within the nodule itself. In many of the older nodules the bacilli seem to have disappeared again completely. But it must be remembered that sections of large tubercles are only fragments; so we cannot conclude that bacilli are absent throughout the nodule because such small portions do not show them. Probably a similar statement to that which was above made with regard to giant-cells, holds good also for tubercles, namely, that near nodules which contain numerous bacilli others occur in which the bacilli have quite disappeared, or are represented only by the spores they have left. If a sufficient number of sections are examined, places rich in bacilli are almost always met with, and it would not be right to form an opinion as to the presence or absence of bacilli in miliary tubercles from the examination of a few preparations.

In the liver and spleen I have found the bacilli almost exclusively in the giant-cells in cases of miliary tuberculosis. In the spleen especially, side by side with fully developed tubercles, giant-cells of considerable size occur almost solitary or surrounded by only a few epithelioid-cells and containing one to three tubercle-bacilli.

The tubercles in the meninges of the brain were almost without exception very rich in tubercle-bacilli. The latter occur frequently in the immediate neighbourhood of small arteries round which lie spindle-shaped masses of epithelioid-cells; between the latter the bacilli are scattered with tolerable uniformity (*cfr.* Plate I., fig. 1). But in many places the bacilli form such dense heaps that their presence can be detected even under a low power by the blue colour of the part. In these cases the bacterial growth takes place chiefly amongst round cells, and therefore in the more recent cell-formations. I have sometimes seen collections of bacilli in the interior of vessels also.

* This view is supported by Watson Cheyne, on the ground of direct observation of giant-cells found in the alveoli of the human lung. *Cfr. Practitioner*, April, 1883

I have to thank Professor Weigert for the only specimen of tuberculosis of the choroid I was able to obtain for examination. Here also were foci without any nuclei, showing complete caseation, surrounded by large giant-cells and many epithelioid cells. Partly within the giant-cells, partly outside them among the epithelioid-cells, were a fair number of tubercle-bacilli.

In every case, with one exception, old caseous masses were found, and chiefly in the lungs and bronchial glands. Where it was possible to carry out an examination, tubercle-bacilli were demonstrated also in these masses, which must be regarded as the starting points of the miliary tuberculosis. Bacilli were often found distributed sparingly in the periphery of the cheesy foci, but occasionally dense masses of them were met with.

It would take too long for me to give a detailed account of all the cases of miliary tuberculosis I examined, I shall therefore select a few that are specially characteristic.

1. Labourer. Age 36. Powerful man, in good health up to fourteen days before admission to hospital. Fell ill with cough, pain in chest and moderate fever. The symptoms observed in hospital were not at all typical, and resembled those of catarrhal pneumonia. Patient sank rapidly with increasing dyspnœa, and died four days after admission. Extract from record of P.M.: Pleura on either side studded with numerous miliary nodules. Both lungs infiltrated, of greyish-red colour, with very many miliary grey nodules, the larger of which show central caseation. Heart—endocardium of conus arteriosus shows several sub-miliary grey nodules; at free margin of mitral orifice eruption of hard miliary tubercles as large as peas. Spleen twice the normal size; in the deep red pulp numerous grey miliary nodules. In the liver only a few nodules. Numerous grey miliary nodules in the medulla and cortex of both kidneys; pelvis of right dilated; shows two tubercular ulcers, 1½ to 2 ctm. in diameter; in one papilla a cheesy deposit of the size of a hazel-nut. Bladder normal. In prostate some cheesy deposits. In urethra numerous miliary nodules. Caseation of the epididymis, with softening in some places. Depressed scars in scrotum. In testicle itself numerous grey granulations. Thoracic duct dilated; cheesy thickening of wall at several places, also a few ulcers.

There was here, evidently, chronic tuberculosis of the genito-urinary organs, followed by tuberculosis of the thoracic duct,

while at a later period still, general miliary tuberculosis set in. This case is shown by its mode of origin to belong to the form of miliary tuberculosis described by Ponfick, and may be taken as a typical example. The microscopic appearances corresponded entirely with the description above given of the behaviour of the bacilli. The tubercles in the lungs were relatively small, and contained bacilli in great numbers. Some were so full of them as to show a blue colour in the middle under low powers.*

In the tubercles from the liver and spleen many giant-cells were found, which mostly contained bacilli. Quantities of bacilli were present in the renal papilli at its margin and in the vicinity of the tubercular mass. At several places in the neighbourhood of this mass the bacilli had formed collections in the uriniferous tubules, and the resulting characteristic grouping of the bacilli reminds one of the forms they assume in cultures carried on in blood-serum, to be mentioned hereafter. It was impossible to say whether, in this case, the bacilli reached the tubules by way of the blood-stream, or by extension from the neighbouring tissue. In another tubercular kidney which I received from Prof. Weigert many of the glomeruli and adjoining convoluted tubules were filled with masses of bacilli, from which it may be concluded that bacilli can pass from the blood into the tubules and thence into the urine.

2. A second case of tuberculosis of the thoracic duct in a man of 48 showed similar conditions. The tubercular process had its origin in cheesy mediastinal glands, and passed thence to the thoracic duct and set up miliary tuberculosis of the lungs, liver, spleen and kidneys. Death occurred later than in the first case; eruption of tubercles less abundant, separate nodules larger, caseation further advanced, and, corresponding to this, bacilli present in relatively smaller numbers.

3. Boy. Age 9. Said to have fallen ill only a few days before. On admission unconscious, very restless and delirious, high fever; after a few days symptoms of bronchitis noticed; death ten days later. P.M. showed cheesy enlargement of bronchial glands; patches of broncho-pneumonia in lower lobes of both lungs, also numerous grey miliary and submiliary tubercles in the lungs, in the enlarged spleen, in the liver and in

* Here follows a reference to drawings which are not reproduced in this volume.—Ed.

the kidneys. Round the vessels at the base of the brain slight opacity and a great number of grey nodules.

In the tubercles in the lungs, liver, spleen and kidneys bacilli were found in varying numbers. They abounded in the tubercles of the pia mater. Fig. 1 represents a specimen from the brain. It shows a part of the coats (*a* intima, *b* media, *c* adventitia) and surroundings of a small artery, the spindle-shaped enlargement of which is due to accumulation of epithelioid-cells. Between the latter, tubercle-bacilli are lying.

Considerable quantities of bacilli were found in the cheesy bronchial glands in this case, and that not only at the margin of the cheesy area but extending also some distance into the interior. Those parts of the gland tissue that had not yet undergone necrosis contained many giant-cells which were remarkable also for the number of radially arranged tubercle-bacilli which they contained. The tubercular process had evidently begun in the bronchial glands only shortly before death and had spread rapidly. The tissue of the glands had soon necrosed and softened under the influence of the numerous tubercle-bacilli. In some part or other a rupture must have taken place into the interior of a blood-vessel, leading to the passage of considerable quantities of bacilli into the blood-stream, and so causing a general eruption of miliary tubercles. The seat of rupture could not, however, be found in this case. The following example will show that its discovery is not always an easy matter.

4. A powerful man, aged 30, died after three weeks' illness with typhoid symptoms. At the P.M. many grey granulations were found in the lungs, liver and kidneys, as well as in the greatly enlarged spleen. The bronchial glands were swollen and of medullary consistence, but not caseous. No old cheesy masses could be discovered in spite of the most careful search, so that one hesitated to make the diagnosis of miliary tuberculosis. There were no changes in the intestine or mesenteric glands.

Microscopic examination gave the following remarkable result. Sections of the bronchial glands showed extensive areas, free from nuclei, but thickly strewn with black pigment-granules and numerous fragments of broken-down nuclei, containing also swarms of tubercle-bacilli. The latter were collected in such

quantity in the immediate neighbourhood of some small arteries that the lumen of the vessel under a low power appeared to be surrounded by a blue areola (fig. 3). Under a higher power this was seen to consist of bacilli. In some places the bacilli had insinuated themselves even into the interior of the vessel, and there could, consequently, be no doubt that they had entered the circulation in this manner, and been carried by it in large numbers to all parts of the body. This discovery pointed to a third way in which general tubercular infection and a resulting miliary tuberculosis could take place; Ponfick having already discovered one channel of infection through the thoracic duct, and Weigert a second, probably by far the commonest, through the veins, into which tubercular masses penetrate.

The miliary tubercles of the spleen and lungs contained a fair number of bacilli, some of them occurring in giant-cells.

But this case was interesting from another point of view also. It appeared, in fact, that many capillaries were blocked for short distances by micrococci. In the double staining process the tubercle-bacilli had taken up the blue colour as usual, while the micrococci had become brown. In many places the blue-stained bacilli and the brown micrococci were to be seen in the same field at a little distance from each other. Capillary emboli formed by micrococci were numerous also in the lungs and particularly in the spleen. They had not, so far, induced any striking changes in the surrounding tissues, such as cell accumulation or necrosis, and had, therefore, probably occurred only a few days before death. The combined invasion by bacilli and micrococci, as we see it here, is due to *mixed infection*, which does not appear to be a very rare thing. A mixed infection of this kind can be artificially produced in animals by inoculating them either at the same time or at intervals with different infective materials; for example, by inoculating a mouse with both the anthrax bacilli and the bacilli of septicæmia. Tuberculosis and splenic fever also can occur together in the same animal. I have inoculated a number of highly tuberculous guinea-pigs with splenic fever bacilli. The animals thereupon developed splenic fever and died. Several of them had large numbers of tubercle-bacilli in the lungs and spleen, and in sections of these organs, when double staining was resorted to, the tubercle-bacilli became blue and the numerous anthrax

bacilli brown. Another example of mixed infection is to be seen in the presence of colonies of micrococci in typhoid fever. Further, Brieger and Ehrlich* drew attention to a combination of typhoid fever and malignant œdema, and they were the first to use the very suitable term "Mischinfektion." Such a mixed infection is evidently present in the case before our notice. The primary infection was shown in tubercular disease of the bronchial glands, which led to general miliary tuberculosis owing to the rapid multiplication of the bacilli and their penetration into the arteries. It was not till this disease had progressed to some extent, so that the strength of the patient was much diminished and a suitable soil probably thus prepared for the invasion of the micrococci, that they made their entrance, as it would seem, by an ulcerating sore on the tongue, and in conjunction with the miliary tuberculosis brought the man's life to a speedy close.

A similar combination of tubercle-bacilli in miliary tubercle of the lungs and micrococi giving rise to emboli in the neighbouring vessels was observed also by Watson Cheyne,† and we are justified in concluding that, if looked out for, this kind of mixed infection will be found tolerably often.

I will now give a short sketch of the other cases of miliary tuberculosis which I examined.

5. Boy. Age 8. Cheesy bronchial glands, numerous miliary tubercles in lungs, spleen, liver and kidneys. The nodules in the lung had all of them large cheesy centres free from nuclei, only in the peripheral parts of which small isolated groups of bacilli were present. Tubercle-bacilli were found also in some giant-cells at the edge of the cheesy area. In the spleen, too, were giant-cells containing bacilli. No bacilli discovered in the nodules of the liver and kidneys. Large numbers in the bronchial glands, arranged in nests.

6. Powerful, well-nourished man. Age 34. Cough for about three weeks before admission. Rather high fever, signs of broncho-pneumonia. Cerebral symptoms soon set in; ophthalmoscope showed tubercle of choroid. Death fourteen days after admission. Confluent cheesy foci in apices of both lungs; fairly large, but not very widely distributed miliary tubercles in lungs, liver and spleen; bronchial glands cheesy. Only a few

* *Berl. Klin. Wochenschrift*, 1882, No. 44.

† *The Practitioner*, vol. xxx., No. IV. (April, 1883), p. 295.

tubercle-bacilli in the periphery of the nodules in the lungs, liver and spleen contained giant-cells, some with bacilli. In the bronchial glands, bacilli detected only in a few places in isolated groups.

7. Baker's apprentice. Age 17. Anæmic, and of feeble build. Cough for half a year. Admitted with right pleuritic effusion. Paracentesis resulted in removal of 500 c.cm. of clear serous fluid. Four weeks later cerebral symptoms set in, and death followed in two weeks from that time. P.M.: tubercular pleurisy, miliary tubercles in lungs, and tubercular meningitis. Tubercle-bacilli in the nodules in lungs and pia mater; very numerous in certain parts.

8. Girl. Age 6. Bronchial glands cheesy and in parts calcified. Isolated lobular masses of red hepatization in lungs, in the interior of which contents of bronchi were purulent. Opaque infiltration of the pia mater at base of brain; numerous miliary and submiliary nodules in the vessels in the fissure of Sylvius. Microscopic examination showed small numbers of tubercle-bacilli in isolated parts of the bronchial glands. In the consolidated portions of lung the alveoli were filled with bacteria of all kinds (*Aspirationspneumonie*). Meningeal tubercles abounded with bacilli, and closely resembled fig. 1.

9. Labourer. Age 34. Heavy drinker. Treated two years previously for caries of the carpal bones, complicated with abscesses on dorsum of left foot and in thigh, resulting from lymphangitis. Death with cerebral symptoms after seven weeks' stay in hospital. P.M.: cheesy infiltration with cavities in both apices. Miliary tubercles in both lungs and at base of brain. Considerable numbers of tubercle-bacilli in tubercles both of lungs and meninges.

10. Boy. Age 5. Extensive caseation of bronchial glands. At left apex a cheesy area, larger than a hazel-nut, with broken-down centre. Moderate number of relatively large miliary tubercles in lungs; a good many grey and yellowish cheesy nodules in liver, spleen and kidneys. Greyish-yellow infiltration of pia mater at base of brain. Numerous bacilli, some within giant-cells, in the bronchial glands; closely packed heaps of bacilli in the meningeal tubercles. Relatively few bacilli in the nodules of the lungs, liver, spleen and kidneys.

11. Very thin child. Age 1. Stated to have had cough

eight days before admission. First examination detected bronchitic symptoms and dyspnœa, which increased in severity. Death two and a half weeks later. Cheesy infiltration of upper lobe of right lung. Caseation of bronchial glands. Numerous miliary tubercles in peritoneum, diaphragm and spleen. Tubercular meningitis. Many tubercle-bacilli present in the meningeal tubercles. Nests of bacilli in the cheesy parts of the lungs and in the bronchial glands. Single bacilli in the tubercles of the peritoneum and diaphragm, occurring exclusively in giant-cells. A fair number of bacilli in the tubercles of the spleen.

2. *Pulmonary Phthisis.*

Twenty-nine cases were examined, and tubercle-bacilli found in all. The number of bacilli was subject indeed to considerable variation, but here, as in miliary tuberculosis, it was possible to recognize some relation between the number of bacilli and the extent of the phthisical process, for the bacilli were most abundant where there was recent caseous infiltration, and in the interior of cavities the walls of which were undergoing rapid softening. Bacilli were met with more sparingly in cavities with firm indurated walls. There were fewest in the cicatricial, contracting and much pigmented lung-tissues. The smaller their number, the more did they seem limited to the interior of giant-cells. It must not, however, be supposed that in each case the distribution of bacilli is uniform, that one phthisical lung shows in all parts large quantities of bacilli, another on the contrary only isolated examples. This is sometimes the case, but more often it is found that in the same lung there are extensive tracts quite free from bacilli, while spots here and there contain closely packed nests of them. For instance, cavities of some extent may appear almost or quite destitute of bacilli until, on examining further, one lights suddenly on one or more nests of tubercle-bacilli situated either in a hidden lateral recess or close to the wall of the cavity, but not yet broken down into it. Here they may be so numerous as to show a dark blue spot even under a low power. Hence it follows that in investigating phthisical lungs it is not enough to examine several sections of any one part, *e.g.*, a piece of the

wall of a cavity, but it is necessary to make preparations of as many different parts as possible, and to examine several specimens of each part. Thus only is it possible to obtain a correct idea of the distribution of the bacilli in the case under consideration.

Figs. 12, 13, 17, 18, 19,* show the distribution of the bacilli when they occur in large numbers in phthisical lungs. Fig. 12 represents a small inclosed cavity of fairly typical character under a power of 100 diameters, which does not permit the recognition of single bacilli, but allows dense masses of them to be seen as blue patches. The contents of the cavity consist of a mixture of cell-detritus and tubercle-bacilli, and are in a softened semi-fluid state, so that the cavity might soon be emptied if any communication with the unobstructed air-passages existed. On the right side the wall of the cavity is of a compact consistence and seems able to withstand the attacks of the bacilli, while on the left the bacilli are growing luxuriantly, adhering to the wall in close masses, penetrating into it and the adjoining lung-tissue, and giving rise to rapid softening. The same conditions are observed in large cavities, naturally on a more extensive scale. Fig. 13 represents a piece out of a large cavity where the alveolar tissue, pervaded by masses of bacilli, has broken down; whilst in fig. 18 [fig. 4] a dense growth of bacilli has attached itself to the wall of the cavity which still in parts offers a resistance to their entrance. Fig. 17 gives some information as to the way in which under some circumstances the bacilli reach the minutest branches of the bronchi and the alveoli. The section in this preparation happens to be taken through the length of several alveoli, only the extreme ends of the alveoli seem filled with collections of bacilli, whilst the remaining parts of the alveoli and the air-passages leading to them contain necrosed masses free from nuclei, thus indicating the direction in which the bacterial growth has travelled.

As the result of my investigations I conceive the relation of the bacilli to the phthisical process to be as follows: in the lung at first only one or two bacilli appear, which, owing to their slow growth, soon get surrounded by a cell-infiltration, and are

* These numbers refer to plates in Dr. Koch's paper which have not been reproduced here with the exception of fig. 18, which will be found on Plate II. as fig. 4. As the remarks are of importance this paragraph has been allowed to stand.—Ed.

so prevented from penetrating more quickly into the tissues adjoining the infected spot. The bacilli meanwhile do not perish in the cell-infiltration, but induce, as in miliary tubercle, necrosis and caseation of the centre of the cell-mass. The beginning of the phthisical process, if it could be examined in this stage, would resemble exactly what is found in a miliary tubercle. The nodule increases by degrees in size, and becomes more and more unlike the miliary tubercle. The analogue of this stage is to be found, however, in those not very rare cases of large solitary tubercles which do not always occur singly, but may also appear in some numbers distributed in different organs. These also I am inclined to regard as developed out of separate miliary tubercles, their number being so small that they do not, as in general miliary tuberculosis, lead to the speedy death of their host; they thus have time for further growth, and may finally grow to cheesy masses of considerable size. Apparently the phthisical process develops in the same manner, starting from a miliary nodule and ending in forming a caseous area which is constantly increasing in extent. The conditions in the lung are, however, of a peculiar nature, because the increasing cheesy area does not remain completely inclosed, but sooner or later makes its way into a bronchus, discharges its contents, and is thus transformed into a cavity. The further extension of the cavity goes on very irregularly according as the growth of the bacilli at various parts ceases altogether for a longer or shorter time or advances, and correspondingly cicatrices or fresh excavations are formed. But on the whole the cavity, however large and irregular it may be, has the essential characteristics of the tubercular caseous focus; necrosed masses in the interior bounded externally by nests of epithelioid cells with interspersed giant-cells containing numerous bacilli. The only difference lies in the fact that, in cavities, tubercle-bacilli are found also within the necrosed mass in relatively large numbers, which is not generally the case with cheesy foci which remain encapsuled. The reason probably is that there is a constant removal of the dead material which has been, so to speak, exhausted by the growth of the tubercle-bacilli, and the secretion from the walls of the cavity provides constantly a fresh nutritive material for them.

The above would be the course taken by the ordinary chronic

form of phthisis where the growth of the bacilli is very slow, their numbers small and limited essentially to the giant-cells in the immediate neighbourhood of the cavity and to the contents of the cavity itself. It is remarkable, also, that even in relatively small tubercular foci the growth and distribution of the bacilli is not uniform, but more or less interrupted. This circumstance, which has been already alluded to, is still more striking in large foci, particularly in the larger cavities. Extensive portions of the cavity may be quite free from bacilli, and occasionally bacilli are to be found only in isolated tracts of very small size; from which we may conclude that the conditions essential to the life of the bacilli are not everywhere present to an equal degree in a tubercular area, and are probably subject to variation from time to time. The bacilli must disappear in parts which do not furnish them with a suitable soil. Under such circumstances it may happen that the disappearance of the parasites is only temporary; for example, if bacilli from neighbouring parts succeed later in gaining an entrance again, or if spores are left, they may develop when the conditions become more favourable. But in another case the disappearance of the bacilli from the diseased part may be permanent, if the conditions mentioned above as necessary for their reappearance do not recur. Contraction, cicatrization and healing will then take place in these parts. But we may conclude that as this is possible to a partial extent in the periphery of the tubercular focus, it may happen throughout the whole extent of the area and lead to complete healing. Analogous conditions are seen in other bacterial diseases, also, which spread centrifugally, but with great irregularity, from the original point of infection, now standing still in one part, now progressing by rapid strides in another, as is the case with erysipelas.

The development of a single tubercular focus following the course of chronic phthisis may be complicated in various ways if the tubercle-bacilli escape in any manner from the original tubercular area into other parts, and cause there the development of secondary foci. This may take place in different ways. The bacilli may reach the interior of the larger blood-vessels and be disseminated by means of the circulation in larger or smaller numbers throughout the body, with miliary tuberculosis as the result. The bacilli spread apparently also by way of the lymphatics,

thus reaching the bronchial glands and setting up secondary tubercular changes in them. But far the most frequent mode in which the bacilli reach and establish themselves in other parts is by passing from cavities into the air-passages. Then they very often take up their abode in other parts of the air-passages, preferably in the larynx. If the sputum is swallowed they may attack the intestinal canal.

But the ordinary course of phthisis is most powerfully influenced when pus from a cavity containing bacilli is about to be expelled through the bronchi, and owing to some unfortunate interruption to the movements of respiration is reinhaled and carried into hitherto healthy parts of the lung. If only a small quantity with few bacilli is inhaled it can lead to the formation of only proportionately few fresh centres of infection. Their situation will correspond with the course of the inhaled material in which the bacilli were contained, and will be either in the immediate neighbourhood of the parent focus or far removed from it, possibly in the hitherto sound lung. They will develop by degrees, like the first tubercular centre, from a very small beginning into cavities. But if, as is not very rare apparently, a larger quantity of the contents of a cavity rich in bacilli is inhaled, and extensive portions of the lung are suddenly saturated to some extent with the infective material, no formation of separate tubercular nodules takes place, but we get tubercular infiltrations, which show immediately by their lobular and even lobar distribution that they started from the bronchi.

The entrance of the tubercle-bacilli in large numbers results, further, in extensive and relatively rapid necrosis of the cell-elements of the affected tissue instead of in the formation of those sequestrated accumulations of cells, with the production of giant-cells, which form around bacilli when they invade the lung-tissue in small numbers. Consequently caseation of large tracts follows, with, in many places, rapid softening of the tissue and development of cavities of a different character from those already described.

Whilst the latter possess hard firm walls in which giant-cells and a few tubercle-bacilli exist, the walls of cavities resulting from the breaking down of extensively caseous lung-tissue are pervaded by a dense growth of bacilli; they do not consist of thickened indurated tissue, which softens but slowly under

the influence of the organisms, but the wall still shows plainly the structure of the alveoli, which are filled with cheesy material rich in bacilli and are on the point of losing their coherence and breaking down. This condition is generally termed caseous pneumonia, acute phthisis, &c.

The highly varied combinations of the two processes just described—that of the tubercular focus arising from a single centre of infection and extending but slowly, and that of caseous infiltration resulting from saturation with infective material—furnish the many-sided picture of tubercular disease of the lungs to which the general name of phthisis has been given.

It must, moreover, be mentioned that the inhaled material which gives rise to caseous infiltration need not necessarily come from a tuberculous mass in the lungs. Certain observations on animals have shown that a caseous ulceration of the tonsils or a tubercular ulcer of the margin of the jaw, developing in a rabbit in consequence of a bite; and in one case, also, a cheesy bronchial gland, communicating with the air-passages, furnished the material containing the bacilli which was inhaled into the lungs. In the human being, too, we must bear in mind that a tubercular process in the larynx, throat or mouth, not to mention cheesy bronchial glands as soon as they begin to discharge their contents into the bronchi, may be the starting-point of caseous infiltration of the lungs.

Particular mention must be made also of the secretion from tubercular lungs, the phthisical sputum. As tubercle-bacilli are not present in any other disease, the demonstration of them is of great diagnostic importance. The first attempts I made with phthisical sputa led me to the conclusion that in about half the cases examined large numbers of bacilli were present in the sputum, in other cases only a few could be seen, and in many they appeared to be entirely wanting. But when I took to using Ehrlich's method of staining and had gained greater experience, in no single case among a not inconsiderable number examined did I fail to find them. It does not follow from this that bacilli may not be found wanting in some few cases after repeated examination of the sputum, but in general it may be considered proved by the results of numerous observations published by other workers, that bacilli are, with few exceptions, present in phthisical sputa, but are absent, on the contrary, in the sputa

connected with other lung diseases, and their demonstration may, consequently, be regarded as an infallible diagnostic sign of tubercular affections of the lungs.

Often the bacilli are present in the sputum in considerable quantity. Apparently these are always cases in which rapid softening of parts of the lung that have undergone caseous infiltration is taking place, and in which the secretion from the walls of cavities, such as is represented in fig. 4, is mingled with the sputum. The familiar cheesy fragments which have been long known as characteristic ingredients of phthisical sputum consist almost entirely of masses of bacilli. We may account for the origin of these cheesy particles by supposing that compact masses of bacilli, such as we sometimes meet with on the inner wall of a cavity (fig. 4), become detached as a whole, and are borne away by the secretion from the cavity. Very often, however, one meets with cases where the sputum is very poor in bacilli, and a series of preparations must be examined, and the examination repeated on several successive days, before any bacilli can be discovered. The observations on sputa carried on for a considerable time by Gaffky* in a number of cases of phthisis give the best idea of the frequency of bacilli in phthisical sputa.

The bacilli in the sputum very often contain spores, and this seems to be the case especially when the bacilli can develop freely and plentifully, as for example in caseous infiltration. This particular circumstance is of the highest importance for the etiology of tuberculosis, and we shall have to return to it later.

As the sputum is always more or less mixed with saliva, it contains, together with tubercle-bacilli, other forms of bacteria in number and variety of forms corresponding to the amount of saliva and buccal mucus present.

If sputum is kept for some time in a vessel the tubercle-bacilli remain unchanged, both as regards number and behaviour with staining reagents. The other bacteria, on the contrary, multiply very rapidly, new forms enter from the air or as chance impurities, and regular putrefaction very soon takes place. Microscopic examination then reveals numberless bacteria of the most different forms, but up to the present I have never found any, either of those from the cavity of the mouth occurring in fresh sputum, or of those present in putrefying sputum, which

* *Mittheilungen aus dem Kaiserlichen Gesundheitsamte*, vol. ii.

give the same colour-reaction as the tubercle-bacilli. The latter, if the staining process is properly carried out, always keep their intense blue colour, while other bacteria appear brown.

It must further be mentioned that bacteria may sometimes penetrate into cavities and multiply in the secretion, so that in such cases other bacteria are seen together with tubercle-bacilli even in the contents of the cavity. In the few cases of this kind to which my observations extended only certain forms were present, so that the conditions differed from those where sputum is exposed to the air and putrefaction results, as it seemed that of the many kinds of bacteria which had chanced to enter the cavity special ones only were able to flourish there. They then either lead a harmless parasitic existence in the contents of the cavity, as, for instance, the bacteria of green pus, which I have repeatedly found in large, old cavities, or they share in the work of destruction initiated by the tubercle-bacilli, as appeared to me to be the case with a particular kind of micrococcus. The latter are distinguished by a peculiar mode of arrangement; they almost always form groups of four individuals, and have, therefore, at first sight some resemblance to sarcinæ, but they are in other ways essentially distinct from them. Gaffky* has traced the characters of these organisms still further, and has found that for many animals they are of a pathogenic nature. In the case in which they were first discovered they seemed to have taken some part in the rapid destruction of the lung-tissue. It is much to be wished that observation should be directed in future to these combinations in phthisis, which would probably lead to the discovery of bacterial forms which ordinarily have no pathogenic action on man, or very little, but which under certain favourable conditions, as, for example, in an ulcerating focus in the lung, thrive and exercise a decided influence on the further course of the process. Of what importance these bacteria which come secondarily into action may be, has been already referred to in speaking of miliary tuberculosis and the mixed form of infection accompanying it in case 4.

In connection with pulmonary phthisis, a few remarks on intestinal phthisis may not be out of place. In addition to parts of the lungs obtained from twenty-nine cases of phthisis which I had the opportunity of examining, I succeeded in eight cases in

* Langenbeck's *Archiv.*, Bd. xxviii. Heft 1.

getting also pieces of small intestine where tubercular ulceration had taken place, and some caseous mesenteric glands. Frequently the ulcers in the small intestine were surrounded by a recent eruption of tubercles following the course of the lymphatics. The tubercles were in some nodules so rich in tubercle-bacilli that blue spots were seen even under a low power. In the remaining cases of intestinal tuberculosis, the bacilli comported themselves in the same way as those of which representations are given. They were present constantly in large numbers, and were found most abundantly in the nodules furthest from the centre, and, therefore, the most recent.

In the mesenteric glands corresponding to these lesions tubercle-bacilli occurred always in dense masses, chiefly at the periphery of the caseous parts.

As far as I have been able to judge from the material at hand for investigation, the bacilli appear to find in the intestine conditions more favourable for their development than are usually present in the lung. Hence it will cause no surprise that tubercle-bacilli are present, and that in relatively large quantities, in the evacuations of phthisical patients with tubercular ulceration of the intestine, as Lichtheim was the first to discover. Among the numerous, principally rod-shaped bacteria of the intestinal contents the microscopical demonstration of tubercle-bacilli would have been almost impossible, had it not been for their characteristic peculiarities of staining, which are more than ever valuable under conditions such as the above. Fig. 7 represents a cover-glass preparation containing tubercle-bacilli taken from the evacuations of a phthisical patient, where tubercular ulceration was demonstrated in the intestine after death some weeks later. As the certainty of proof of the presence of bacilli in the evacuations has been called in question in many quarters, I requested Dr. Gaffky to make a series of observations on this point. The results obtained showed that neither in the evacuations of healthy subjects, nor in those of patients suffering from non-tubercular disease, was any kind of bacterium present which stained like the tubercle-bacillus. The presence of tubercle-bacilli could not be demonstrated in the evacuations of all phthisical patients having bacilli in their sputum, which were examined with special regard to this point; but they were always found in phthisical cases where distinct symptoms of ulceration

of the intestine existed. An observation made by Gaffky in the course of these experiments deserves special notice. He found that in the intestinal contents large spore-bearing bacilli not infrequently occur, the substance of which, like all other bacteria, stains brown, whilst the spores show a more or less deep blue colour. The younger the spores the darker blue do they appear. When the bacillus itself perishes, and the spores only remain, they may easily be mistaken at first sight for micrococci, as they are about the same size as a large micrococcus; for example, when several spores are lying near together they look very like a colony of large micrococci. The forms described by Lichtheim as blue-stained micrococci are, therefore, probably identical with these spores, a small group of which is represented at *a* in fig. 23. There seem to be yet other spores present in the intestinal tract which become blue by Ehrlich's method, for Gaffky found in the evacuations of a tuberculous monkey, along with tubercle-bacilli, other bacilli of still larger dimensions than those just mentioned. Their spores were not oval, but elongated, indeed almost rod-shaped. The spores were terminal, and, in many-jointed bacilli, so arranged that in contiguous segments the ends containing the spores were together, and followed each other in the manner here indicated by dots (spores) and lines (segments of the bacillus) —· ·— —· ·—, forming a peculiar disposition of the spores that I have already remarked upon elsewhere.* The spores of the anthrax, hay and potato bacilli, which have also been tested by Ehrlich's method of staining, did not give the reaction, but it is very probable that spores of other bacilli may do so, and that the aniline reaction will furnish a means of easily distinguishing these forms of bacilli from others. The animal in which the bacilli with rod-shaped spores were found died soon after, and the P.M. revealed, in the small intestine, several tubercular ulcers containing quantities of tubercle-bacilli, besides numerous tubercles in lungs, spleen, &c.

I will mention only a few of the cases of phthisis examined from which material was taken for inoculation and cultivation of the tubercle-bacilli.

1. Woman. Age 30. Mother died also of phthisis; cough and expectoration for six months. Great emaciation, occasionally slight fever. Death three months after admission. P.M.: left

* Cohn's *Beiträge zur Biologie der Pflanzen*, Bd. ii. Heft 3.

lung partially adherent, several communicating cavities in both upper and lower lobes. Right lung also adherent, large ramifying cavity in upper lobe, several smaller ones in middle lobe. No tubercular changes in spleen, liver or kidneys. Microscopic examination showed only a moderate number of bacilli in the contents of the cavities. In the neighbourhood of the firm walls of the cavities giant-cells were grouped round small foci, in which nuclei were absent, and most of them contained tubercle-bacilli.

2. Man. Age 23. Mother apparently suffered from phthisis. In hospital a year ago for pleurisy. Repeated hæmoptysis, also diarrhœa, for several months. On admission, thin, anæmic; dulness and bronchial breathing over right apex; cough with purulent expectoration. Death after four months. In right lung large cavities with walls, indurated in some places, in others caseous. Tubercular ulceration of vocal cords. Commencing amyloid degeneration of spleen. Numerous ulcers in the intestine. Enlargement and caseation of the mesenteric glands. Tubercle-bacilli found in relatively small numbers in contents of cavities and in lung-tissue, in large quantities in floor of intestinal ulcers and in caseous mesenteric glands.

3. Labourer. Age 43. Fairly powerful build. No heredity discovered. Cough, expectoration and increasing weakness for three months. Disease has gained ground latterly; dyspnœa especially increased. Death after twelve days in hospital. P.M.: in both apices cavities of moderate size, extensive caseous infiltration with softening and formation of cavities in places in the middle and lower parts of the lung. Ulceration in larynx. Large numbers of bacilli in the cavities and in the lung-tissue where caseous infiltration had taken place.

4. Man. Age 32. No hereditary taint. Illness said to be of four weeks' duration. On admission anæmic, emaciated. Death after six weeks' stay in hospital. In both lungs numerous cavities of various sizes, round which for some distance was caseous infiltration. A few smaller cavities situated more externally, forming slight projections on the surface; they were used for obtaining pure cultivations. Fig. 4 belongs to this case.

5. Servant girl. Age 19. Mother died of phthisis. Cough for one year. Of feeble build, emaciated, short of breath, and sweats profusely. Death after seven weeks' stay in hospital. In upper

lobe of left lung a fairly large cavity. Remaining part of lobe infiltrated with lobular cheesy foci situated close together, some showing central necrosis. Almost the whole of right lung infiltrated with greyish-yellow caseous masses; softening going on in many parts. In trachea shallow ulceration. In ileum and upper part of colon numerous ulcers with sinuous margins. Mesenteric glands show recent caseous infiltration in places. In the interior of the cavities and in the cheesy infiltrated parts of both lungs extraordinary numbers of bacilli, mostly in nests. Tubercle-bacilli in considerable numbers in intestinal ulcers and mesenteric glands also.

3. *Tuberculosis of Various Organs.*

The cases which I have examined and am about to refer to under this heading were examples of tuberculosis affecting single organs, some of which were removed by operation, whilst others were sent from the post-mortem room without any account of the course of the disease or of the remaining post-mortem appearances. I can, therefore, give only a short summary of them.

Two cases of tubercular ulceration of the tongue. In the floor of the ulcers, and penetrating in places deeply into the substance of the tongue, dense masses of tubercle-bacilli were found.

The tubercle-bacilli were just as numerous in four cases of tuberculosis affecting the pelvis of the kidney, in one case of tuberculosis of the bladder and urethra, another where the suprarenal capsules were attacked, and in one case where the uterus and tubes were the seat of tubercle.

On the other hand, tubercle-bacilli were very sparingly distributed in five tubercular testicles that had been excised. Only isolated individuals could be demonstrated, and these were confined to the numerous giant-cells present.

It was the same in two cases of large solitary tubercular foci from the brain. Here too the presence of the bacilli was limited to the giant-cells.

In the only one of the cases belonging to this division in which no tubercle-bacilli could be demonstrated, it was the pus from a tubercular abscess of the kidney which was examined. Inoculation with this material had given positive results, and therefore infective germs must have been present in it. I shall

have to refer to this case later, and shall then explain why the microscopic examination had a negative issue.

4. *Scrofulous Glands.*

For the scrofulous glands which I examined I have chiefly to thank Herr Geheimrath Bardeleben, who placed them at my disposal as soon as they were extirpated. In all, twenty-one cases were examined in which the glands were evidently tubercular. I understand by this term the presence of epithelioid-cells grouped together in masses and inclosing a greater or less number of giant-cells. With few exceptions, in which necrosis and caseation of the diseased gland-tissue had not yet set in, these nests of epithelioid-cells were in close proximity to the cheesy deposits present, and formed their immediate covering. Tubercle-bacilli could be found only in glands where this tubercular structure was to be seen. In several cases, on the contrary, where the glands were enlarged, softened in places, and contained collections of pus, but no epithelioid or giant-cells and no necrosed tissue, bacilli were altogether wanting.

The tubercular, *i.e.*, scrofulous, glands examined were obtained from twenty-one different patients. Of these, eleven were between the ages of 10 and 20; seven between 20 and 30; and three others aged 37, 39 and 3, respectively. As to situation, fifteen were in the neck and submaxillary region, three in the suboccipital region, two in the axilla, and one in the neighbourhood of the elbow; in the latter case, a boy of 3 years old, there was also caries of the wrist on the same side. In three cases recurrence had taken place after the first operation and necessitated a second removal of glands. In many of these cases a phthisical family history was given.

The relative number of tubercle-bacilli present in the tubercular glands was pretty constant in the interior of the cheesy centres. I found bacilli in only two cases, and then they were very few. Between the epithelioid-cells also bacilli were exceptionally present, and then only very few. Among the giant-cells, on the other hand, some constantly, and a good number occasionally contained one or two tubercle-bacilli. In scrofulous glands I did not succeed in finding the giant-cells with numerous bacilli which are so frequent in bronchial and mesenteric glands.

In the three cases where a second extirpation of glands took place after some time, two of the glands showed the same conditions as at the first examination; the third, which was altogether a remarkable case, presented the following details: man, aged 34, powerful build. A year previously large glandular tumours developed in neck and both axillæ, accompanied by extreme anæmia. No tuberculosis in lungs. Excised tumours were of the size and form of potatoes, of soft, almost pulpy consistence, and showing no caseous change in interior. Microscopic examination revealed numerous small collections of epithelioid-cells imbedded in the substance of tumour and inclosing one or more giant-cells. In many of these giant-cells was one bacillus, or at most two. In a single instance a bacillus was situated in the interior of an epithelioid-cell quite close to the nucleus. Hardly a year after the ablation of these glands, fresh tumours of nearly as large size had developed at the site of operation. These also were extirpated and showed the same microscopic appearances, except that the number of giant-cells containing bacilli had increased considerably.

5. *Tuberculosis of the Joints and Bones.*

I examined thirteen tubercular joints, namely, three hips, five knees, three elbows, one ankle and one finger, also ten cases of tubercular disease of bone, in three of which the carpus was affected, in five the tarsus, and in two the vertebræ (in one of the latter the pus only was examined). I have to thank Herr Geheimrath Bardeleben for most of these also.

The granulation-tissue formed in the neighbourhood of tuberculous bones and joints shows no essential difference in the various cases. The same picture is constantly repeated in the structure of the tissue and the arrangement of the bacilli, and it resembles exactly that presented by scrofulous glands. Here, again, more or less densely packed and often confluent masses of epithelioid-cells inclosing giant-cells are found; and, again, the bacilli are limited almost entirely to the giant-cells. In the caseous parts where no nuclei are left and also in the puriform fluid solitary bacilli were found in only a few cases. In this respect also the conditions in tuberculosis of bones and joints much resemble those present in scrofulous glands.

Bacilli could be demonstrated in all these cases. Only in the pus from the abscess due to vertebral caries tubercle-bacilli could not be found. But inoculation with this pus gave a positive result, like the pus from a tubercular renal abscess mentioned above.

6. *Lupus.*

After the anatomical investigations of Friedländer, and the positive results of inoculation obtained by Hüter and Schüller, it seemed probable that lupus too belonged to the group of true tubercular diseases. I therefore accepted the opportunity offered me by Herr Direktor Hahn, Professor Küster and Professor Lewin soon after my first publication on the etiology of tuberculosis, and examined a number of cases of lupus in order to obtain some certainty on this point.

Seven cases were investigated, all of which had been marked by the most definite symptoms, and had been under observation in hospital for some length of time so that there could be no doubt about the correctness of the diagnosis. From four of these cases I obtained excised pieces of skin, from the remaining three, scrapings of the lupoid growth. The excised portions of skin were alone suitable for direct microscopic examination. Tubercle-bacilli were found in all four cases, though only sparingly distributed in each, and that within the giant-cells. They occur so far apart in lupoid tissue that in two cases they were not found till at one time 27, and another time 43 sections had been looked through. But it happened repeatedly that when not a single bacillus had been observed in a series of sections, I came upon several sections one after another where one to three bacilli were present. In lupus I have never seen more than one bacillus in a giant-cell.

I may remark here that all seven cases furnished material for inoculation of the anterior chamber of the eye in rabbits, resulting, without exception, in tuberculosis of the iris, and, where the animals were allowed to live for some time, in the development of general tuberculosis with numerous tubercle-bacilli in the tubercles. From one case (excision of a portion of the skin of the cheek in a boy of 10 with lupus hypertrophicus) I succeeded

in obtaining a pure culture of the bacillus, which in its turn furnished material for the successful inoculation of animals.

B.—TUBERCULOSIS IN ANIMALS.

In studying the appearances presented by tuberculosis in the different kinds of animals, the remarkable fact comes to light that the disease appears under a different aspect in each species. However extraordinary this fact may appear at first, it accords with what has been observed in other bacterial diseases. Splenic fever, in the same way, manifests itself very differently in different animals. Another example is furnished by septicæmia in the mouse; this is due to the presence of very small bacilli, which, when inoculated, cause the death of mice, but in rabbits produce only an erysipelatous affection limited to the skin.

Up to the present, no warm-blooded animal is known which is quite insusceptible to the tubercular virus, and we should therefore expect that there would be many variations in the anatomical picture of tuberculosis as it is seen in the different kinds of animals.

However different the symptoms of tuberculosis may appear in the separate species, and however little inclined one may be to acknowledge the resemblance between phthisis in man and tuberculosis resulting from inoculation in the guinea-pig, there are between these two extremes, sometimes even in the same species, but more often in other species, transitional forms of tuberculosis which bridge over the gulf. But the perfect identity and unity of the tubercular process in different kinds of animals cannot be doubted, when attention is diverted from the macroscopic condition of tubercular organs and from the secondary changes in them, as caseation and calcification, to the primary structure of tubercle, which, as we have already seen, recurs with typical regularity in all the different processes in man, and such is the case also in the apparently widely different forms of tuberculosis in the various species of animals. The most striking differences in the appearance of tuberculosis in the different species have to do only with those secondary changes which lead in one case to extensive coagulation-necrosis without caseation (liver and spleen of guinea-pigs), in another to rapid

softening and formation of a thin puriform secretion (tubercle in the monkey), in a third to transformation into pappy cheesy material (human tuberculosis), in a fourth to simultaneous calcification and caseation (perlsucht of cattle), in yet another to the formation of tough tumours containing chalky concretions (tuberculosis of fowls), &c. The primary changes in all these cases are precisely similar histologically. Suppuration in different animals presents somewhat parallel varieties. Thus pus due to simple inflammation in a rabbit or fowl has properties quite different from those of pus of Man; yet no one speaks, in this case, of different kinds of suppuration.

For brevity I shall give only a short description of the different forms of tuberculosis of animals.

1. *Bovine Tuberculosis.*

Bovine tuberculosis almost always runs its course with the formation of nodules which do not exactly undergo caseation and break down, but calcify, lying together in such masses that they may at length form large tumours. There may be at the same time extensive firm caseous infiltrations of the lung-tissue, and cavities in the lungs filled with pappy cheesy masses.

Four cases of the kind last mentioned were examined. The cheesy contents were of such consistence that they could be squeezed out of the cavities, when these were cut into, as sausage-shaped masses. The cavities themselves appeared to have had their origin in dilated bronchi; in their walls were a good number of giant-cells, some of which contained one or more tubercle-bacilli. The cheesy mass had infective properties, as the results of inoculation with it proved, but no bacilli could be discovered in it. At those places where the bronchiectasic cavities approached the surface of the lung the ordinary pearl nodules were frequently found on the pleura, showing the close relationship of the pulmonary with the ordinary form of bovine tuberculosis.

Of the common form, eleven cases were examined, where the development of the pearl nodules was not limited to the lungs but extended also to the diaphragm, peritoneum and omentum. In several cases the mesenteric glands showed tubercular changes, and were filled with firm cheesy deposits. Tubercle-

bacilli were present in all cases, though their number varied extremely. In some cases, just as in scrofulous glands and the afore-mentioned cheesy foci in the lungs of cattle, the pearl nodules contained but few bacilli, and these few were in giant-cells. I cannot therefore support the often expressed view that pearl nodules, in contradistinction to tubercles in the human subject, are always very rich in bacilli. Side by side with these cases, which run a slow course and never show more than a few bacilli, are others where the number of bacilli may be permanently or temporarily very considerable.

In the same lung too at one place there may be very few, at another a great many bacilli. The preparations represented in figs. 5 and 6 were taken from a case of this kind. Sections taken from large, hard, and consequently old nodules in this lung contained often only solitary bacilli in the giant-cells. The more recent nodules, on the contrary, appeared extraordinarily rich in them, and showed very clearly the relations between bacilli and giant-cells, to which reference has been already made. The giant-cells were so filled with tubercle-bacilli that they appeared as blue spots and circles. In the neighbourhood, among the small cells, bacilli appeared in such numbers that they gave the preparation a blue colour in places. Fig. 5 shows a single giant-cell, and fig. 6 a collection of bacilli remaining after the disintegration of a giant-cell, such as are found towards the interior of a nodule.

The cheesy mesenteric glands of cattle with *perlsucht* which I obtained for examination were always extremely rich in bacilli.

The bacilli were fewer, on the other hand, in some villous tubercular growths containing many small hard nodules from the pericardium of an ox, and also in some nodules found in the kidney of another animal.

In all, seventeen cases of *perlsucht* were examined, and bacilli were absent in none.

2. *Equine Tuberculosis.*

Four cases were investigated. I did not get all the organs to examine, but it was easy to see that tuberculosis in the horse holds a middle place between bovine and human tuberculosis. The tubercular new formations in the peritoneum and omentum bore the greatest resemblance, in places, to the pearl nodules of cattle, whilst in the same cases the lungs were filled with extremely numerous miliary tubercles which gave to the surfaces of section appearances exactly similar to those of a human lung attacked by miliary tuberculosis. In one case the way in which the tubercular virus had entered the blood-stream and set up miliary tuberculosis could be seen. The retro-peritoneal glands were here transformed into a large tumour, containing firm cheesy collections, which partly surrounded the inferior vena cava, and formed irregular projections into the interior of the vessel. Sections taken through this mass of glands, and particularly through the nodules projecting into the vena cava, contained enormous quantities of tubercle-bacilli, partly free, partly distributed in the numerous giant-cells. Many of the protuberances were softened on the surface, and had evidently thrown a great number of tubercle-bacilli into the blood of the vena cava. Miliary tuberculosis, therefore, arose here in the way pointed out by Weigert as occurring in the human subject.

In the remaining cases of equine tuberculosis tubercle-bacilli were found in the nodules of the peritoneum and omentum, in the enormously enlarged bronchial glands, and in the tubercles of the lungs, liver and spleen; here and there they were present in large numbers.

3. *Tuberculosis of Swine.*

The disease seems relatively very common. Caseous changes in the lymphatic glands of the neck, which are probably always of a tubercular character, occur frequently in swine. In four cases where I examined the glands I found numerous tubercle-bacilli in each, some free, others in giant-cells.

Further, in swine a peculiar form of caseous pneumonia occurs, when lobular greyish-red or greyish-yellow masses form through-

out large tracts of the lungs, which become almost devoid of air. I examined five cases of this kind. The alveoli were filled in places with dense accumulations of tubercle-bacilli. In other parts, the bacilli had already penetrated into the surrounding tissue, and here many giant-cells containing bacilli had developed. In two cases several giant-cells with one or more bacilli were seen free in the alveolar spaces. Evidently in all these cases of caseous pneumonia it was a question of tuberculosis arising from the inhalation of large masses of bacilli. In one case where the infection of the lung was recent it seemed to have had its origin in the tonsils, which were converted into deep ulcers, the floors of which were caseous and contained tubercle-bacilli.

Once I obtained pieces of muscle from a hog, which were filled with numerous small and for the most part calcified nodules. The microscope showed these also to be of a tubercular nature; they contained giant-cells which inclosed tubercle-bacilli.

4. *Tuberculosis of Goats and Sheep.*

I had only once the opportunity of examining a lung with tubercular nodules and the accompanying partially caseous and calcified bronchial glands from a sheep. The pulmonary tubercles contained giant-cells with few tubercle-bacilli. In the bronchial glands, on the contrary, the bacilli were more abundant.

Only one case in the goat came under my notice, which is however of special interest, as in both right and left lungs there was a cavity almost the size of a man's fist, proving that under some circumstances a condition may arise in animals analogous to pulmonary phthisis in the human being. The cavities were partly filled with cheesy pus; their inner wall was irregular, rough and shreddy. In the surrounding tissue were numerous giant-cells with tubercle-bacilli; single bacilli were demonstrated also in the purulent contents of the cavities. Further, the lung-tissue in the neighbourhood of the cavities for a considerable distance contained miliary tubercles in which also were giant-cells containing bacilli. The same conditions were present in some large nodules from the spleen and liver, and in the greatly enlarged and caseous bronchial glands.

5. *Tuberculosis of Fowls.*

This disease usually occurs endemically, and sometimes destroys almost every fowl in a poultry yard. In the intestine and liver of the diseased birds are found a varying number of knobby, or sometimes quite smooth tumours, in size varying from that of a pea up to a walnut. In one of the cases examined there was a mass in the liver almost as large as a small apple. These tumours are of firm consistence, look whitish in section with yellowish spots, and are partially calcified at the yellow parts. In one case tubercular nodules almost as large as a hemp-seed were found in the marrow of the long hollow bones. All these tubercles were extremely rich in tubercle-bacilli, which were collected chiefly in the immediate neighbourhood of the calcified parts. In the intestinal nodules the tubercle-bacilli could be followed as far as the intestinal villi, and it is therefore not improbable that they made their way from the intestine into the more internal organs, particularly as only once were a few small nodules found in the lungs. On the other hand it may be concluded from this discovery that the bacilli, as in intestinal tuberculosis in man, pass out in the excreta and so give rise to further infection.

6. *Tuberculosis of Monkeys.*

Tuberculosis in the monkey presents in many ways a different aspect from the same disease in Man. It does not generally remain confined for long to one organ, but early spreads over the whole body. When this is the case it does not appear under the form of numerous nodules of uniform size, like human miliary tuberculosis, but leads to the formation of a larger or smaller number of tubercular foci of various sizes containing, especially in the liver, spleen and glands, instead of the firm cheesy substance found in tubercular centres in the human subject, rather thin pus, so that they look more like multiple abscesses than tubercles. We see also cases where the typical form of grey tubercle with a yellowish centre occurs in the lungs, pleura and omentum. But here, again, the tubercles are of very varying size and give one the idea that,

in the monkey, the tubercular virus is not distributed once for all, as is the case in human miliary tuberculosis, but continuously and only in small quantities at a time.

I examined eight tubercular monkeys. In all the disease had arisen spontaneously, and the earliest infective focus in each appeared to have been in the lung. Only in one case had the tuberculosis originated in the nasal cavity. An ulcer had formed in the nasal passages, occasioned probably by a scratch of the nostril, and had spread slowly to the septum and turbinated bones. The submaxillary lymphatic glands enlarged and suppurated. The animal, which had been brisk and strong up to that time, then began to suffer from dyspnœa and to lose flesh. The necropsy revealed very numerous tubercles of varying size in the lungs, spleen, liver, and omentum.

In every case tubercle-bacilli were demonstrated in the tubercles of the most different organs. But the bacilli were not very abundant.

7. *Spontaneous Tuberculosis in Guinea-pigs and Rabbits.*

Out of many hundreds of guinea-pigs and rabbits bought for purposes of research upon which autopsies were performed after the desired experiments had been carried out, there was not a single animal which was tubercular. It was not until attempts at infection by means of tubercular substances had been made and a large number of tubercular animals placed in separate cages, but in the same room with other animals used for scientific purposes, that solitary cases of spontaneous tuberculosis arose in the latter. But distinct symptoms of tuberculosis rarely appeared in these animals till they had been associated with tubercular animals for three or four months in the same room. It was very remarkable also that when the number of artificially infected animals decreased, the cases of spontaneous tuberculosis became correspondingly fewer, and *vice versâ*. During a long period, when but few tubercular animals were kept in the chamber for animals experimented on, no case of spontaneous tuberculosis occurred among the many guinea-pigs and rabbits there.

The changes found in animals that died of spontaneous tuberculosis are characteristically different from those where the

disease has been imparted artificially, so that the mode of infection can be recognized with certainty.

For instance, in animals with spontaneous tuberculosis one or a few large tubercular centres, in which caseation was already far advanced, were met with in the lungs, simultaneously with enlarged and cheesy bronchial glands. Sometimes the larger foci in the lungs were wanting, but the bronchial glands were extraordinarily large and filled with cheesy matter. The tubercular changes in the remaining organs, on the contrary, were relatively but little advanced.

The artificially infected animals showed different conditions according as the introduction of fluids containing bacilli was effected by subcutaneous inoculation or by inhalation. Inoculation was generally done on one side of the abdomen, and then the nearest lymphatic glands were always considerably swollen and caseous. The bronchial glands, on the contrary, were nearly always so small that they could hardly be discovered. In these cases the liver and spleen showed the most advanced tubercular changes, whilst the tubercles in the lungs were still relatively small. In those animals infected by inhalation, where large quantities of bacilli had entered the lungs, there was seen neither one nor a few large foci, but a great number of small tubercles in the lungs. If we review the observations made on artificially infected animals we shall find ourselves forced to the conclusion that spontaneous tuberculosis, as it appeared under the above-named conditions in guinea-pigs and rabbits, was due to the inhalation of one or a few infective germs or bacilli.

I examined seventeen guinea-pigs and rabbits with spontaneous tuberculosis, amongst the latter a wild rabbit which was the only one out of ten animals of the same kind that died in a tubercular state after about three months' captivity. They all showed a great many, sometimes an enormous number of bacilli in the neighbourhood of the pulmonary caseous foci. The bacilli were mostly fewer in tubercles of secondary origin.

I deem it worth mentioning that central disintegration had in many cases made great way in the larger cheesy foci in the lungs, and that, consequently, perfect cavities, although of limited extent, had formed. Spontaneous inhalation-tuberculosis in these animals thus leads to conditions resembling to a certain

extent those found in phthisis of Man. But the infection does not remain localized sufficiently long; it passes on to other parts of the body, and leads to the death of the animal before large cavities have had time to form, as they do in the human being.

Tuberculosis of the remaining organs runs a quite peculiar course in rabbits and guinea-pigs, and is not the same in both animals.

In the first instance, the tubercular nodules of the liver and spleen in both animals have the usual characteristic appearance seen in the lung. They are miliary grey transparent nodules with yellowish centres of tolerably firm consistence.

The spleen in the guinea-pig is considerably enlarged and of a dark red colour, from which the grey nodules stand out very clearly. The tubercles soon become confluent and larger whitish-grey islands result. These increase in size, so that the spleen acquires a marbled appearance, the colours being pale greyish red and dark red. Finally, the light parts get the upper hand, and the spleen may then have quite an unusual appearance, which does not recall in the least the tubercular origin of this condition, especially when small ruptures and hæmorrhages occur into the friable splenic tissue, giving to the spleen a still more variegated appearance. In any case it is extraordinary that tuberculosis of the spleen in the guinea-pig should lead to such extensive coagulation-necrosis but never to true caseation, whilst in the lymphatic glands of this animal distinct caseation occurs. Exactly the same thing takes place in the liver of the guinea-pig. First of all grey, disseminated nodules are formed, then lighter patches appear, which increase in size, run together and become rather deep yellow. The liver increases enormously in size, and becomes marbled with large tracts of yellow and brown. In the darker parts of the organ recent grey nodules are generally to be seen.

At the autopsy of a highly tubercular guinea-pig, the lungs pervaded with grey nodules, the extraordinarily enlarged spleen, of a dark red colour mottled with light grey, and the yellow and brown marbled and equally enlarged liver, offer a striking picture which cannot be mistaken for that of any other disease to which these animals are subject.

On microscopic examination of such a liver or spleen, it

becomes evident that in the light-coloured parts there are no stained nuclei, indicating that the cells are dead and that coagulation-necrosis has really taken place. A large part of the organ is dead, but no further destruction occurs; the organ retains its form, and has changed its colour only. In these dead masses, as a rule, only solitary bacilli are found. In a few cases I noticed a peculiar multiplication and disposition of the bacilli in the necrosed liver-tissue, about which I shall speak later. Bacilli are seen in greater or less numbers at the margin of the necrosed parts, and are then frequently contained in giant-cells.

In the kidneys of the guinea-pig I have never observed tubercles visible to the naked eye.

In the rabbit the liver and spleen appear enlarged, but not to anything like the same extent as in the guinea-pig. But the tubercles in these organs always remain small and insignificant, and we never see in the rabbit such changes as were described in the guinea-pig. On the other hand, the kidneys generally show a number of whitish nodules which may grow to the size of a pea. In these nodules tubercle-bacilli in plenty are usually present, and are arranged for the most part in nests. I have sometimes found the uriniferous tubules containing bacilli.

8. *Artificially Induced Tuberculosis of Animals.*

Tuberculosis artificially induced presents on the whole the same aspect as that arising spontaneously. It produces, in the same way, different effects in different kinds of animals; for example, in fowls there is development of hard knotty tumours in the intestine and liver; in rabbits formation of small grey tubercles with a yellowish centre in lungs and spleen, and larger whitish nodules in the kidneys; and in guinea-pigs considerable enlargement of the spleen and liver with the peculiar grey or yellow mottling of these organs.

Naturally, however, the different modes of infection occasion certain differences in the course of the disease and in the pathological changes. It is, for instance, of the greatest importance whether infection is established by means of a very few bacilli or by a great many. The difference thus produced is best studied in the rabbit's eye. If as few bacilli as possible are introduced into the anterior chamber, isolated grey nodules result, but

miliary tubercles, which become yellowish in the centre. Their number gradually increases, they run together, and lead, after the lapse of some time, to general caseation and destruction of the eye, as well as to the development of tubercles in other organs. If, on the contrary, a large number of bacilli are in the first place introduced into the chamber of the eye, no isolated nodules are formed, but that condition arises which was described as diffuse caseous infiltration in the account of pulmonary phthisis due to the aspiration of material rich in bacilli. The eye in this case undergoes diffuse caseous infiltration and is quickly destroyed, while general infection, with the development of many grey nodules in spleen and lungs, takes place early, generally within three weeks.

Almost the same difference in the effect produced by infective material with few or many bacilli is seen when it is introduced into the peritoneal cavity of guinea-pigs. In the one case disseminated tubercular nodules in the peritoneum and omentum with slow progress of the disease; in the other case considerable thickening, puckering and caseation of the omentum, and diffuse infiltration of the peritoneum with numberless minute tubercular granulations.

The conditions are different again when the bacilli are introduced immediately into the blood-stream, when they reach the lungs in large numbers by inhalation or when infection takes place only from a small wound on some part of the surface of the body. In each of these cases the first stage of the change corresponds to the particular mode of infection. But the disease in its further course always presents the same typical picture of tuberculosis. Those secondary tubercular nodules, especially, which develop at some distance from the original point of infection are always of one and the same character. They begin as small grey nodules, consist of accumulations of epithelioid cells, and contain giant-cells and tubercle-bacilli like tubercular nodules of spontaneous origin, from which they in no way differ.

There is no need therefore to describe specially the relations of the tubercle-bacilli in tuberculosis artificially induced, and I will content myself with giving a summary of the cases examined. These were 273 guinea-pigs, 105 rabbits, 3 dogs, 13 cats, 2 marmots, 10 fowls, 12 pigeons, 28 white mice (variety of the house mouse), 44 field mice (*Arvicola arvalis*), and 19 rats.

In all these animals, without exception, tubercle-bacilli were found in the tubercles. Owing to the large number of animals it was not possible, to examine all the tubercular organs in each case, and in most cases I had to limit myself to crushing and spreading some of the tubercular nodules from the lungs or spleen on a cover-glass, and demonstrating the bacilli.

The following is a short summary of the results of the microscopic examination of tubercular objects detailed in previous pages:

In all those morbid processes characterized as true tuberculosis by their course, their peculiar microscopic structure and the infective properties of their products, rod-shaped forms, which can be demonstrated by special methods of staining, occur in the tubercular foci. This holds good for human tuberculosis as well as for the same disease in different kinds of animals.

The number of individual cases which were examined, considered collectively and with special reference to the separate forms of tuberculosis, was large enough to justify the conclusion that we were dealing with a constant, not a chance phenomenon, and that the tubercle-bacillus, therefore, is a typical element in tubercle and its products. The only two cases in which bacilli could not be found were those in which a microscopic examination was made of the pus from a tubercular renal abscess and the pus from an abscess due to spinal caries. But it may not, therefore, be affirmed that bacilli were really absent, because tubercular products were examined in which, as other investigations had proved, the bacilli probably present at an earlier stage had by that time disappeared. There is no doubt that in these cases also they would have been discovered, could the source of the pus have been examined.

Although careful search has often been made for the bacilli characteristic of tubercle in other diseases both of Man and animals, they have never up till now been discovered. Where the contrary has been stated the assertion has been proved to be erroneous owing to an improper use of the methods of research.

A second important point is that the appearance of the tubercle-bacilli betokens the beginning of the tubercular process. They are observed as soon as the changes in the cell elements become visible. It is not until the tubercle-bacilli are present

that the accumulation of epithelioid cells with the formation of giant-cells takes place; later still, as a result of the breaking down of the cell elements, the cheesy products formerly regarded as specially characteristic develop. Further, the presence and number of the tubercle-bacilli are in the closest relation with the march of the tubercular process. For where tuberculosis runs a chronic course there are but few scattered bacilli; where, on the contrary, it makes rapid progress, bacilli are present in great numbers and closely packed together; and where the tubercular process is at a standstill, or has come to an end, there the bacilli disappear.

From these three facts, viz., that tubercle-bacilli are always present in tuberculosis and occur nowhere else, that they precede, both as to place and time, all the peculiar pathological changes, and that their number, appearance and disappearance are in direct relation with the course of the disease—the conclusion may be drawn with great probability that the tubercle-bacillus is not a chance accompaniment of tuberculosis, but stands in the position of the direct cause of the disease.

Such far-reaching consequences depend on this question, however, that it was impossible to be content with a probable solution of it without endeavouring to obtain perfect certainty. Besides, closer investigation of the life-history of the parasite and of the conditions favourable for its development promised further important conclusions as to the etiology of tuberculosis and the prevention of this disease, so deadly to the human race.

The only possible way of attaining this end lay in following the same method adopted in the investigation of other diseases. It was necessary to isolate the tubercle-bacilli from the diseased organs, to cultivate them outside the body and study their behaviour under these conditions; and finally to produce tuberculosis artificially by inoculations with the bacilli thus freed from all admixture with the products of disease.

II.—ISOLATION AND CULTIVATION OF TUBERCLE-BACILLI.

It was to be expected that there would be some difficulty in obtaining pure cultivations of tubercle-bacilli, and therefore from the beginning the method of cultivation upon a solid transparent

soil was employed, because it surpasses all other methods in certainty and ease of manipulation. The principle, peculiarities and advantages of this method are described in the *Mittheilungen a. d. kaiserl. Gesundheitsamte*, vol. i. p. 18.

First of all an endeavour was made to cultivate the bacilli in a crushed tubercle from the lung on nutrient jelly (meat-infusion with peptone, rendered solid by gelatine), but without success. These attempts were made at the ordinary temperature of the room, as the gelatine liquefies at a higher temperature, and thus the advantages of a solid culture material are lost. Since it seemed probable that the experiments failed because a temperature of about 20° C. was not high enough for the growth of the bacilli, it was necessary to prepare another solid and at the same time transparent culture material which should contain all the elements necessary for the nourishment of the bacilli. The desired thing appeared to offer itself in solidified blood-serum. I had found in the course of experiments directed towards the sterilization of blood-serum by the method of repeated heating first recommended by Tyndall for hay-infusion, that when serum is kept for some time at about 65° C., it becomes solid but remains transparent. This culture material can be exposed for a considerable period to a temperature equal to that of the body without undergoing any change. Substances containing bacilli were now spread out on this solidified transparent blood-serum and kept in an incubator at 37° C.; an examination of the preparations under a low power was frequently made, and revealed after a few days the presence of peculiarly formed colonies, which under higher powers and by the employment of staining processes were found to consist entirely of tubercle-bacilli. But before going on to a more detailed description of these cultures of bacilli, I must describe the mode of preparation of solidified blood-serum, which experience has shown to be the most suitable.

Certain precautions must be taken in obtaining the blood. The most convenient vessel for this purpose is a fairly tall cylindrical glass with a glass stopper. It must be washed, rinsed out with a solution of corrosive sublimate (1 to 1,000) to destroy any bacterial germs that may be adhering to it, and again washed in alcohol to get rid of the corrosive sublimate. The blood of the slaughtered animal is then allowed to flow

directly from the wound into the purified vessel, but it is better to allow the blood that first comes and that contains bits of hair and particles of dirt from the skin and hide to escape. The vessel is nearly filled, stoppered and placed at once in a refrigerator. When coagulation has begun the vessel must not be moved again, for fear of preventing the formation of a firm clot and allowing red blood-corpuscles to get mixed with the serum. The vessels containing blood remain twenty-four to thirty hours, or even longer, in the refrigerator, until a good layer of perfectly transparent amber-coloured serum has formed above the clot. If the serum has more or less the colour of blood, it contains too many red blood-corpuscles, and will not remain transparent when heated. The serum is transferred by means of a pipette to test-tubes plugged with cotton wool. The pipette, as well as the test-tubes and wool plugs, have been already purified from bacterial germs by exposure for at least an hour to a heat of 150° to 160° C. in a double-cased sheet-iron hot box. The test-tubes are filled about one-third full with serum, and the cotton wool plugs immediately re-inserted. In spite of all these precautions there are always some bacteria present in the blood-serum which have entered from the air, the hair of the animal, &c., and which would soon occasion putrefaction and decomposition of the serum if not destroyed. Other fluids used for pure cultivations of bacilli can be easily and certainly sterilized, *i.e.*, rendered free from germs, by boiling. This method cannot be applied to blood-serum, however, because it becomes quite opaque at high temperatures. There is nothing left for it then but to employ Tyndall's method of sterilizing hay-infusion, and to raise it repeatedly to a temperature of 55° to 60° C., instead of boiling it once for all. Bacteria, unless they contain spores, are killed pretty quickly in fluids at a temperature of 55° C. The spores, on the contrary, are known to resist a temperature of that degree, and are killed only by boiling heat. A single heating of the fluid kills, therefore, only bacteria containing no spores, and leaves untouched any spores that may be present. In this favourable medium the spores germinate sooner or later, become transformed into bacilli and are no longer able to resist a temperature of 55° C., so that they are killed by the next heating before they have had time to form spores anew. But as the spores come to maturity at different times, and some of them not till several days have

elapsed, it is necessary to repeat the heating. Experience has shown that it is almost always enough to heat the blood-serum for an hour daily to 58° C. for five successive days in order to obtain it free from germs capable of development. The heating can take place in an open water-bath. But it is safer to use a specially designed tin vessel with double walls and roof, the space between the walls being filled with water to insure the equal distribution of heat.

The sterilized blood-serum has next to be rendered solid, and as we wish to obtain as large a surface for inoculation as possible, the tubes must be placed in a very slanting position. For this purpose also a tin box with a double floor and glass cover may be suitably used; it must be placed a little sloping. The water in the floor of the box is heated till a thermometer placed among the test-tubes in the box registers 65° C. At this temperature the serum solidifies in from half an hour to an hour. The serum from different animals is not alike in this respect; the serum of a sheep solidifies the most quickly, that of a calf the most slowly. If the serum is more strongly heated, *i.e.*, to 70° C., the solidification takes place more rapidly, but it is more difficult to preserve the transparency. A well-prepared specimen of blood-serum ought to be almost perfectly clear, transparent and amber-coloured, at most whitish and somewhat opaque at the bottom of the test-tube in the thickest layer. Further, it must not be too soft, but of about the consistence of a hard-boiled egg.

During the heating more or less vapour gets condensed on the upper, cooler part of the surface of the test-tube, forming drops which trickle down when the test-tube is restored to the upright position and collect between the lowest part of the surface of the serum and the glass wall. A small portion of the inoculation surface is covered by the liquid. But this is useful, as soluble substances pass by diffusion out of the solidified serum into the liquid, which is thus transformed into a very good nourishing medium. If the bacteria to be cultivated are spread out on the surface of the solidified serum quite close to the upper edge of the liquid, they develop simultaneously and side by side in the solid culture ground and in the culture fluid, so that their special modes of growth in fluids and on a firm soil can be readily compared.

When the test-tubes with solidified serum are kept for some

time, as the cotton wool plug does not prevent evaporation the serum dries up by degrees, beginning from the top, but this takes place so slowly that for months a sufficiently large surface suitable for culture experiments remains between the upper dried part of the serum and the lower part covered by the fluid.

If the sterilization of the serum has failed, it is discovered a few days after the solidification when the serum is placed in the incubator by way of test. Whitish spots then appear, which may be solitary or in larger numbers, and which soon increase in size. Occasionally the serum liquefies also under the influence of the bacteria, and then becomes turbid and covered with a whitish serum. Microscopic examination shows that in such a case bacilli are constantly present which have evidently developed from spores late in coming to maturity. Obviously, for pure cultures only those tubes of serum can be used which show no trace of such impurity after many days in the incubator, but remain perfectly clear and transparent.

For many purposes, particularly when it is wished to examine pure cultures under the microscope direct with a low power, it is convenient to place the serum in watch-glasses or other suitable glass vessels. These must be provided with a glass cover to protect them from the entry of germs from the air; they are then placed under bell-glasses provided with moist blotting paper, and can be exposed in this way to the incubating temperature. This arrangement, indeed, does not offer such certain protection from the entrance of impurities as test-tubes plugged with cotton wool, and when pure cultivations of tubercle-bacilli are to be carried on through many generations, they can be obtained only by using solidified blood-serum in test-tubes.

Quite as much care as was required to prepare sterilized solid blood-serum is necessary in inoculating the prepared soil, and in preventing the entrance of extraneous germs into the cultures and the contamination of the latter by bacteria and fungi.

As to the material to be used for inoculation, the most suitable, naturally, is one containing many bacilli and of soft consistence, so that the bacilli may be easily spread over a large surface, and quite fresh, *i.e.*, still free from putrefactive bacteria. If the latter are limited to the surface of the organ from which cultivations are to be made, it is still always possible by taking

certain precautions to obtain pure cultures of tubercle-bacilli. But when extraneous bacteria have already penetrated into the deeper layers, all efforts to separate the tubercle-bacilli from them in the cultures are vain, because the putrefactive bacteria grow much more quickly than the tubercle-bacilli, and have spread over the whole surface before the latter produce any visible growth.

There is some difficulty in carrying out the cultivations also when the material whence the seed is obtained contains few bacilli and is of hard consistence. Under these circumstances, the substance containing the bacilli cannot be crushed so that the bacilli may be spread out free on the upper surface of the serum; they remain hidden in the material, develop there, and as the growing colonies are few in number they may easily be overlooked.

Pure cultivations are obtained with the greatest certainty by using as seed a tubercle rich in bacilli or material of the same nature from the interior of lymphatic glands, not far advanced in caseation, taken from a recently killed tubercular guinea-pig. To this end we proceed as follows. A number of knives, scissors and forceps are heated in a flame strongly enough to free them from any adherent bacteria and laid ready in such a manner that no further contamination of the instruments can take place. Meanwhile the animal immediately after being killed is fastened to a dissecting board. In order to avoid the flying off of particles of dirt, hairs, &c., when the skin is severed, the fur of the animal is freely moistened with a 1 to 1,000 solution of corrosive sublimate. With a pair of scissors and forceps, both still hot, the skin is now divided and turned back on each side sufficiently to free the lymphatic glands of the axilla and groin; but the glands, if they are to be used to start cultivations, must not be touched with the instruments employed for cutting through the skin. With another pair of scissors, also heated, a piece 1 to 2 square ctm. is cut out of the side wall of the thorax, and the surface of the lung laid bare. A number of tubercular nodules are thus rendered accessible, and a few are removed as quickly as possible with fresh instruments, which must, however, be cooled for this purpose. In order to set free the bacilli contained in the nodules, the latter are cut in pieces or crushed with the scissors, or,

better still, between two scalpels that have just been heated and allowed to cool. The substance thus subdivided and crushed is removed by means of a platinum wire fused into a glass rod (which, immediately before use, has been heated and allowed to cool), introduced into the test-tube, spread out on the surface and well rubbed about. During this operation the test-tube must be held obliquely or almost horizontally between the thumb and forefinger, and the cotton wool plug held meantime between the other fingers of the same hand in such a way that no contamination of it by other objects can take place. The transference of the substance into the solidified serum, which may for brevity be designated inoculation, must take place as quickly as possible in order that no germs of extraneous organisms from the air may alight on the inoculation material or enter the test-tube. It is desirable also to conduct the experiment in a room where no dust is flying about, and in the same way all unnecessary movements by which dust from the clothing, &c., is mingled with the air are to be avoided, as experience has shown that it is to particles of dust that the germs of micro-organisms suspended in the air adhere.

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

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

When the organs of a recently killed or dead animal could be obtained, and the inoculation with substances containing tubercle-bacilli was done in the way just given, I invariably succeeded in

obtaining pure cultures. The result was uncertain, on the contrary, when material from human corpses or from cattle with *perlsucht* was used, as it was always impure on the surface, and, moreover, was not always quite fresh when it reached me. In these cases I first washed the surface of the object repeatedly with a solution of corrosive sublimate (1 to 1,000) and then cut away the upper parts in layers with red-hot instruments, which were changed repeatedly; finally, I took the material for inoculation from a depth which justified me in concluding that it would be free from the bacteria which had entered the tissue post-mortem. In this way I generally succeeded in obtaining pure cultures even from this kind of material, particularly from small superficial pulmonary cavities, the outer wall of which, after treatment with solution of corrosive sublimate, was removed with hot instruments.

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

For the first few days no alteration is to be observed in the cultures in the incubator. If, however, there is a change, and drops or spots of white or other colour form on the surface of the serum, increase more or less rapidly in size, render the fluid at the bottom of the glass turbid or cause the serum to liquefy, it is a sign that the culture is not pure, and that foreign bacteria have entered and choked the growth of the tubercle-bacilli. If these drops or spots are examined they are found to consist of bacilli or micrococci which, by Ehrlich's method of staining, assume a

different colour from the tubercle-bacilli, and are distinct from them also in size and shape.

In the glasses free from these impurities, the first signs of the growing colonies of tubercle-bacilli are not visible to the naked eye for ten to fifteen days. They then appear as whitish points and small spots lying on the surface of the serum; they have no lustre, and consequently stand out clearly from their moist surroundings. They are best compared to tiny dry scales adhering loosely to the surface of the serum. The number of the scales and the extent of surface covered by them vary with the richness of the infecting material in bacilli, and with the extent of the surface over which it was rubbed or spread out.

The individual scales attain only a limited size, so that if few are present they remain distinct; but when numerous and closely packed, they coalesce finally and form a very thin, greyish-white, lustreless covering on the serum. After a fragment of the tubercular lung of a guinea-pig has been rubbed on serum small whitish colonies of tubercle-bacilli appear, close to the greyish-red bit of lung, and also in its neighbourhood wherever it has been pushed over or pressed on to the surface of the serum by means of the platinum wire used to distribute the bacilli as widely as possible. The colonies in some cases are relatively few in number, because only a few bacilli were present in the pulmonary tubercles, as the examination of sections shows. In other cases the little colonies are much more numerous; in many, as especially after inoculation with the contents of cavities very rich in bacilli, they soon coalesce and form a coherent membranous mass.

An entirely different picture from that just described was presented by cultures started from substances containing only solitary bacilli. As already mentioned, we cannot in these cases set free the bacilli and distribute them on the surface by rubbing and crushing the substance. They remain within the substance, and there form colonies which may grow almost to the size of a poppy-seed. In cases of this kind there can be no doubt that each of these little colonies originated from one bacillus, or at most two, because microscopic examination showed that a giant-cell from the tissue in question never contained more than one or two bacilli. We may go further, and conclude that in the first-described examples also the

separate scales developing on the serum originated in single bacilli.

When a pure cultivation of the tubercle-bacillus has been started in the manner described, the continuation of the process presents no difficulty. For this purpose some of the whitish scales are transferred to a test-tube by means of a platinum wire (which has been first heated and then allowed to cool) and spread out as far as possible on the surface of the serum. In this second inoculation a far larger number of bacilli reach the surface of the serum, and they can be more easily and evenly distributed than was possible with the original inoculation substance; consequently, in this and succeeding cultivations we get no longer separate scales but coherent membraniform colonies. They take, on the whole, the course followed by the platinum wire in the inoculation, and can therefore be grown in streaks having a vertical or horizontal direction, or in any other figure limited to some chosen part of the surface of the serum. Luxuriantly growing cultures, however, overpass more or less the region to which the inoculation was originally limited. But this extension is not the result of any power of locomotion in the bacilli, for this, as I said before, they do not possess; but it is owing to the fact that the increase in the mass of the bacilli as they multiply does not take place in thickness but in superficial extent. The growing bacilli do not get piled up one on the other, but they tend to spread horizontally, and cause extension of the fully formed coherent bacillary membrane over the surface of the serum. This point becomes most striking when the film of bacilli reaches the fluid at the bottom of the test-tube. It does not then penetrate into the fluid, but spreads over the surface in the same way as before, forming a fine, perfectly defined covering on the surface of the fluid. Very often it spreads on to the opposite side of the glass to the height of a few millimeters. Cultures of this nature are represented in fig. 8. It is difficult to depict fairly the contrast presented by the peculiar colour and the lustreless surface of the cultures of bacilli to the shining surface of the serum. The reflection of light by the fluid in the lower part of the test-tube is not well shown either. However, the effects are sufficiently well caught to illustrate what has been said. Fig. 8 represents a test-tube plugged with cotton wool containing solidified blood-serum, where a culture of bacilli,

appearing as a whitish filmy layer, has not yet reached the upper edge of the fluid.

Cultures of these bacilli have other striking peculiarities which serve to distinguish them macroscopically from cultures of other bacteria if observed attentively. First of all, they never cause liquefaction of the serum, while some kinds of bacteria invariably do so. They do not penetrate into the serum, but always remain on the surface, loosely attached to it. Consequently, when the test-tube is inclined and the fluid lying at the bottom flows out over the surface of the serum, the membranous growth of bacilli can be lifted off and washed away. Other bacterial colonies are of a crumbling consistence, and get distributed equally in fluids, which they render turbid. This is not the case with tubercle-bacilli. The thin membranes that they form do not break up completely in the fluid, but owing to their firm consistence they divide into larger or smaller flakes, which are carried away by the fluid, and finally sink to the bottom. The peculiar, stiff and brittle consistence of these colonies is best shown in that part of the culture which covers the fluid in the test-tube. If the fluid is set in movement the film on its surface breaks up into laminæ and flakes, which sink slowly to the bottom. The fluid remains clear when the membrane of bacilli is growing over it, and when masses of bacilli that have been washed off the surface of the serum are floating in it, or when it has been primarily and intentionally inoculated. From this phenomenon also we should conclude what direct observation had already shown—viz., that tubercle-bacilli possess no power of motion, for moving bacteria distribute themselves in all directions throughout the nourishing material, and give it a turbid aspect.

Further, within certain limits the macroscopic conditions presented by cultures of tubercle-bacilli depend upon the consistence of the solidified blood-serum on which they grow. The firmer it is, the more marked are the conditions just described in the colonies of bacilli. But on very soft gelatinous serum, on the contrary, the development varies a little. The distribution of the bacilli at the time of inoculation is not uniform, because the hard and compact masses of bacilli cannot be crushed on the soft serum. Consequently, the inoculation substance remains upon the serum in small isolated fragments. The growth of the colonies does not then spread so evenly over the surface as on

firm serum, but forms thicker and more compact masses, which adhere firmly to the soft serum. Even when the serum is rather less soft, so that the colonies begin to spread out on the surface, the film of bacilli is still attached closely to its surface. Under these circumstances it is impossible to wash the membrane off, or to lift it up with the platinum wire, without removing with it some of the serum.

If the characters of cultures of tubercle-bacilli observable even macroscopically enable us to distinguish them from cultures of other bacteria, and so to judge of their purity, much more is this the case when they are examined microscopically under moderate powers, for instance with Zeiss' AA, ocular 4, and drawn-out tube (about 80 diameters). We then see that the colonies of bacilli assume peculiar forms shown by no other kind of bacteria. Of course the development of the colonies can be seen much earlier with the microscope than with the naked eye. As early as five to six days after the inoculation, when the culture is kept in an incubator, very pretty, peculiar forms appear on the surface of the serum. They assume the aspect of fine strongly curved and crooked lines. The smallest generally have the form of an S. Longer colonies show the most multiform serpentine windings and curves which often remind one of letters intertwined. While these lines end in sharp points, they are swollen in the middle somewhat in the shape of a spindle—the smaller, more recent colonies being extremely thin and delicate, the older ones being thicker and plumper. By degrees, as they widen, their windings coalesce and the colonies become more and more lamelliform, the mode of origin of the membranes being shown by undulatory markings upon their surfaces and by the passage of their edges into the characteristic wavy lines of the separate colonies. Finally, a blending of the lamellæ takes place, leading to the formation of the membrane-like colonies of bacilli before described, whilst the lamellæ, originating as they do from single colonies, are represented by whitish scales barely visible to the naked eye. For examining the colonies under the microscope and watching their development, a square glass box with a glass cover is specially suitable. The solidified serum contained in it becomes covered on its upper surface with colonies of bacilli which develop in the course of fourteen days. In fig. 9, part of the margin of these colonies is represented under Zeiss' AA and ocular 4

It is soon seen that these colonies are formed only of tubercle-bacilli, if they are stained according to Ehrlich's method and examined with a powerful objective. This is best managed by pressing a cover-glass firmly down on the surface of the serum where the colonies occur, and lifting it off again. Numerous colonies then adhere to the cover-glass in their natural arrangement and form, dry on to it, and can now be stained, as was described above for cover-glass preparations.* The bacilli are not scattered irregularly in the colonies, but are arranged with their long axes more or less parallel to the long axis of the colony. It is further remarkable that the bacilli are not in immediate contact, but are separated from each other by a very small space. This circumstance supports the view promulgated above, that the bacilli are surrounded by a cementing substance, and are united together by means of it, as the firm coherence of the colonies shows. In colonies further advanced it is common to find that most or almost all of the bacilli contain spores.

The cultures generally attain their maximum development at the end of four weeks, and remain unchanged after that time. Further cultures are therefore best made at intervals of two to four weeks, but cultures which have been in existence for months are still capable of development, and may be used for obtaining fresh crops of bacilli.

I obtained in the way described in the foregoing pages a number of pure cultures of tubercle-bacilli from all kinds of material, and carried them on for a varying number of generations.

Many culture-experiments, more especially the earliest, were made from guinea-pigs, which had been rendered tubercular by

* Klebs has stated in several recent publications (*Archiv. f. exper. Pathol.*, vol. 17) that he has constantly found along with the tubercle-bacilli a finely granular mass which does not take up the aniline colours, and which he regards as consisting of micrococci. Further, that he has found these micrococci in a pure culture which he obtained from me. The culture which I gave Klebs, at his request, was one made on very soft serum. Under these circumstances, as mentioned in the text, the mass of bacilli cannot be lifted off the surface of the serum without tearing away some of the serum at the same time. Now, solidified serum appears, under the microscope, as a finely granular mass, upon which aniline dyes have little effect. Klebs was evidently looking at some of the serum when he thought he discovered micrococci. In the numerous microscopic examinations I have had to make of my pure cultures of tubercle-bacilli I have never come across any organisms but the true tubercle-bacilli.

inoculation from Man and various animals. Other cultures were obtained direct from the original tubercular material.

The pure cultures obtained indirectly by first inoculating guinea-pigs were from the following cases :—

1. Pulmonary phthisis in man ; carried through 34 cultures in 22 months (almost 2 years, therefore).

2. Pulmonary phthisis in man (cheesy mass from the lungs) ; 5 cultures in 2½ months.

3. Pulmonary phthisis in man (contents of cavity) ; 6 cultures in 3 months.

4. Human miliary tuberculosis (tubercle from the lungs) ; 12 cultures in 7 months.

5. Human miliary tuberculosis (tubercle from the pia mater) ; 5 cultures in 3 months.

6. Human miliary tuberculosis (tubercle from the spleen) ; 4 cultures in 2½ months.

7. Tuberculosis of uterus in human subject ; 6 cultures in 4 months.

8. Intestinal tuberculosis in man (cheesy mesenteric glands) ; 9 cultures in 6 months.

9. Pulmonary phthisis in man (sputum) ; 7 cultures in 4½ months.

10. Scrofula in man (extirpated cervical glands) ; 12 cultures in 7 months.

11. Tuberculosis in monkey (pulmonary tubercle) ; 12 cultures in 6½ months.

12. Tuberculosis in monkey (tubercle from the spleen) ; 13 cultures in 7 months.

13. Tuberculosis in monkey (cheesy bronchial glands) ; 6 cultures in 4 months.

14. Perlsucht in ox (pleural nodule) ; 5 cultures in 3 months.

15. Perlsucht in ox (pleural nodule) ; 5 cultures in 3½ months.

16. Perlsucht in ox (peritoneal nodule) ; 29 cultures in 21 months.

17. Perlsucht in ox (peritoneal nodule) ; 5 cultures in 3 months.

18. Perlsucht in ox (nodule from the diaphragm) ; 6 cultures in 4 months.

19. Perlsucht in ox (crumbling cheesy mass from lung, first case) ; 13 cultures in 8 months.

20. Perlsucht in ox (crumbling cheesy mass from lung, second case); 5 cultures in 3 months.

21. Culture of tubercle-bacilli (No. 1, 5th generation); 7 cultures in 4 months.

The following cultures were obtained direct from tubercular material:—

22. Human miliary tuberculosis (tubercle from the lung); 24 cultures in 19 months.

23. Human miliary tuberculosis (tubercle from the lung); 10 cultures in 6 months.

24. Pulmonary phthisis in man (contents of a cavity); 11 cultures in 7 months.

25. Pulmonary phthisis in man (contents of a small cavity at the apex); 10 cultures in 7 months.

26. Pulmonary phthisis in man (contents of a closed cavity); 24 cultures in 18 months.

27. Caseous pneumonia in man (lung-tissue); 7 cultures in 5 months.

28. Caseous pneumonia in man (lung-tissue) 9 cultures in 7 months.

29. Scrofulous glands; 8 cultures in 6 months.

30. Scrofulous glands; 7 cultures in 5 months.

31. Scrofulous glands; 3 cultures in 3 months.

32. Scrofulous glands; 4 cultures in 3 months.

33. Tubercular testicle; 6 cultures in 4 months.

34. Fungous arthritis; 19 cultures in 15 months.

35. Lupus; 21 cultures in 16 months.

36. Lung from a case of perlsucht (cheesy mass); 8 cultures in 6 months.

37. Lung from a case of perlsucht (calcified nodule); 7 cultures in 5 months.

38. Pearl-nodule from diaphragm; 15 cultures in 9 months.

39. Pearl-nodule from pericardium; 23 cultures in 18 months.

40. Caseous pneumonia in hog; 8 cultures in 5 months.

41. Spontaneous tuberculosis in guinea-pig (pulmonary nodule); 9 cultures in 6 months.

42. Spontaneous tuberculosis in guinea-pig (spleen); 5 cultures in 3 months.

43. Spontaneous tuberculosis in guinea-pig (pulmonary nodule); 7 cultures in 4 months.

Such an expenditure of time and labour is necessary to obtain the cultures, that more than a certain number could never be undertaken at the same time. I allowed most of them to lapse when, after carrying them on for several months, and using them for inoculation experiments, I had conclusively proved that they were possessed of vegetative and pathogenic properties. Only the cultures Nos. **1**, 16, 22, 26, 34, 35 and 39 are being carried on at the present time, and will still be kept up, in order to learn whether any change takes place in the properties of tubercle-bacilli cultivated thus for a long period outside the animal body.

It may cause some surprise that so relatively large a number of cultures was set on foot when a few would have sufficed for observing the behaviour of the bacilli in cultures. It seemed to me, however, not improbable that though bacilli from varying forms of tuberculosis, perlsucht, lupus, phthisis, &c., presented no difference microscopically, yet that in cultures differences might become apparent between bacilli from different sources. But although I devoted the greatest attention to this point, I could find nothing of the kind. In all the cultures, whether taken from miliary tubercles, the contents of a cavity, lupus or perlsucht, the tubercle-bacilli behaved exactly the same. Nor was there any change visible in the cultures carried on for a longer time, say 16 to 18 months.

In support of the assertion which I made above, that cultures of tubercle-bacilli possess characteristic properties by means of which the tubercle-bacilli may be distinguished from other bacteria with almost greater certainty, and in any case on more important grounds than by their staining peculiarities, I can appeal to a very extensive series of observations. Once the favourable qualities of solidified blood-serum were known, countless experiments were made by inoculating blood-serum, either from pure cultures of all kinds of bacteria, or with the most diverse animal substances, but in no case with the production of vegetations resembling the cultures of tubercle-bacilli. These attempts, although belonging properly to other series of investigations, form control-experiments which prove that it is only from substances containing tubercle-bacilli that the above-mentioned characteristic cultures can be obtained.

Another point of great etiological importance had to be now

decided, viz., whether tubercle-bacilli could grow and multiply under conditions which ensured for them an independent existence apart from the body of man or animals.

In order to settle this question it was necessary first to ascertain whether the bacilli grow only upon the solidified serum, or whether they flourish also on other soils.

Experiments with fluid sterilized serum showed that particles from cultures of bacilli, placed upon its surface, developed there in the way described on the surface of the fluid lying near the solidified blood-serum, and formed over it a thin whitish film of hard brittle consistence, which broke up and sank to the bottom when the serum was shaken. The serum remained clear in every case. If the seed was not kept floating on the surface of the serum, but sank into the fluid, there was no visible increase in the fragments.

The blood-serum of different animals in a fluid, as in a solid state showed no essential difference as regards its suitability to serve as nourishing soil for tubercle-bacilli. They seemed, indeed, to flourish best in the serum of sheep, oxen, and calves, but the serum of horses and swine gave luxuriant cultures. Even in the serum of dog's blood there was no marked falling off in the growth, although these animals are tolerably insusceptible to tuberculosis. But on coagulated egg-albumen, on the contrary, tubercle-bacilli would not grow.

In fluids other than blood-serum I could not at first succeed in obtaining a growth of the tubercle-bacilli. When one or more fragments of a culture were placed in a glass with neutralized meat-broth, the fragments seemed certainly to have increased somewhat in the course of 4 to 5 weeks, but it was difficult to decide whether true growth had taken place. It was not until I had broken up bits from a culture of bacilli into fine fragments by trituration, added them to meat-infusion, and distributed them thoroughly by shaking, that an unmistakable development took place. For the success of this experiment it appears to be of some importance to put the cultures into glass flasks with a flat wide bottom, the so-called Erlenmeyer's flasks, and to pour into the flask only enough fluid to cover the bottom to the depth of $\frac{1}{2}$, or at most 1 centimetre. The meat-infusion always remains clear, but in the course of 4 to 5 weeks a finely granular, sandy, whitish-looking deposit forms at the bottom of the vessel. The

separate grains, which have probably developed from the almost invisible particles of the material introduced, consist exclusively of tubercle-bacilli.

If the conditions presented by these cultures in fluid nourishing materials, namely, their slow growth and the continued transparency of the fluid, are compared with the accounts given of former culture experiments by Klebs, Schüller, and Toussaint, who observed a turbidity of the culture-fluid after one to three days, we are forced to the conclusion that these authors did not succeed in obtaining pure cultures at all.

With regard to meat-infusion, also, we find that the flesh of different animals, even those that are only slightly susceptible to tuberculosis, as dogs, rats, and house-mice, favour the development of the cultures in an almost equal degree.

Another suitable solid culture-ground for tubercle-bacilli is furnished by neutralized meat-infusion, solidified by the addition of Agar-Agar; this material can be kept at the body temperature without liquefying. This is not nearly so good a material as solidified blood-serum, because the bacilli cannot be easily spread out on the slippery surface, and the cultures do not therefore present the characteristic membraniform appearance, but are grouped in more compact irregular masses.

As some pathogenic bacteria, *e.g.*, anthrax-bacilli, typhoid-bacilli, the bacilli of glanders and the micrococci of erysipelas, grow very freely on vegetable substances, such as boiled potato, experiments were made in this direction with tubercle-bacilli, but they have not so far led to any positive results.

On the whole, then, with regard to soils, tubercle-bacilli have no very wide scope.

It is the same with another condition essential to the existence of bacteria, the limits of temperature within which growth can take place.

Often repeated experiments proved that at a temperature of 42° C. no growth occurs in the course of three weeks. Further, at 30° C. there is very little development, and between 28° and 29° C. it ceases completely. The cultures succeed best at a temperature of 37° to 38° C.

Other pathogenic bacteria have a much wider range of temperature, within the limits of which they can grow. For example, anthrax-bacilli grow luxuriantly between 20° to 24° C., and

form spores in a short time; but they develop even at 43° C. If one now reflects that the anthrax-bacilli can pass through their whole cycle of development up to the formation of spores in twenty-four to forty-eight hours at a temperature often reached by the surface of the earth in summer, and that they can do this on dead vegetable substrata, there is ground for the conjecture that they really do, in suitable places, complete their cycle of development external to the bodies of animals. Hence the etiology of splenic fever takes a very different position from that which it would occupy if the bacillus anthracis could find a suitable habitat only in the animal body.

The same would hold good for the tubercle-bacillus if it could grow on soils such as are found in Nature, and if it could develop in a relatively short time, and form spores at a temperature corresponding to summer heat. But this is not the case. The lower limits of temperature at which the tubercle-bacilli are just able to grow are not reached by summer heat, and besides, the growth of these bacteria goes on so slowly that, before their life-cycle was completed, they would be choked by other kinds of bacteria, which are present everywhere and develop much more rapidly. So that if other soils more easily reached than that afforded by the animal body were to be found upon which the tubercle-bacilli would flourish, the last-mentioned reasons would be quite sufficient to decide the impossibility of an existence for the tubercle-bacilli outside the animal body. We are therefore obliged, so far as our investigations have yet reached, to regard the tubercle-bacilli not as occasional, but as true parasites, *i.e.*, as finding the conditions necessary to their existence only in the animal or human organism.

III.—INFECTION-EXPERIMENTS.

Infection-experiments have formed until now the most important part of the experimental investigation of tuberculosis. But although an extraordinarily large number have been made, all but a few series are deficient in those precautionary measures necessary to make them unimpeachable.

There are three kinds of error which may invalidate an infection-experiment: first, the confusion of spontaneous tuberculosis with the artificially induced form; secondly, the confusion of the

products of true tubercular disease with materials which more or less resemble them macroscopically, or even microscopically, but which result from other morbid processes; thirdly, unintentional infection with tubercular virus by means of infected instruments, impure inoculation material, &c., in short, by neglect of antiseptic precautions.

How are these sources of error to be avoided?

To escape errors arising from spontaneous tuberculosis it has been proposed to experiment only on animals in which tuberculosis rarely or never occurs. But animals not subject to spontaneous tuberculosis are distinguished by their greater or less insusceptibility to the disease; they are not at all with certainty affected by the tubercular virus, so this suggestion is not practicable. In making experiments upon inoculability of splenic fever, no one would choose dogs, which are known to enjoy almost entire immunity from the disease, as the only animals to experiment upon, but, on the contrary, animals particularly susceptible to the contagium of splenic fever would be selected. The same thing holds good for experiments on tubercular infection.

The more sensitive an animal is to infection by the tubercular virus, the better suited it is for the infection-experiments under consideration. But only with the proviso that it is possible to distinguish in the animal experimented upon between the artificial and spontaneous modes of infection. With a little attention, however, this is not very difficult. The diagnostic signs by which they are recognized were given above in detail. But although by help of these diagnostic signs spontaneous tuberculosis can be excluded from the list of experimental errors, all precautions must, nevertheless, be taken to limit, as much as possible, the occurrence of the spontaneous disease. This may be done by isolating tubercular animals in special cages, by abundant ventilation, and frequent cleansing and disinfection of their stalls. It is never advisable to keep rabbits and guinea-pigs for a long time in the same place with tubercular animals; they are hardly likely to remain free from tuberculosis longer than eight to ten months in infected inclosures. In one case a number of animals was kept as long as possible in order to obtain information as to their immunity; but, in spite of the greatest care, only a few escaped tuberculosis for more than a

year, and these became tubercular some months later. According to this evidence it would seem that all the numerous experiments, in which tuberculosis is said to have appeared after a lapse of three months or more, are of little or no weight, if it were not possible from the appearances found to affirm positively either that spontaneous tuberculosis was present or that it was absent.

As to the second danger, that of mistaking non-tubercular nodules for those of a true tubercular nature, nothing is simpler than to exclude this source of fallacy. The true tubercles are infective, and contain tubercle-bacilli; with non-tubercular nodules this is not the case. Even though some refuse to recognize the diagnostic importance of the tubercle-bacillus, all must distinguish between infective and non-infective nodules. Supposing, therefore, that we succeed in an infection-experiment, *e.g.*, in producing some grey nodules in the lung of a dog as an effect of the inhalation of some substance or other, we must not rest content with this simple result, and maintain on the strength of it that they are true tubercles. In every case the infective nature of such nodules must be demonstrated. In most cases of true tuberculosis we are spared the trouble of giving special proof of its infective character by further inoculations with the nodule, for under these circumstances the pathological process is rarely limited to the seat of infection; it has almost always extended to other organs of the body, and thus proves its infective nature by its power of spreading. Hence when we find the formation of tubercles spreading beyond the original seat of infection to lymphatic glands, lungs, liver and spleen, we may conclude from that fact that it is infective; but if, as happens in the case of the lungs when solid particles containing no virus are inhaled, and in the case of the peritoneum when non-infective granular masses are injected in the abdominal cavity, the nodules produced are limited to the seat of infection, to the lung or peritoneum as the case may be, and show no tendency to more extensive infection of the body, there is strong *a priori* reason to believe that we are not dealing with true tuberculosis, and special proof of the infective character of the process must be given. If this is not done, and, strange to say, it was omitted in many recent investigations directed against the infective character of tuberculosis, the experiment furnishes no conclusive proof.

The third error mentioned, viz., unintentional infection by instruments, &c., appears to have attended almost all the earlier experiments on tuberculosis in a greater or less degree, both those made to prove and those directed against the infective nature of tuberculosis; and yet this error can be avoided without much difficulty if the rules laid down for the performance of antiseptic operations are attended to, above all taking care to thoroughly disinfect the instruments for each separate experiment. All metal instruments, as scissors, forceps, knives, inoculation-lancets, must be heated. Special care must be employed with regard to the syringes used for injection. Syringes of ordinary construction cannot be disinfected with sufficient certainty because they cannot be strongly heated without injury, and experience has shown that disinfecting fluids do not infallibly destroy infective material in the interior of the syringe, particularly any that may be adhering to the piston. Hence a syringe of peculiar construction is required to render disinfection by heat possible. It must be made of glass and metal. The lower end is united to the setting for the needle by means of an air-tight interposed perforated cork plate, and the piston made to fit closely by winding a soft cotton thread round it. A syringe so constructed can be rendered free from infective germs before each experiment by heating it for an hour to 150° to 160° C. The piston is then moistened by sucking up boiled distilled water, and if the cotton has been carefully wound round it, it fits just as accurately as the ordinary leather or caoutchouc piston.

The hands of the operator and the part to be operated on are to be disinfected with a solution of corrosive sublimate (1 to 1,000), and everything must be avoided which might cause an unintentional infection of the animal either during or after the operation.

In all the following infection-experiments the precautions mentioned were stringently observed. Accordingly, to recapitulate briefly, for each experiment several newly bought animals were used and kept in separate cages, while the effect of the infection was ascertained at so early a period that there could be no danger of confusing artificial with the much later developing spontaneous tuberculosis; further, in the tubercular changes occurring after infection the presence of tubercle-bacilli was always sought for, and where necessary, a special test of

their infective properties made; the experiment itself was conducted under antiseptic precautions, and particularly with instruments disinfected in a reliable manner.

The infection-experiments made in the course of my researches fall into two groups—in one tissues containing tubercle-bacilli, in the other pure cultures of the organisms were used as infection-material.

1. *Infection-experiments with Tissues containing Tubercle-bacilli.*

These served partly for studying the effect of the products of different forms of the tubercular process, and partly to obtain suitable material with which to start pure cultures. As inoculation-material were used fragments of tissue from various organs in cases of human miliary tuberculosis, from phthisical lungs and different forms of localized tuberculosis—strumous joints, scrofulous glands, lupus—and from tuberculosis in various animals. The inoculation-substance was always examined for tubercle-bacilli.

The inoculation was effected by making a small incision in the abdominal wall of a guinea-pig with the scissors, inserting the point of the scissors to form a pocket-like subcutaneous wound about a half ctm. deep. Into this little pocket a fragment of the inoculation substance about the size of a millet or mustard-seed was pushed as deeply as possible. On the following day the inoculation wound was always united, glued together, and showed no reaction. Generally it was not till after a couple of weeks that a visible swelling of the lymphatic glands next the seat of inoculation occurred, usually the inguinal glands on one side, and at the same time induration and the development of a nodule took place in the inoculation wound, which up till then had remained perfectly healed. After this the lymphatic glands enlarged rapidly, frequently to the size of a hazel-nut. The nodule at the seat of inoculation then generally broke and became covered with a dry crust, beneath which was a flat ulcer with a cheesy floor, discharging very slightly. The animals began to lose flesh about this time, their coat became bristly, dyspnœa set in, and they died generally between the fourth and eighth weeks, or they were killed within the same space of time.

In some instances the inoculation substance was inserted into a pocket-like wound in the skin of rabbits also. But as the course of the disease was not so constant and rapid as in guinea-pigs after subcutaneous inoculation, I inoculated rabbits afterwards only in the anterior chamber of the eye. Tuberculosis of the iris resulting from this inoculation has been often described, therefore I need not give a special account of it.

The following inoculations were carried out in the way above described:—

1. Miliary tuberculosis. Tubercles of the pia mater, very rich in tubercle-bacilli: 6 guinea-pigs. Of these one died 5, two 6, and two 7 weeks after inoculation. The sixth was killed in the 8th week. In all the animals the lungs, liver and spleen were highly tubercular, and the inguinal glands had undergone caseation.

2. Miliary tuberculosis. Grey nodules in the lungs, with fairly numerous tubercle-bacilli: 6 guinea-pigs. Three died in the 6th week; the rest were killed some days later. All tubercular, as in No. 1.

3. Miliary tuberculosis. Greyish yellow nodules from the spleen and kidneys, with not many tubercle-bacilli: 6 guinea-pigs. Died in the 6th and 7th weeks. All tubercular, as in No. 1.

4. Miliary tuberculosis. Grey nodules from the lung, fairly rich in bacilli: 3 guinea-pigs. Two died in the 6th, one in the 7th week. All tubercular, as in No. 1.

5. Miliary tuberculosis. Grey nodules from the lung containing few bacilli: 5 guinea-pigs, 2 rabbits at the root of the ear. One guinea-pig died after 8 weeks, the remainder were killed some days later. All tubercular. The rabbits killed after 10 weeks had caseous lymphatic glands at the root of the ear and in the neck, tolerably numerous grey nodules in the lungs, a few in the kidneys and spleen. 5 more guinea-pigs were inoculated with tubercles from the spleen of one of the guinea-pigs. 3 of them died in the 8th week, the 2 remaining were killed the same week, and all found tubercular. Some of the cheesy glandular substance from a rabbit was rubbed up with water and injected into the peritoneal cavity in two rabbits. When these animals were killed after 8 weeks, tuberculosis of

the omentum, spleen and liver was found, together with a fair number of grey nodules in both lungs.

6. Caseous pneumonia and tuberculosis of the meninges: 2 guinea-pigs inoculated with cheesy substance from the lungs in which were numbers of bacilli, 1 guinea-pig with a fragment from the tubercular pia mater which contained numerous bacilli. The animals died in the 5th and 6th weeks. All tubercular.

7. Lungs showing caseous infiltration with many bacilli: 6 guinea-pigs. The first died after 6 weeks. The remainder were very ill at the time and were killed a few days later. All tubercular.

8. Phthisical lungs with cavities, intestinal ulcers and cheesy mesenteric glands. 2 guinea-pigs were inoculated from the contents of a cavity containing a fair number of bacilli, and 4 more from the mesenteric glands, which were very full of bacilli. The latter died in the 5th and 6th weeks; of the first two, one died in the 6th week, and the other was killed a few days later. All tubercular.

9. Caseous bronchitis and intestinal tuberculosis. 5 guinea-pigs were inoculated from the lung-substance, in which there were a good number of bacilli. 2 of them died in the 8th week, the remainder were killed before the end of the same week. All tubercular.

10. Phthisical lungs with cavities. 4 guinea-pigs inoculated from the consolidated lung-tissue, in which were only a few bacilli. 3 of them died in the 7th and 8th weeks, the last not till the 12th week. All tubercular.

11. Phthisical sputum. 9 guinea-pigs were inoculated at different times with fresh sputum containing a varying number of tubercle-bacilli taken from 3 different patients. Some of the animals died before the 8th week, some were then killed. They were all tubercular.

12. Phthisical sputum dried for 2 weeks: 3 guinea-pigs. 2 died in the 6th week, the third was killed at the same time. All tubercular.

13. Phthisical sputum dried for 2 months: 3 guinea-pigs, killed after 5 weeks, and tubercles found in lungs, liver, and spleen.

14. Tuberculosis of the uterus and tubes. 6 guinea-pigs inoculated with cheesy material from the tubes. 2 animals

died after 7 weeks. The others were killed in the 9th week. All tubercular.

15. Pus from a tubercular renal abscess: 2 guinea-pigs inoculated subcutaneously, and 2 more by injection into the peritoneal cavity. The animals were killed at the end of 5 weeks. In the guinea-pigs inoculated subcutaneously the inguinal glands were swollen and beginning to undergo caseation, the enlarged spleen showed many, the lungs a few grey nodules. The injected guinea-pigs had many tubercular nodules in the peritoneum and omentum; spleen more tubercular than in the inoculated animals, and tubercles in the lungs also larger and more numerous.

16. Pus from a congestive abscess due to vertebral caries. 5 guinea-pigs were injected in the peritoneal cavity; a control experiment was made on another guinea-pig by injecting some of the boiled distilled water, used for diluting the pus, into the peritoneal cavity; it was left in the same cage with the other animals. In the 7th week the animals were killed. The control animal showed no trace of tuberculosis either in the peritoneal cavity or in the lungs. In those into which the pus had been injected there was marked tuberculosis of the peritoneum and omentum, and in addition more or less advanced tuberculosis of the spleen and lungs.

17. Strumous inflammation of the elbow joint. 4 guinea-pigs inoculated with material containing very few bacilli. Killed in the 10th week. All tubercular.

18. 10 guinea-pigs inoculated at different times from scrofulous glands taken from 3 different cases. The inoculation material contained few bacilli, and the tuberculosis ran a correspondingly slow course. Nevertheless, in these animals also the first symptom to appear was swelling of the inguinal glands followed by caseation, leaving no doubt that the inoculation wound had formed the point of entrance for the tubercle-bacilli. 4 of the animals died between the 10th and 12th weeks, the remainder were then killed. In all, the lymphatic glands near the seat of inoculation were caseous, the spleen, liver and lungs markedly tubercular.

19. Scrofulous gland. Gland-tissue containing few bacilli introduced into the anterior chamber of the eye in 4 rabbits. In all four, tuberculosis of the iris began to develop in 3 weeks,

leading finally to caseation of the globe. The rabbits were killed in the 10th week, and in addition to the destruction of the globe of the eye, caseation of the cervical lymphatic glands and numerous grey nodules in the lungs were found.

20. 18 rabbits were inoculated in the anterior chamber of the eye from 5 different cases of lupus. The course was exactly that described under No. 19. A tardy development of tuberculosis of the iris, leading by degrees to caseation and suppuration of the globe, and finally to general tuberculosis. The inoculation did not fail with any of the rabbits. Some were killed when tuberculosis of the iris had just developed, others after swelling and caseation of the cervical glands had taken place, and others again died in the end with extensive tuberculosis of lungs, liver, spleen and kidneys. In the iris-tubercles as well as in the tubercular glands, lungs, &c., tubercle-bacilli were demonstrated more or less plentifully. From a sixth case of lupus 3 guinea-pigs, and from one of the cases mentioned above 5 guinea-pigs were inoculated subcutaneously. In these animals also swelling and caseation of the inguinal glands occurred. They died between the 7th and 10th weeks after inoculation, were highly tubercular, and had numerous tubercle-bacilli in lungs, spleen, liver and glands.*

21. Lung from a case of *perlsucht*. 8 guinea-pigs inoculated from partially calcified nodules containing a fair number of bacilli. They died within 5 to 8 weeks, and were all highly tubercular. From one of these guinea-pigs 4 others, and from a second 3 more were inoculated. Of the second set 5 died in the 6th and 7th weeks, the two last were killed in the 8th week. In all tuberculosis was found. Further, from the perlsucht lung used for this experiment a cat also was inoculated, and died tubercular after 7 weeks. A second cat inoculated with lung tubercles from the first, became emaciated and short of breath in 6 weeks; it was killed, and numerous tubercles found in the lungs and spleen.

* Demme, Pfeiffer, and Dontrelepont, have lately published observations upon the presence of tubercle-bacilli in lupous skin, and in the tubercles of animals inoculated from cases of lupus. My researches upon lupus, which are not limited to the simple demonstration of bacilli in lupous skin and in tubercles resulting from inoculation, but embrace long-continued pure cultures of lupus-bacilli, and successful inoculations with them, were complete several months before the publication of those observations, so they could have exercised no influence upon my work.

22. 6 guinea-pigs were inoculated with pearl-nodules from the peritoneum; 3 of them died in the 5th and 6th weeks, the remainder were killed some days later. All tubercular.

23. Pearl-nodules from the lung, some with cheesy contents, and not very rich in bacilli. 7 guinea-pigs; 5 of them died up to the 7th week, the two last were killed in the 8th week. All tubercular.

24. Calcified pearl-nodules from the peritoneum with many bacilli. 3 guinea-pigs; they died by the 6th week. All tubercular.

25. Caseous pneumonia in a hog. Consolidated lung tissue containing many bacilli; 5 guinea-pigs. They died in the 5th and 6th week, and were tubercular.

26. 4 guinea-pigs inoculated with pulmonary tubercles from a guinea-pig that died of spontaneous tuberculosis. 2 of them died in the 7th week, 2 were killed in the 8th week. They were all tubercular. From the first of these animals again 4 guinea-pigs, from the second 2 guinea-pigs and 4 rabbits, from the third 2 rabbits, and from the fourth 1 guinea-pig and 1 rabbit were inoculated, the guinea-pigs subcutaneously, the rabbits in the anterior chamber of the eye. The guinea-pigs died up to the 9th week of tuberculosis, the rabbits all developed tuberculosis of the iris; 2 died in the 9th and 10th weeks of tuberculosis, the remainder were then killed, and more or less numerous pulmonary tubercles found in them likewise.

27. 2 guinea-pigs and 2 cats were inoculated with lung tubercles from a monkey that died of spontaneous tuberculosis. The guinea-pigs died in the 6th week, one cat at the end of 7 and the other at the end of 13 weeks, all tubercular.

From one of the guinea-pigs again, 6 guinea-pigs and 1 rabbit (in the anterior chamber of the eye) were inoculated, and all likewise became tubercular before the 8th week (some died, some were killed). Finally, from 2 animals of the second group tuberculosis was induced in 7 more guinea-pigs. From one of the cats also 4 guinea-pigs were successfully infected.

Further, 5 guinea-pigs were inoculated from the spleen of this monkey, the organ having been dried for 56 days, and 4 guinea-pigs from lung-tubercles, which had been lying in absolute alcohol for 57 days. These animals showed no change for 4 months, they were then killed and found free from tuberculosis.

28. With lung-tubercles from a second monkey, dying of spontaneous tuberculosis, 2 guinea-pigs were inoculated, and died of tuberculosis in the 8th and 9th weeks. From these guinea-pigs again 2 guinea-pigs and 1 rabbit were inoculated. They were killed in the 6th week, as they seemed already ill, and they were found to be tubercular.

2 more guinea-pigs were inoculated from the same monkey with lung-tubercles which had been dried and kept for 3 days. They too were killed in the 6th week, and found tubercular.

For the infection-experiments just detailed 79 guinea-pigs, 35 rabbits and 4 cats were used altogether, and the inoculation of these animals resulted in tuberculosis without exception. The occurrence of tubercular changes was not limited to isolated nodules of doubtful nature in any one organ, but in each case as certain proof of tuberculosis as could be desired was furnished, first of all by the development of the characteristic symptoms of the disease, such as glandular swelling, caseous ulceration at the seat of inoculation, emaciation and dyspnœa, and later at the autopsy by the presence of marked tubercular changes starting from the inoculation wound, and affecting the neighbouring lymphatic glands; as also the lungs, spleen, and liver. In addition, the characteristic structure of tubercle and the presence of tubercle-bacilli were always demonstrated microscopically.

Other workers have had less favourable results from inoculation with tubercular substances. In comparison with these the uniform effects I obtained will seem less striking if it is remembered that I inoculated only with material in which tubercle-bacilli had been shown to be present, and that I used for my experiments animals of species particularly subject to tuberculosis. Further, my results were possibly not a little influenced by the fact that the operation itself was carried out very carefully and exactly.

It may be considered an omission that no control experiments were made by inoculation with non-tubercular substances. But it did not seem to me necessary to undertake special control experiments of that nature, because in the course of my investigations I never found tuberculosis develop as the result of hundreds of inoculation experiments made in the same rooms, and likewise on guinea-pigs and rabbits with all kinds of material, which, however, contained no tubercle-bacilli. For instance,

non-tubercular material was several times introduced into the anterior chamber of the eye in rabbits without ever producing tuberculosis of the iris, which, however, never failed to develop after inoculation with true tubercular substances. Control experiments are furnished too by the unsuccessful inoculations under No. 27 made with tubercles, some dried and others preserved in alcohol, from a monkey's lung, where the loss of virulence was evidently due to the death of the bacilli. It was an experiment made with indifferent material in fact.

My researches justify me, therefore, in the conclusion that inoculation only with substances containing tubercle-bacilli can induce true tuberculosis in animals experimented on.

I was not able to distinguish any difference in the effect of inoculation with material derived from varieties of the tubercular process, as miliary tuberculosis, phthisis, scrofula, fungous inflammation of joints, lupus, *perlsucht* and other forms of animal tuberculosis. So that in this respect also the different forms of tuberculosis resemble each other exactly.

2. *Infection-experiments with Pure Cultures of Tubercle-bacilli.*

This second group of infection-experiments completes the proof that tuberculosis is an infective disease, caused by tubercle-bacilli.

So far it has been proved that tubercle-bacilli are present in all tubercular processes, and in these only; further, that only substances containing tubercle-bacilli are capable of inducing tuberculosis. But as in both cases the bacilli are mixed up with elements of the body, it was justifiable to surmise that with the bacilli some other material of importance was present, in which possibly the real infective power might lie, whilst the bacilli played only a secondary part. This point could be decided only by inoculating with bacilli in a pure state isolated from all elements of the body. If under these circumstances tuberculosis still developed, bacilli would, necessarily and beyond all doubt, be the sole infective virus of tuberculosis. The great importance of this part of the investigation necessitated the observance of the most stringent precautions in order to exclude all error. Accordingly, several recently bought animals were used for each

experiment, as in the earlier infection researches, and special control-experiments were undertaken besides in most cases. The animals belonging to each experiment were placed in a separate cage, and were strictly isolated from other tubercular animals; they were killed also as early as possible, to avoid, as far as one could, the possibility of a confusion with spontaneous tuberculosis. Further, the most different methods of infection possible and different species of animals were used, in order to learn in this way, also, the effect of pure cultures. The greatest attention was paid to the disinfection of the vessels and instruments to be used, particularly the syringes. The cultures employed for infection consisted entirely of tubercle-bacilli, as was specially proved in almost every case. They were taken from the solidified blood-serum with great care by means of a heated platinum wire, which can easily be managed, as before stated, without tearing away the smallest particle of serum with them. It is not, therefore, too much to say that in most of the experiments absolutely pure masses of bacilli were used, to which no trace of the soil on which they were grown adhered. Again, in several experiments, sterilized blood-serum was injected into the control animals, without any trace of tuberculosis ever resulting. We may therefore affirm with all certainty that when true tuberculosis develops in consequence of infection from a pure culture of tubercle-bacilli, which has been carried on through many generations, it is due to the action of the tubercle-bacilli alone.

1st Experiment. Pure culture from miliary tubercles in the human lung (No. 22 in the earlier account of the pure cultures), carried through 5 generations in 54 days; subcutaneous inoculation of 4 guinea-pigs; 2 animals in the same cage not inoculated. In the inoculated animals swelling of the inguinal glands took place after 14 days, ulceration occurred at the seat of inoculation, and the animals began to lose flesh. One of them died at the end of 32 days, the others were killed on the 35th day. The inoculated guinea-pigs, the one that died as well as the three killed, showed extensive tuberculosis of the spleen, liver and lungs; the inguinal glands were much swollen and caseous, to a markedly greater degree on the inoculated side; bronchial glands slightly enlarged. Neither of the animals that had not been inoculated showed any trace of tuberculosis.

2nd Experiment. Pure culture from the tubercular lung of a

monkey (No. 11), carried through 8 generations in 95 days; subcutaneous inoculation of 6 guinea-pigs, 2 control animals not inoculated. After 32 days all the animals were killed, and the 6 inoculated appeared highly tubercular, the 2 uninoculated remaining healthy.

3rd Experiment. Pure culture from a lung from a case of *perlsucht* (No. 37), carried through 6 generations in 72 days; subcutaneous inoculation of 5 guinea-pigs; 1 control animal not inoculated. All the animals killed after 34 days; the inoculated were tubercular, the uninoculated healthy.

4th Experiment. Pure culture from the tubercular lung of a monkey (No. 11), carried through 9 generations in 113 days; subcutaneous inoculation of 2 guinea-pigs, 1 marmot, 6 white rats, 5 white mice, 4 field-mice, 2 hedgehogs, 6 hens, 4 pigeons, 2 sparrows, 3 eels, 1 gold-fish, 5 frogs and 1 tortoise. Of the above animals only the guinea-pigs, the marmot and the field-mice became visibly ill; these were killed 53 days after inoculation, and were all found to be highly tubercular. Tuberculosis in the marmot apparently resembles very closely the same disease in the guinea-pig; the spleen is much enlarged, and has a greyish-red, mottled appearance; the liver also is beset with large yellowish foci. The organs of the field-mouse that have undergone tubercular change have a very characteristic aspect too. The inguinal glands are markedly swollen and caseous, the lungs full of grey nodules, of sizes varying from that of a poppy-seed to that of a pin's head; while many whitish nodules as large as a millet-seed are distributed uniformly throughout the liver and spleen, so that they have a very pretty speckled appearance. All the remaining animals of this experiment were killed 2 months later, and on examination it was discovered that one of the 5 white mice had a few grey nodules in the lung, while the others were healthy, the rats and the hedgehog also; 3 of the hens had the large tubercles in the intestine and liver characteristic of the disease in these birds. The other animals experimented on were healthy.

5th Experiment. Pure culture from a closed cavity in a phthisical lung (No. 26), carried through 16 generations in 12 months; 17 guinea-pigs inoculated subcutaneously; 2 control animals not inoculated. With these animals observations were set on foot as to the influence of means calculated to prevent the

development of the tubercle-bacilli, so that they could not be killed. Although arsenic in some cases, carbolic acid in others, was administered to the guinea-pigs in as large quantities as possible, the tuberculosis took the same course as in the animals experimented on earlier; the lymphatic glands became considerably enlarged, emaciation set in, all the animals died between the 4th and 6th weeks, and all were extremely tubercular. Both control animals were then killed, and found healthy.

6th Experiment. Each of the following pure cultures was employed for the subcutaneous inoculation of field-mice, 4 animals being inoculated from each cultivation: 1st, from lupus (No. 35), carried through 8 generations in 5 months; 2nd, from strumous arthritis (No. 34), carried through 7 generations in 4 months; 3rd, from a scrofulous gland (No. 29), carried through 7 generations in 5 months; 4th, from miliary tuberculosis (No. 22), carried through 12 generations in 9 months; 5th, from a cavity in a phthisical lung (No. 25), carried through 9 generations in 6 months; and 6th, from pearl-nodules (No. 39), carried through 11 generations in 9 months. The mice were placed in couples in roomy glasses. Some of the animals died after the lapse of a few days, apparently from the effect of the captivity. All the others became visibly ill, the inguinal glands began to swell, the animals lost flesh and became short of breath. In the course of 4 to 6 weeks they all died. The examination of some of these animals was rendered impossible, or partially so, by the fact that the surviving field-mice, although liberally supplied with vegetable food, gnawed at their dead companions and consumed the internal organs with great avidity. However, several animals from each division of this experiment were available for examination, and it was proved that they all succumbed to extensive tuberculosis of lungs, liver, and spleen. No difference could be detected in the character of the tuberculosis resulting from the various pure cultures. The collective picture of the pathological changes was identical in all the animals, and the macroscopic appearance of the individual nodules was similar, as was also their microscopic structure, especially as regards the presence of tubercle-bacilli. One other point in this experiment was that the animals had been only a few days in confinement before they were inoculated, and that a large number of other field-mice were kept for months in glasses under

the same conditions without a single one of them becoming tubercular.

7th Experiment. As field-mice respond so certainly and conveniently to tubercular infection, they were the animals chosen for some experiments I made in conjunction with Dr. Gaffky as to the influence exerted by germicidal substances on tubercular animals. 24 were inoculated subcutaneously from a phthisical lung (No. 1), from which 12 successive cultivations had been made in 7 months. These animals were made to inhale volatile substances, but some of them died of pneumonia; after a few days in all the others tuberculosis developed and ran the same course as in the mice belonging to the foregoing experiment. The autopsy showed in every case marked tuberculosis of lungs, spleen and liver.

8th Experiment. For the same purpose 5 guinea-pigs were inoculated with a pure culture from caseous pneumonia (No. 28), carried through 8 generations in 6 months; also 4 guinea-pigs with a pure culture from a phthisical lung (No. 24), carried through 10 generations in 6 months; and 6 guinea-pigs with a pure culture from a tubercular testicle (No. 33), carried through 5 generations in 3 months; all subcutaneously. The animals had different gaseous germicidal substances to inhale; but became ill in spite of them, lost flesh, died within 4 to 6 weeks, and were all found to be tubercular on examination.

9th Experiment. Pure culture from lupus (No. 35), carried through 15 generations in 12 months; 5 guinea-pigs inoculated subcutaneously. This experiment was made in order to see whether a year's cultivation of the tubercle-bacilli from the skin in a case of lupus had had any effect on their virulence. It was not so, however. The animals inoculated fell ill just as certainly and quickly as in the earlier experiments; two died in the 4th week, the remainder were killed about the same time, and all found to be highly tubercular on dissection.

10th Experiment. With the same view 4 guinea-pigs were inoculated subcutaneously with the pure culture of longest duration (No. 1), which was started from a case of pulmonary phthisis in the human subject, and had been carried through 26 generations in 18 months. The course was exactly the same as in the 9th experiment. The animals died tubercular in the 4th and 5th weeks after inoculation.

11th Experiment. On former occasions an essential difference in the susceptibility of house-mice and field-mice to tubercular inoculation had been noticed. Hence 12 white mice were inoculated with a pure culture from a case of miliary tuberculosis (No 22), the same which had been used for the inoculation of the field-mice in the 6th experiment, and the inoculation was done at the same time. The field-mice, as already stated, became tubercular, whilst the white mice remained for 2 months without any symptoms of illness at all; they were then killed, and in not a single one were tubercular changes found.

The 11 experiments here classed together have this in common, that the inoculation substance was introduced subcutaneously in all. The effect was on the whole the same as that obtained by subcutaneous inoculation with fragments of fresh tubercular tissues. The small skin-wound united and healed in the first few days; then followed swelling of the glands, emaciation and death. The autopsy revealed an extensive and copious eruption of tubercles in lungs, spleen and liver, combined with the attendant characteristic changes that usually follow in these organs. The only difference lay in the quicker course of the disease after inoculation with pure cultures than after inoculation with tubercular tissues. In guinea-pigs this difference was estimated to be on the average two weeks. The phenomenon is most naturally accounted for by the fact that when tubercular tissues are used as the inoculation material, the bacilli are not able to act until the tissues in which they are inclosed are absorbed, whilst from pure cultures they enter the subcutaneous tissue of the animal experimented on unimpeded, and can at once begin their work. It is the same with the iris tuberculosis resulting from inoculation of the anterior chamber of the eye in rabbits, but still more strikingly because the development of the tubercles can be followed directly with the naked eye.

Microscopically, the tubercles obtained after inoculation with pure cultures resembled in every respect those resulting from inoculation with truly tubercular tissues and also those of spontaneous tuberculosis. They consisted of accumulations of cells having mostly an epithelioid character, contained giant-cells and, in addition, tubercle-bacilli in varying numbers. Their virulence was proved by the fact that in every case they had spread from the subcutaneous tissue to all the organs liable to be affected by

that solitary yellowish-white nodules appeared in the iris in the neighbourhood of the inoculation puncture, and from this point a typical tuberculosis of the iris developed. Fresh nodules continually appeared in the iris, which fell into radiating folds; by degrees, however, the cornea became muddy, so that the further changes could not be watched. At the end of thirty days the animals were killed. The first was perfectly healthy; in the second, besides the changes described in the eye, the lymphatic glands below the jaw and near the root of the ear were swollen and beset with yellowish white foci; the lungs and remaining organs were still free from tuberculosis. The two last rabbits had countless tubercles in the lungs.

15th Experiment. A pure culture of a miliary tubercle from a human lung (No. 4), carried through 8 generations in 4½ months, was rubbed up with blood-serum and the canula of a syringe full of it passed into the anterior chamber of the eye in 6 rabbits, but no injection was made. In all the animals tuberculosis of the iris developed; and in some there was infiltration of the conjunctiva with tubercular nodules, which spread slowly round about the inoculation puncture. Two animals of this series that were killed after 4 weeks showed already cheesy infiltration of the cervical lymphatic glands, but no tubercles as yet in the lungs. The remaining rabbits were killed after 8 weeks, and then more or less numerous tubercles were found in the lungs.

Other rabbits at different times received injections of pure cultures in the anterior chamber of the eye, in order to test on these animals the effect of substances which interfere with the development of tubercle-bacilli. Information as to these experiments, in which Dr. Gaffky was my coadjutor, will be given on a later occasion. I will now merely mention in passing that besides numerous other things, arsenic,* helenin, sulphuretted hydrogen, all in as large doses as possible, were administered to

* The use of arsenic as a remedy for tuberculosis has been often recommended in former times, and has been already tried by many physicians. It was, therefore, important to ascertain the effect of this remedy on tuberculous animals. Our experiments were made almost a year ago, before the recommendation of arsenic by Buchner appeared, and, therefore, they were not performed as the result of his publication. Helenin, according to the statement of Korab, should have prevented the development of tuberculosis in animals, and sulphuretted hydrogen was very strongly recommended by Proschauer.

these animals for some weeks. But we could not affirm that any means tried acted favourably in a single case. All the animals succumbed just as quickly to tuberculosis as those which had not been treated in this way. The infection was accomplished in various ways—in some cases by simple inoculation (compare experiments 7 and 8), in others by injection into the anterior chamber of the eye, in others again by injection into a vein. The rabbits infected from the anterior chamber of the eye included the following cases :—

16th Experiment. A pure culture of miliary tubercle from a human lung (No. 22), carried through 10 generations in 8 months, was rubbed up with distilled water and injected into 2 rabbits; a pure culture from a phthisical lung (No. 1), carried through 21 generations in 3 months, was similarly injected into 15 rabbits; and 6 rabbits were injected from the same culture a month later. All these rabbits died rapidly with the symptoms before given, and had without exception numerous tubercular nodules in the lungs.

In all the cases in which very small quantities of the pure culture were introduced into the anterior chamber of the eye, the effect was exactly the same as after implantation of the natural tubercular virus in the eye. Isolated tubercles appeared in the iris, which increased in number, and led first to caseation of the globe, and finally to general tuberculosis. There was a distinction, certainly, in that the eruption of nodules took place earlier than after inoculation with tubercular tissues. The probable reason of this difference has already been mentioned. The experiments brought to light another important fact, viz., the marked difference there was in the effect according as a very small or a large number of bacilli reached the anterior chamber of the rabbit's eye. In the first case a slowly creeping process arises, in which the infective material spreads first over the iris, then reaches the lymphatic glands, leading to their caseation, and, penetrating at last into the circulation, gets distributed to other organs of the body. But if, on the contrary, a large number of bacilli are in the first instance introduced into the anterior chamber of the eye it seems as if the bacilli escaped part of this circuitous route, in fact as if the lymphatic glands, which as a rule oppose a barrier to the further progress of the bacilli and detain them for a longer or shorter time, were passed

over entirely. The appearance of large numbers of tubercles in the lungs, spleen, &c., takes place as early by this method of infection as when tubercle-bacilli are injected immediately into a vein. The number of nodules also is not much less after injection into the anterior chamber of the eye than is found after injection into the blood-stream. I cannot undertake to decide whether the explanation of this is that considerable numbers of the bacilli can in some way really reach the blood-stream direct from the anterior chamber of the eye, or that they flood the intervening lymphatics and glands in such quantities that most of them break through the barrier and only a few are held back. In any case these observations are calculated to throw light upon the enigmatical and uncertain course of tuberculosis with regard both to its duration and to its limitation for a longer or shorter period to local manifestations.

Injection of Pure Cultures into the Peritoneal Cavity.

The pure cultures were rubbed up with blood-serum or distilled water and a disinfected syringe was filled with this mixture, the part of the animal's abdomen to be operated on was purified with a solution of corrosive sublimate, the canula of the syringe was pushed slowly through the abdominal wall to avoid injuring the intestine, and the fluid was injected into the abdominal cavity. This operation, so simple in itself, is easy enough to carry out on animals whose intestines are not constantly full of solid unyielding food, and I have always succeeded with guinea-pigs, rats, mice, cats, &c., without causing injury to the intestine or the development of traumatic peritonitis. Rabbits are less suitable for this experiment on account of the well-filled cæcum. Large quantities only of the pure cultures were injected into the animals in order to obtain as rapid results as possible. The effects in the peritoneal cavity, as in the anterior chamber of the eye, depend on the quantity of the tubercular virus introduced. After an injection of pus containing few bacilli a disseminated eruption of tubercles appeared on the peritoneum of the guinea-pigs, as we have previously seen (p. 160), and in addition there was development of nodules in the omentum and spleen. But when quantities of tubercle-bacilli are injected into the peritoneal cavity of guinea-pigs, they are

taken up especially by the great omentum, which becomes drawn together into a thick sausage-like roll lying horizontally across the abdomen, and presenting on section a strong resemblance to a greatly enlarged lymphatic gland which has recently undergone caseation. These yellowish-white, rather tough foci in the omentum contain huge numbers of tubercle-bacilli, most of which have reached the spore-bearing stage. Microscopic examination shows that the enlarged spleen, the liver and the peritoneum contain abundance of tubercle-bacilli, but the animals die so early that there is not time for the development of tubercles visible to the naked eye. There is no effusion into the peritoneal cavity in guinea-pigs as there is in dogs and cats; but in guinea-pigs, on the contrary, the pleural cavity is always filled with a clear straw-coloured fluid to such an extent that the lungs are compressed and death follows from this cause. The guinea-pigs generally die 10 to 20 days after the injection. When a smaller quantity of the culture is introduced, the disease is of longer duration, and tubercles visible to the naked eye develop in extraordinary numbers, particularly in the peritoneum, omentum, spleen and liver. Animals less susceptible to tuberculosis, as dogs, rats, white mice, do not succumb even to very copious injections until after the lapse of some months. One then finds, however, an abundant eruption of tubercles in the abdominal organs, but fewer nodules in the lungs.

17th Experiment. Pure culture from the tubercular lung of a monkey (No. 11), carried through 11 generations in 6 months, rubbed up with blood-serum and injected into the abdominal cavity in 10 guinea-pigs, to the extent of ½ c.cm. to each animal. 2 control animals were observed, one having received a similar injection of pure blood-serum, the other, which had been bitten recently and rather severely in the abdomen, remained without any injection. Of the animals injected, some died at the end of 10, 13, 16, 17 and 18 days respectively. The remainder, with the control animals, were killed on the 25th day. In the guinea-pigs that died first the great omentum was rolled up, much thickened and infiltrated with areas of a yellowish-white brittle substance; no nodules visible in liver and spleen. Those dying or killed later showed an eruption of tubercles in the spleen and liver besides infiltration of

the omentum. The control animals were found to be perfectly healthy.

18th Experiment. Pure culture from the tubercular lung of a monkey (No. 11), carried through 10 generations in 5½ months, rubbed up with serum and injected into the abdominal cavity of 2 full-grown and vigorous cats. One of them died at the end of 19 days. The omentum was rolled up, much thickened and infiltrated with a white hard substance. The serous surface of the intestine and peritoneum had lost its brilliancy, the spleen was greatly enlarged. The infiltration of the omentum consisted, as in the guinea-pigs of the previous experiment, of closely packed masses of tubercle-bacilli embedded for the most part in cells. Macroscopically no nodules could as yet be discovered in lungs, spleen and liver, but microscopically a very plentiful eruption of tubercles was seen in these organs. The second cat was killed after 43 days, and tubercles of the size of a millet-seed were found in great numbers distributed pretty equally through lungs, spleen and omentum, but occurring relatively more sparingly in the liver. It was intended that both cats should be injected with an equal quantity (a syringe full) of the infective fluid. But the second was very restless during the operation, so that only a small amount of the fluid could be introduced; consequently the course of the disease was much slower in this case, and fewer tubercles developed, which, however, had the opportunity of growing to a considerable size.

19th Experiment. Pure culture from miliary tuberculosis (No. 22), carried through 5 generations in 3 months, and rubbed up with blood-serum; 2 c.cm. of the fluid were then injected into the abdominal cavity of a bitch several years old, while ½ c.cm. of the same fluid was injected into a dog several months old. No change was observed in the animals during the first weeks after the operation. After the 3rd week the bitch lost all liveliness, ate less, and a distinct swelling of the abdomen took place. At the beginning of the 5th week the animal was killed. A fairly abundant effusion of a clear straw-coloured fluid was found in the peritoneal cavity. The omentum, mesentery, uterine ligaments and peritoneum were beset with a large number of tubercles, and also the surface of the intestine and bladder. The enlarged spleen, liver and lungs showed

countless miliary tubercles containing tubercle-bacilli. Nothing could be seen of the inoculation puncture. The second dog seemed ill for a long time, had distinct effusion into the peritoneal cavity and lost flesh; finally it recovered and became strong. Five months later this dog, together with a bitch of the same litter, received an injection of the same pure culture, but this time 2 c.cm. were used. The consequences were the same in both animals; for some weeks they showed no symptoms of illness, then lost flesh and ascites appeared. After 5 weeks one animal died, and the other, already very weak, was then killed. The post-mortem appearances were exactly like those described in the first dog. Omentum, peritoneum, spleen, liver and lungs all showed an extraordinary number of tubercles.

This experiment is of particular interest in that one dog after an injection of ½ c.cm. of bacillary fluid became decidedly ill, but recovered. This is the only case of tuberculosis in animals where I saw recovery take place. The hope has often been expressed that a preventive inoculation with attenuated virus will be accomplished against tuberculosis as against splenic fever. But if one successful battle with tuberculosis affords protection from a second attack of the disease, which view, by the way, is not borne out by clinical experience, this dog should have been immune against further infection. But this was not the case, and the circumstance has a bearing against the fulfilment of the hopes referred to.

20th Experiment. Of 5 cats experimented on, the first received an injection of pure blood-serum; the second, a mixture of the same serum with the pure culture No. 23 (human miliary tuberculosis, carried through 8 generations in 5 months); the third, the pure culture No. 1 (pulmonary phthisis in the human subject, carried through 12 generations in 7 months); the fourth, the pure culture No. 16 (pearl-nodule, carried through 9 generations in 5½ months); the fifth, the pure culture No. 13 (tuberculosis in a monkey, carried through 5 generations in 3 months). Exactly the same process was carried out on 5 guinea-pigs. Of the latter, four died, on the 12th, 14th, 15th and 21st days respectively; the control animal was killed on the 22nd day. Of the cats, the fourth died on the 22nd day, the third on the 27th, the others were killed on the 28th day. All the animals injected with bacillary fluid

tubercular changes with which former experiments have familiarized us, at a stage of development corresponding to the time that had elapsed after the injection. The cat and guinea-pig which had pure serum injected into the peritoneal cavity were found to be perfectly free from tuberculosis. This experiment, like many previous ones, was undertaken with the view of discovering whether there was any difference in the action of cultures of bacilli obtained from different forms of tuberculosis. But here too the expectations entertained were not fulfilled, for the tuberculosis resulting from the different pure cultures was always of the same form, both in cats and guinea-pigs.

21st Experiment. Pure culture from tuberculosis in a monkey (No. 11, carried through 10 generations in 5 months), injected into the abdominal cavity in 5 rats. These animals had been fed previously for a considerable time with portions of tubercular guinea-pigs. In other rats fed after the same manner which had been killed, solitary grey nodules had occasionally been found in the lungs. But when the rats on which the injection of tubercle-bacilli had been practised were killed at the end of 3 weeks, countless tubercles were seen in the lungs and greatly enlarged spleen, and also in the liver and omentum.

22nd Experiment. Pure culture No. 24 (from a phthisical lung, carried through 9 generations in 5 months), rubbed up with water and injected into the peritoneal cavity. The following animals were experimented on: 6 guinea-pigs, 3 cats, 4 white mice, 4 hens, 8 pigeons. The guinea-pigs died within 10 to 17 days, one cat on the 15th, another on the 23rd, and the last on the 24th day. The post-mortem appearances in these animals were the same as in the previous experiments. The mice, hens and pigeons remained alive certainly, but became rough and thin, and seemed ill. As they did not recover they were all killed at the end of 10 weeks. The mice showed the same state of things as the white rats, a good number of tubercles in the lungs and a great many in the much enlarged spleen. In the hens and pigeons the nodules before described were found in the intestine and liver.

Injection of Pure Cultures into the Veins.

This is the most certain and fruitful method of infecting animals.

The body can be saturated suddenly by way of the blood-stream with as large a quantity of the infective material as is desired; not having, as in the other experiments, to overcome the barrier offered by the lymphatic glands, &c., it spreads immediately to all the organs and causes an extensive and pretty equally distributed eruption of tubercles. This mode of infection evidently resembles closely that of human miliary tuberculosis, where the tubercular virus likewise makes its way into the blood, which carries it to all parts of the body. By injection into the veins, tubercular nodules are produced in all the organs within a much shorter time and in much greater quantity than is ever the case with spontaneous tuberculosis; hence it is impossible to mistake one form of the disease for the other. The fluid in which the pure cultures of tubercle-bacilli were distributed as finely as possible, was filtered through fine gauze to keep back the coarser particles and then injected into the jugular vein by means of a disinfected syringe, or, following Aufrecht's method, direct into an exposed vein of a rabbit's ear.

23rd Experiment. Of 12 rabbits 2 had ½ c.cm. of pure blood-serum injected into a vein of the ear; 4 were treated in the same way with serum to which was added the pure culture No. 11 (tuberculosis in a monkey, carried through 4 generations in 6 months, compare experiment 17); 3 with serum containing the pure culture No. 1 (from a phthisical lung, carried through 10 generations in 6 months); and 3 with serum containing the pure culture No. 19 (from a lung taken from a case of perlsucht, carried through 7 generations in 4 months). Nothing particular could be remarked in any of these rabbits for the first few days after the operation. The first two rabbits remained brisk and vigorous throughout, but all the rest began as early as the second week to breathe with difficulty and to lose flesh rapidly. After 18 days the first rabbit died (injection from culture No. 1), after 19 the second and third (injection from culture No. 11), after 21 the fourth (culture No. 19), after 25 the fifth (culture No. 1), after 26 and 27 days the sixth and seventh (culture No. 11), on the 30th and 31st days two other animals. The last and the two control animals were killed on the 38th day after the injection. As before, no difference could be detected in the condition of the lungs and other organs of the animals treated with the different cultures. In all the animals there were countless

miliary tubercles in the lungs. The liver and spleen also in all contained an extraordinary number of tubercles. In those that died first the nodules were smallest but most numerous. Evidently, the large number of tubercles had caused early death. In the animals dying later the number of nodules was less, but the tubercles themselves were larger. The 2 control animals were found on dissection to have no tubercles in any of the organs.

24th Experiment. Pure culture from lupus, No. 35 (carried through 8 generations in 5 months), rubbed up with distilled water, and injected into the auricular vein in 5 rabbits. They died between the 13th and 18th days after the injection, and showed the same post-mortem appearances as the rabbits of the previous series.

25th Experiment. Pure culture from tuberculosis in a monkey, No. 11 (carried through 12 generations in 6 months), rubbed up with distilled water, was injected into the jugular vein in 10 rabbits selected for inhalation experiments with germicidal substances. They all died in the course of 2 to 3 weeks after the injection, and had large tubercles in lungs, liver, and spleen. The tubercle-nodules produced by the injection of bacilli into the circulation differed in no way from nodules of the spontaneous disease, and the same is true of the nodules in all the earlier infections produced by means of pure cultivations. They contained tubercle-bacilli in greater or smaller numbers, and had virulent properties, for when they were inoculated into other animals, as they often were, they gave rise to tuberculosis in just the same way as inoculations from true spontaneous tubercles.

Inhalation of Pure Cultures of Tubercle-bacilli.

In order to introduce tubercular material into the lungs of animals for experiment, either an injection was made into the bronchi from a tracheotomy wound, or the animals were made to inhale an atmosphere in which the infective material first suspended in a fluid had been dispersed. The first method does not resemble sufficiently the natural mode of infection, and is complicated unfavourably by the operation wound. I chose the

second method, therefore, which, for obvious reasons, is certainly not without danger for the experimenter, and necessitates in consequence special precautions.

The experiment was conducted as follows. A very roomy box, having on one side an opening for the orifice of the spray apparatus, was placed in a garden at a good distance from any habitation. The spray apparatus was placed outside the box with its orifice projecting into the interior. The apparatus was connected by means of elastic tubing and a suitable length of lead pipe, which passed through the woodwork of a closed window, with the india-rubber bellows, and so could be worked from the room beyond the region of the spray.

26th Experiment. Pure culture from a phthisical lung in the human subject, No. 1 (carried through 23 generations in 15 months), rubbed up with distilled water, and the fluid diluted to such an extent that it looked almost clear. Any visible fragments present in the fluid subsided after standing a short time, the upper layer, which showed hardly any opacity, was poured off, and used for inhalation. 50 c.cm. were dispersed in the course of half an hour on three successive days, and inhaled by the animals in the box as follows: 8 rabbits, 10 guinea-pigs, 4 rats and 4 mice. After the inhalation the animals were kept in separate roomy cages, and well looked after. In some of the animals dyspnœa appeared after 10 days; and 3 rabbits and 4 guinea-pigs died in the course of 14 to 25 days. All the remaining animals were killed 28 days after the last inhalation. All the rabbits and guinea-pigs had numerous tubercles in the lungs, the size of the tubercles being proportionate to the length of time the animals had lived after inhalation. In the animals which were the last to die, as well as in those killed, tubercles were found in the liver and spleen. The pulmonary tubercles resembled in every respect those discovered in rabbits and guinea-pigs as the result of inhalation of phthisical sputum in experiments instituted for other purposes. For instance, the tubercular nodules occurring in consequence of the inhalation of sputum, and of pure cultures of tubercle-bacilli, had this in common, that when they had reached a certain size their extension by way of the alveoli could be plainly recognized by the naked eye. They did not seem sharply rounded and defined, but embraced mostly the centre of a lobule. They had a

faintly granular appearance, as the separate alveoli were filled with a cheesy substance, and looked like whitish dots, and at their margins the yellowish-white spots of the caseous alveoli stood out very clearly against the dark greyish-red areola. The largest tubercles embraced a whole lobule, and coalesced in some cases with neighbouring foci, forming in this way larger thickened yellowish-white places in the lung, which had exactly the ordinary aspect of caseous pneumonia. Spontaneous tuberculosis in rabbits and guinea-pigs shows the same constitution of the primary tubercular nodules, viz., the alveolar extension of the tubercular process. This supports the view already mentioned, that spontaneous tuberculosis in these animals is the result almost exclusively of inhalation.

The rats and mice that were killed had very numerous grey nodules in the lungs as large as a hemp-seed, many of which had a yellowish-white centre, but caseation was not nearly so far advanced as in the lungs of the guinea-pigs and rabbits. In the spleen of the rats and mice solitary grey nodules only were found. These animals, as has been already insisted upon, are much less susceptible to tuberculosis, the separate tubercles develop in them much more slowly, and the disease does not spread so readily to other organs.

Microscopically, too, the tubercles resulting from the inhalation of pure cultures resemble true tubercles in the arrangement of the epithelioid-cells and giant-cells, and in containing tubercle-bacilli. In order to test their infective properties, 22 guinea-pigs, in all, were inoculated subcutaneously in the abdomen with tubercles from different organs of both guinea-pigs and rabbits, and from the lung of a rat and of a mouse. All these animals developed swelling of the inguinal glands on the same side as the inoculation, lost flesh, and died of tuberculosis in the course of 5 to 8 weeks.

If we now glance back over the whole series of infection-experiments with pure cultivations, we may draw the following conclusions:—

Those animals operated on which belonged to species susceptible to tuberculosis, viz., guinea-pigs, rabbits, field-mice and cats, became tubercular, without exception, in consequence of infection with tubercle-bacilli. The number of animals was 217 (94 guinea-pigs, 70 rabbits, 9 cats and 44 field-mice). On the

other hand, a certain number of control animals treated in the same way with indifferent fluids, and kept under similar conditions, remained, without exception, free from tuberculosis. Of the less susceptible animals only hens, and then no more than half the number inoculated, became tubercular as the result of simple subcutaneous inoculation. But even dogs, rats and white mice, which are as a rule little subject to tuberculosis, could not resist infection with large quantities of tubercle-bacilli from pure cultures, and they all became tubercular.

The various methods of infection had the same effect when pure cultures were used as with natural tubercular substances, only the former operated rather more quickly.

The products of infection too were like those obtained with natural infective material in their microscopic structure, the tubercle-bacilli they contained and their virulent properties.

By carefully attending to all the precautions necessary to prevent mistakes when experimenting on tuberculosis, errors were excluded with certainty in these experiments. It may here be mentioned that a very large number of trials were made with other pathogenic and non-pathogenic bacteria in the same way as with pure cultures of tubercle-bacilli. These various organisms also were introduced into the anterior chamber of the eye and injected into the veins of rabbits, inoculated subcutaneously upon guinea-pigs, rabbits, mice and other animals, injected into the peritoneal cavity and used for inhalation experiments in the manner above described. But in no case did they cause tuberculosis in the animals experimented on.

In the experiments made with pure cultures, therefore, tubercle-bacilli only, freed from all contamination with the original morbid products, can have been the cause of tuberculosis. But that proves the truth of the proposition that tuberculosis is an infective disease depending on the presence of tubercle-bacilli. It may certainly be said, and indeed it has been said, that tubercle-bacilli are one cause of the occurrence of tuberculosis, but that other things, *e.g.*, other micro-organisms, may also have the power of inducing tuberculosis. This statement is erroneous, because, as we have seen, in all cases of true tuberculosis tubercle-bacilli are present, and the manner of their appearing further proves that they stand to the disease in the position of the cause. If a special tubercular virus

is to be granted in addition to the tubercle-bacilli, then some specific and hitherto unknown agent must be assumed as the infective material in the case of trichina and acarus scabeii. So we may say with good reason that the tubercle-bacillus is not merely a cause of tuberculosis, but the only cause, and that without the tubercle-bacillus there can be no tuberculosis.

Tuberculosis is thus seen to be allied etiologically to splenic fever. The tubercle-bacillus stands in exactly the same relation to tuberculosis as the bacillus anthracis does to splenic fever.

THE RELATIONS OF THE TUBERCLE-BACILLI TO THE ETIOLOGY OF TUBERCULOSIS.

The researches detailed in the foregoing pages have furnished us with so much information about the biological characteristics of tubercle-bacilli, and their peculiar behaviour in the body attacked by them, that we are enabled to determine the etiology of tuberculosis in its broad outlines with some degree of certainty. In time, we shall no doubt get to know the properties of the tubercle-bacilli more thoroughly, and to discover new facts about them, with the effect of enlarging and in some respects correcting our views on the etiology of tuberculosis. Meanwhile, this conviction cannot restrain us from already expressing an opinion upon the relations of tubercle-bacilli to the disease caused by them.

If we start with the proposition, resting on experimental proof, that tubercle-bacilli alone can give rise to true tuberculosis, and if we try to follow out the way in which they infect animals and Man, the first question that meets us has reference to the origin of the bacilli. Do they occur anywhere in Nature independent of the human or animal organism, as we must suppose to be the case, for instance, with anthrax bacilli and the micrococcus of erysipelas? The answer to this question is of the highest importance, not only etiologically, but also with regard to prophylaxis. For if tubercle-bacilli could exist in the universally distributed putrefying animal and vegetable matter, and there multiply and form spores, it would be impossible to keep these parasites away from human beings. Happily things are other-

wise. Experience has shown that tubercle-bacilli grow much more slowly than any other bacteria, further, that they grow only in serum and meat infusion, and, most important of all, require a temperature above 30° C. to grow at all. But if all these conditions were present together, and yet the tubercle-bacilli were not protected from being choked by other quickly growing bacteria, the same thing would happen that is often seen in cultures rendered impure by extraneous bacteria, viz., the tubercle-bacilli would be very soon overpowered and would perish. As a matter of fact, the conditions favourable to the development of the tubercle-bacilli, especially a temperature of over 30° C. lasting day and night for weeks, do not exist together outside the animal body; hence no view is possible, but that the tubercle-bacilli are limited in their existence entirely to the animal and human organism. They are therefore true parasites, which cannot live without their host, not occasional parasites like the splenic-fever bacilli, which ordinarily complete their developmental cycle outside the animal body, and only at times invade it. An essential point of difference between the splenic-fever and tubercle-bacilli is that the former only multiply in the body but do not form spores, for which they need to regain the outer air, whilst the tubercle-bacilli pass through the whole cycle of their existence in the body, and in no way need an open-air life to enable them to assume the "resting" form necessary for the preservation of the race.

Another question is whether the ordinary widely distributed bacteria which often enter the body may, as the result of adaptation and cultivation, develop into tubercle-bacilli, and whether tubercle-bacilli on their part can change back again into harmless bacteria, either in the body itself or after they have left it. An invasion by specific bacteria would not then be necessary for the development of tuberculosis, but everything would depend on the existence of the conditions necessary for the change of harmless into noxious bacteria, and such conditions would constitute what is at present commonly called liability to the disease. This idea of a development of tubercle-bacilli from other bacteria is quite in accord with the highly exaggerated views now widely prevalent as to the mutability of bacteria, and has already found some supporters. Its only worth as yet is that of a pure hypothesis; for no facts support it, while many bear strongly against

it. No certain example of the transformation of harmless into virulent bacteria is known; there is therefore no reason for specifically ascribing to tubercle-bacilli the power of developing from indifferent organisms; and it is the more unreasonable to do so, as among the countless experiments on animals with pathogenic and non-pathogenic bacteria not one single instance has occurred—in spite of the highly favourable soil afforded for tubercle-bacilli by the bodies of rabbits and guinea-pigs—where these organisms have developed out of other bacteria. On the contrary, all experiments in which the necessary precautions were taken show that tuberculosis arises only when true, *i.e.*, already developed, tubercle-bacilli gain an entrance into the animal body.

It is otherwise as regards a possible attenuation of the tubercle-bacilli, since the attenuation of the anthrax bacilli can be cited as an example. Although the possibility of such a modification of virulence is not to be disputed, yet it must be remembered that the attenuation of anthrax bacilli is accomplished under conditions which can be furnished only artificially, and which do not come into play under ordinary circumstances either in the animal body or outside it. Another thing against this view is that tubercle-bacilli, after cultivation for almost two years outside the animal body and on a lifeless nourishing soil, did not show the slightest change in their properties particularly their virulence. In Fischer and Schill's experiments too, no modification of virulence was observed after tubercle-bacilli had been exposed for six weeks to the influence of putrefaction. All this speaks decisively against the supposition of an easily induced change in the virulent properties of tubercle-bacilli. We can, indeed, hardly think otherwise than that tubercle-bacilli originated from other bacteria at some time or other. But once having become true parasites, they appear to have this peculiarity in common with others of their kind, that they retain their properties with great persistency.

The only source of origin remaining is, therefore, the animal or human organism; and remembering the extremely wide distribution of tuberculosis it is evident that this parasite does not lack opportunities of multiplying enormously in suitable individuals, of forming resting spores of reaching the outer air and of attacking other victims. Among the various forms of tuber-

culosis there are, certainly, only a few which allow of an easy transmission of the bacilli. But they are just the forms that occur most commonly, viz., phthisis and tubercular disease of domestic animals. The other kinds of tuberculosis take hardly any part in infection, as the tubercle-bacilli are present in some cases in such small numbers, in others so deeply hidden that they could only in exceptional cases give rise to infection.

If we now inquire how far phthisis may occasion the transference of tubercle-bacilli from diseased to healthy subjects, it is very evident that all the conditions for the distribution of the infective material in very large quantities are here present. It is necessary only to remember that on an average one-seventh of mankind die of phthisis, and that most phthisical patients eject for at least some weeks, often for whole months, large quantities of sputa containing immense numbers of spore-bearing tubercle-bacilli. Most of these countless infective germs, which are scattered everywhere, on the floor, on articles of clothing, &c., perish, without finding an opportunity of settling again in a living host. But if we further bear in mind the results of Fischer and Schill's experiments, from which it is seen that tubercle-bacilli may retain their virulence for 43 days in putrefying sputum, and for 186 days in sputum dried at the ordinary temperature of the air, *i.e.*, if we remember the immense number of tubercle-bacilli derived from phthisical patients, and, as we have just seen, their tenacity of life both in a moist and in a dry condition, a sufficient explanation is afforded of the very wide distribution of the tubercular virus.

There can likewise be no doubt as to the manner in which the tubercular virus is carried from phthisical to healthy subjects. By the force of the patient's cough particles of tenacious sputum are dislodged, discharged into the air, and so scattered to some extent. Now numerous experiments have shown that the inhalation of scattered particles of phthisical sputum causes tuberculosis with absolute certainty, not only in animals easily susceptible to the disease, but in those also which have much more power of resisting it. It is not to be supposed that Man would be an exception to this rule, but, on the contrary, we may surmise that any healthy person brought into immediate contact with a phthisical patient, and inhaling the fragments of fresh sputum discharged into the air, may be

thereby infected. But probably infection will not often take place in this way, because the particles of sputum are not small enough to remain suspended in the air for any length of time. Dried sputum, on the contrary, is much more likely to cause infection, as, owing to the negligence with which the expectoration of phthisical patients is treated, it must evidently enter the atmosphere in considerable quantity. The sputum is not only ejected directly on to the floor, there to dry up, to be pulverized and to rise again in the form of dust, but a good deal of it dries on bed-linen, articles of clothing, and especially pocket handkerchiefs—which even the cleanliest of patients cannot help soiling with the dangerous infective material when wiping the mouth after expectoration—and also is subsequently scattered as dust. Examination of the air for bacteria capable of development has shown that they are not suspended separately in the air, but that they dry on the surface of objects, and do not enter the air until the dried mass breaks up, or unless the object on which the dried fluid rests is itself so light as to be carried away by the slightest breath of air. Such readily distributed carriers are particles of dust, consisting of bits of vegetable fibre, animal hair, epidermis scales and such like. Hence we have to fear chiefly the soiling with phthisical sputum of materials consisting of vegetable products or animal hair, such as bed-linen, coverlets, clothes, handkerchiefs. Sputum that has dried in spittoons or on the floor gets detached only in larger pieces which do not readily float in the air. On the other hand, one can hardly imagine a more favourable contrivance for the distribution of the sputum as dust than that of allowing it to dry rapidly on stuff garments, from which at each movement fibres fly off and carry the infective material into the air, where they remain suspended for some time; and when at last they fall to the ground the particles are easily caught up again by the slightest breath of air. The examinations of air undertaken by Hesse are very instructive on this point, and confirm fully what I have just stated.

As already mentioned, dried sputum may retain its virulence for months, perhaps for much longer under some circumstances. The time probably depends on whether or not the tubercle-bacilli contain well-developed spores, capable of germinating. But supposing the virulence of the dried sputum to last only for

some weeks, a phthisical patient is bound, under the circumstances in which these patients are at present generally found, to scatter around him a large quantity of infective material, and that in the form most likely to give rise to infection.

When tubercle-bacilli are inhaled in the form of dust they may, like other inhaled particles, remain in the upper part of the air-passages or penetrate into the alveoli. The depth they reach in the respiratory tract will depend essentially on the style of breathing. They penetrate farthest when deep breathing is going on with the mouth open. Breathing through the nose on the contrary will furnish some protection against the entrance of carriers of infective material, as a good deal of dust is kept back from the air entering the lungs by the nasal mucous membrane. Whether the tubercle-bacilli, having once entered the bronchi and alveoli, succeed in gaining a footing and entering the tissues will depend on various circumstances. The slow growth of the tubercle-bacilli has a decided influence in the matter. Other pathogenic bacteria, *e.g.*, the anthrax bacilli, as we learn from Wool-sorters' disease, and especially from that form of the affection known as laryngeal anthrax, appear, as a result of their rapid multiplication, very soon to cover a large area, and thus so speedily to exercise a directly injurious influence on the cells in their neighbourhood that the ciliated epithelium of the respiratory mucous membrane is no longer able to overcome and expel them ; so they can settle even in the upper part of the respiratory tract and give rise to the pathological processes peculiar to themselves. The conditions are quite different with tubercle-bacilli. They need just as many days as the anthrax bacilli do hours to attain any noticeable development, and under ordinary circumstances are banished from the respiratory passages by the movement of the cilia long before this is accomplished. Hence they need specially favourable conditions to enable them to establish themselves, and these may no doubt be furnished by many circumstances. But the most common and important for facilitating infection are found in diseases, such as measles, in which the respiratory tract is for a time denuded of its protecting epithelium, or which produce a secretion difficult to dislodge, and suitable as a nidus for the tubercle-bacilli. It has also been pointed out, and with reason, that adhesions of the lungs and deformities of the thorax, hindering full expansion of the lungs,

are particularly liable to cause circumscribed collections of bronchial secretion, and thus favour the development of tuberculosis, *i.e.*, the establishment of the tubercle-bacilli.

If there is a clear idea as to the necessity for such auxiliary forces in order to enable the bacilli to make their way, it will no longer seem so remarkable that many people, in spite of free intercourse with phthisical patients, do not become infected, that others are evidently attacked on the first opportunity, and that others again, after being exposed to infection for a long time without evil result, succumb to it in the end. In the first case circumstances did not come to the aid of the tubercle-bacilli, which were undoubtedly inhaled often enough, and hence they were expelled from the respiratory tract; in the second there was, to begin with, some weak spot in the respiratory organs where the bacilli were able to settle, and the only thing required was that the infective germs should reach this spot; in the last case no defect appeared until much later, but then immunity against tuberculosis was lost to some extent. The obstacles opposed to the establishment of the tubercle-bacilli are still greater in the upper part of the air-passages, and this explains the rare occurrence of primary disease of these parts.

Since by far the largest number of cases of tubercular disease have their origin in the lung, it may be assumed that infection has taken place in all after the manner just mentioned, viz., by inhalation of dried and scattered phthisical sputum. On account of the copious supply of infective material, and of the frequent contact into which it must come with other parts of the human body, it is not improbable that infection is accomplished at other places beside the lungs. I am inclined to think that primary disease of superficial lymphatic glands is caused by the entry of tubercle-bacilli into scratches and eruptions on the skin, whence the bacilli are conveyed by the lymphatics to the lymphatic glands. Then when the original place of infection has healed, it looks as if the pathological process had arisen primarily in the glands. I could explain a number of cases where extirpation of cheesy cervical lymphatic glands containing tubercle-bacilli had been performed in otherwise healthy adults, only by supposing that they were due to infection through abrasions of the scalp. As the alvine dejecta of phthisical patients not infrequently contain a good number of bacilli there is the same danger of

infection from them as from sputum if drying and pulverization take place. But the opportunity for this is not often present. At the same time the possibility of spreading infective material by this means must not be forgotten.

The second chief source of the tubercular virus, tuberculosis of domestic animals, seems to be by no means of such great importance as phthisical sputum.

So far as is known, animals produce no sputum, hence no tubercle-bacilli are liberated by them during life from the respiratory tract. In the evacuations too of tubercular animals, tubercle-bacilli seem to be only occasionally present. On the other hand it is certain that the milk of tubercular animals may give rise to infection. With the exception of this one channel, therefore, the tubercular virus does not come into action till after the death of the animal, and can cause infection only when the flesh is eaten. Accordingly if we except the probably very rare occurrence of direct infection of small wounds and excoriations of the skin of healthy people from tubercular parts with which they may come into contact, absorption of the infective material from animals takes place exclusively by means of the digestive organs, which should accordingly manifest the first symptoms of the disease. But primary intestinal tuberculosis is not at all common; in comparison with pulmonary tuberculosis it is quite a rare affection. Hence we must conclude that the aforementioned infection from eating the flesh of tubercular animals does not often occur. It would probably be more common if the visibly diseased portions of flesh were not, as is usual, rejected; or if, when eaten, they were not previously cooked in almost every case. Another point of importance to remember is that tuberculosis in animals used for food, especially *perlsucht* in cattle, remains more or less localized, so that there would be no danger except in eating the tubercular lungs, glands, &c. Meanwhile the possibility of infection of the intestinal canal is shown by the frequent occurrence of secondary intestinal tuberculosis in phthisical patients, where it must be referred to the swallowing of their own sputum. It is indeed remarkable that although every phthisical patient must be supposed to swallow more or less of the secretion, loaded with bacilli, from his lungs, intestinal ulceration does not always result. I explain this as follows: in the first place the intestine seems to offer in itself a still less

favourable point of attack for the slowly growing tubercle-bacilli than do the lungs. Then experiments on the effects of feeding with anthrax bacilli and their spores have shown that anthrax bacilli containing no spores are destroyed in the stomach, whilst the spores of these bacilli are able to pass the stomach uninjured. Consequently only substances containing spores can cause infection by means of the alimentary canal. In this respect the tubercle-bacilli most probably resemble the antharx-bacilli, and do not give rise to intestinal tuberculosis unless they contain spores, and then only on condition that they do not pass through the alimentary canal quickly but that they are able to germinate and invade some part of the intestinal mucous membrane. The same holds good of course with reference to the danger of infection by tubercular meat, and may explain the relative infrequence with which infection results from eating meat of this kind.

The same conditions hold with regard to infection by the milk of cows suffering from *perlsucht*. First of all, for infection to take place, the milk must contain tubercle-bacilli. This appears to happen only when the mammary glands themselves are affected with tubercular disease. But as pearl-nodules do not commonly occur in the udder, the milk of cows with *perlsucht* will very often have no infective properties. This explains the contradictory statements of various authors who have instituted feeding experiments with the milk of cows suffering from *perlsucht*. Some affirm that they have obtained positive results, and their testimony is of such a nature as to leave no room to doubt the correctness of their observations. Others, on the contrary, could not get any infection of the animals experimented on. These results, too, were no doubt correct. The positive effects were obtained with milk which happened to contain tubercle-bacilli, the negative with milk in which there were none.

However, although infection from domestic animals does not on the whole appear to be very common, it is by no means to be underrated. Bovine tuberculosis and caseous changes in the lymphatic glands of swine are so common an occurrence that they deserve full consideration.

If we note the behaviour in the body of the tubercle-bacilli, which have entered the lungs by inhalation, the skin through wounds, the intestinal canal by being swallowed, we see that they often

remain for some time, perhaps permanently, in the place where they first settled. They give rise to nodules, which consist of clusters of epithelioid cells, and contain giant-cells, and which finally undergo coagulation-necrosis from the centres outwards. The appearances resulting from the gradual growth of such a focus, and the constant, regularly advancing retrogressive changes that take place there, have been discussed in detail under one of the foregoing divisions. The first sign of the extension of the tubercular process to surrounding parts is a new formation of similar nodules in the neighbourhood of the primary focus. I have already pointed out, also, the way in which we may imagine the bacilli to migrate, in connection with this part of the process, from the primary focus towards the place where the secondary nodules arise. The most simple explanation of this occurrence appears to me to be as follows: the tubercle-bacilli, since they possess no power of locomotion of their own, can only be carried away by other elements endowed with such a power, or by currents in fluids. But as tubercular nodules are non-vascular, and it is not easy to see how other fluids in motion can reach the focus and bear away bacilli from its interior, there is nothing remaining but to accept the wandering cells, which are known to perform the office of carriers for other pathogenic bacteria, as the elements which assist in the dispersion of the bacilli. The cell laden with a bacillus travels on only until the influence of the parasite causes it to lose its power of locomotion. In the place where the cell comes to a standstill another fresh tubercular nodule must develop. In this way groups of tubercles are formed, which break down and give rise to the destruction with which we are familiar.

The view that wandering cells may be carriers of the bacilli, explains most naturally the longer excursions which the tubercle-bacilli make in the body in most cases. When the wandering cell is moving in the lymph-spaces of the tissue, and is dependent on its own powers of locomotion, it can travel but a short distance, and the new focus of infection is necessarily close to the point whence the cell started. But if the wandering cells travel by way of the lymphatic vessels, and are helped on by the lymph-stream, they go much further, as can be often seen in tubercles spreading along the course of the lymphatics. Then indeed tubercle-bacilli are often carried as far as the nearest

lymphatic glands, where they cause the formation of nodules and caseation, as at the primary seat of infection. The changes thus produced in the glandular tissue seem generally to prevent any further penetration of the bacilli by way of the lymphatics. But that does not furnish an insurmountable barrier to the further progress of the bacilli. For instance, under special circumstances they may enter the blood-stream. This happens, as Ponfick has shown, when tuberculosis attacks the thoracic duct and reaches its interior; then the tubercle-bacilli pass from the lymph-stream direct into the circulation. A second, and certainly the most common way in which the tubercle-bacilli enter the blood was discovered by Weigert, viz., by the formation of tubercles in the walls of veins and the bursting of the disintegrating nodules into the lumen of the vessel. A third possible way is mentioned in the case described on p. 105, where the bacilli grew into the lumen of an artery. In all these cases the bacilli are quickly carried away by the blood-stream, distributed to the most different organs of the body, and deposited there. When a great many bacilli enter the blood at one time, the conditions are exactly the same as in the experiment in which a rabbit had a considerable number of tubercle-bacilli injected into an auricular vein. In both the artificial and natural experiments tubercular nodules occur in large numbers, especially in the lungs, spleen and liver. Why these are the organs specially chosen we do not yet know. The discoveries of Ponfick and Weigert give incontestable proof of the connection between localized tubercular processes and acute miliary tuberculosis, which used to seem so mysterious, and was consequently pronounced by many to be impossible. This example of the multiformity of one disease is a strong warning to us not, in the absence of weighty reasons, to base our conceptions of pathological processes, especially of infective diseases, merely upon anatomical grounds, but to regard their etiological relations as of primary importance.

The tubercle-bacilli do not always enter the blood-stream in large quantities at once. It may happen that only relatively few bacilli are borne away by the blood-stream. Then correspondingly fewer tubercular foci develop, which, however, as the patient lives longer in this case, attain greater dimensions than when death is speedily induced by a copious eruption of tubercular nodules. Here, too, the natural mode of infection is just like

the artificial. Occasionally only a few bacilli enter the blood, and solitary tubercles then appear, which, however, in course of time grow to a considerable size. This process, which may be repeated by starts, has been well named by Weigert chronic miliary tuberculosis, in contradistinction to the acute form, which soon leads to a fatal termination, owing to the tubercles developing in large numbers to begin with.

Closely related to the forms of miliary tuberculosis just mentioned, are those morbid processes in which a local tuberculosis develops in parts of the body apparently inaccessible to a direct invasion of bacilli from without, and yet there is no evidence of the existence of a primary focus through which the infection has been brought about. Such processes, among which fungous and carious affections must be placed, originate in a strictly localized manner. Their existence can hardly be explained otherwise than by supposing that a single infective germ, a single bacillus, was deposited at the affected spot by the blood-stream. But how should a single bacillus get into the blood? Could it, on being inhaled into the lung, pass direct into the lung-capillaries without first having given rise to the formation of a tubercular focus in the lung itself? This view seems to me very improbable. The almost invariable presence of caseous or calcified bronchial glands in the diseased states just mentioned, suggests rather that the lymphatic glands are not always an insurmountable barrier to the further progress of the bacilli, and that just as single bacilli are carried into the lymphatic glands by means of wandering cells and the lymph-stream, so in the same way, by the help of the wandering cells, they may again leave the lymphatic glands and, taking a centripetal direction, reach the blood by way of the lymph-stream. I do not doubt that as in almost every case of miliary tuberculosis the starting point of the infection can be made out, so also in all necropsies on cases of localized tuberculosis of internal organs, as well as of bones and joints, we shall succeed in finding some old tubercular focus, perhaps most often caseous bronchial glands, whence single bacilli might have escaped into the blood.*

* The following case is of great importance in connection with the etiology of local tuberculosis. It is narrated by Dr. E. A. Tscherning in the *Fortschritte des Medicin*, vol. iii. No. 3, 1885:—

"Maria P. Age 24 years. Cook in the house of the late Prof. H., is of a completely healthy and strong constitution. She has never been affected with any

Very probably the tubercular form of basilar meningitis in children belongs to this group, for in these cases the lungs,

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scrofulous or tubercular disease. There is not the slightest trace of hereditary predisposition to tuberculosis in her family.

"Prof. H. died at the end of July (1884) from acute phthisis, which had lasted 5 or 6 months. His sputum towards the end of his life was almost a pure cultivation of tubercle-bacilli in pus. A few days before the Professor's death the patient wounded the palmar side of the first phalanx of her middle finger with a fragment of a broken vessel containing sputum. I first saw her 14 days after the accident, when there were signs of commencing whitlow. For 8 days carbolic fomentations were employed with subsidence of the symptoms without suppuration; but a small nodule about the size of half a pea could be felt in the subcutaneous tissue. During the next few weeks the nodule remained somewhat painful and the tissue around was œdematous. At the end of August I made an incision and removed by means of a sharp spoon a small granulation-tumour scarcely as large as a pea, which lay between the skin and the sheath of the tendon. Healing took place in 8 days by first intention under a dressing of iodoform and corrosive sublimate. For the time improvement occurred; but when I saw the patient at the beginning of October she complained of pain on bending the finger. The skin and subcutaneous tissue were slightly swollen and also the palm of the hand close to the phalanx. I could not make out any circumscribed swelling of the sheath of the tendon. By the advice of Prof. Studsgaard she used local vapour baths for some weeks and presented herself again in the middle of November. We could then feel, through the somewhat œdematous skin, a very distinct swelling of the sheath of the flexor tendon. The movements of the finger were interfered with and there was considerable pain and tenderness. There were also two swollen glands above the elbow and two in the axilla. In other respects the patient was well; there was no trace of lung disease.

"On November 21st Prof. Studsgaard removed the swollen axillary and cubital glands, and amputated the middle finger at the metacarpo-phalangeal articulation, splitting up the palm and removing the tendon with its sheath as far as the middle of the palm. The subcutaneous granulation tissue was widely removed and scraped out. The operation was performed with the usual antiseptic precautions (corrosive sublimate 1 per 1,000) and dressed with a sublimate wool and gauze dressing. The wounds healed by first intention in 11 days. The patient was well when discharged.

"The following pathological changes were found. The sheath of the tendon was filled with granulation tissue, its wall thickened. In the serous covering of the tendon were petechial hæmorrhages; but there was no pus nor caseous masses. There was no affection of the joints or bones. The granulation tissue showed under the microscope (after hardening in alcohol and staining with picro-carmine) very numerous young tubercles with, in many cases, central caseous degeneration, frequently large cells and very beautiful giant cells often in the centre of the nodules. The extirpated glands presented to the naked eye the appearance of simple hyperplasia without pus or caseous deposits. Under the microscope I found large numbers of large cells with here and there small tubercles. Both in the granulation tissue and in the lymphatic glands I found in all the sections, stained by Ehrlich's method, well marked tubercle-bacilli partly in the large cells, more especially in the giant cells, and partly at the margin of the necrotic places. The bacilli as a rule lay singly, but here and there there were 2 or 3 together, especially in V-shaped groups. Many of them contained the so-called spores.

"I saw the patient again at the end of January, 1885. She was quite well and without chest symptoms. No fresh lymphatic enlargements; linear scars with little tenderness; no sign of spread of the disease either locally or generally.

"The microscopic appearances described here correspond entirely to what I have

spleen and liver are very often free from tubercle, whilst the bronchial glands are almost always caseous; from which it may be concluded that they are to be regarded here also as the primary focus of the disease. But it still remains most remarkable that in this form of tuberculosis, in which obviously not single, but numerous bacilli are deposited from the blood, the pia mater should be so singled out.

Although we have already shown, in the earlier sections, that the different forms of tuberculosis must be regarded as varieties of one disease, on the grounds that all contain the same bacilli, yield similar cultures, and when inoculated produce identical results, still this view derives further support from an increasing knowledge of their mode of origin. The various forms of consumption of the lungs which at first sight appear so different, acute and chronic miliary tuberculosis, the affections of glands and mucous membranes which rank as scrofulous tuberculosis, of bones and joints, localized tuberculosis of single organs, as the kidneys or intestine, are grouped all together quite naturally, when they are regarded from the point of view of their origin. Lupus alone offers some difficulty as to its identification with tuberculosis, because clinical observation shows a difference not to be ignored between lupous and undoubtedly tubercular affections of the skin and mucous membranes. Nevertheless the etiological grounds in favour of the unity of these two diseases are so important that they must be allowed to outweigh this difference, which may possibly be explained by individual peculiarities.

The relation between human and animal tuberculosis, particularly *perlsucht*, is a similar one. Here, too, in spite of differences in the anatomical conditions and clinical course, they must be regarded as identical diseases on account of the identity of the parasites to which they are due. Much has indeed been made of the absence of complete proof that *perlsucht* is transmissible to Man. But, on the other hand, the following must be remembered. Owing to the extremely slow development of the disease, by the time the first symptoms come to

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formerly found in about 30 cases of surgical tuberculoses (joints, sheaths of tendons, spondylitis, pyogenic membranes, lymphatic glands, testicles, tongue and tuberculous kidneys) which I investigated from this point of view, formerly as Prosector and lately as assistant physician."—ED.

light the place and time, and therefore the source of infection, can generally be no longer determined or not in a satisfactory manner. Common as tuberculosis due to inhalation is, the mode of infection can be fixed with scientific accuracy in only relatively few cases. Much less will this be possible in the considerably rarer cases of intestinal tuberculosis resulting from the consumption of the flesh or milk of cows with perlsucht, because here the uncertainty will be still more increased by the ease with which other and commoner modes of infection may be mistaken for the true one. It is therefore much to be doubted whether a single case of tuberculosis in the human subject will ever be referred unquestionably to the partaking of the flesh or milk of tubercular animals. But when we consider that by inoculation of the most different kinds of animals (cats, rabbits, guinea-pigs, field-mice) with pearl-nodules and the pure cultures proceeding from them, a disease is produced with the greatest regularity, which exactly resembles in its anatomical features the disease occurring in these animals as the result of inoculation with tubercular material, and which just as certainly leads to the death of the animals, we cannot imagine that man is an exception with regard to this pathological poison. So that if it should yet be proved in the course of further researches that there is any other difference between the bacilli of tubercle and *perlsucht*, which would compel us to regard them as nearly related yet belonging to different species, we should then nevertheless have every cause to consider the *perlsucht*-bacilli as in the highest degree suspicious. From a hygienic point of view the same precautions must be adopted against them as against infection by tubercle-bacilli until it is proved that in the human being *perlsucht*-bacilli may with safety be brought into contact with wounds of the skin, and that they or their spores can be inhaled or swallowed without giving rise to tuberculosis.

Against the view of the unity of all the forms of disease caused by tubercle-bacilli is the material difference in the course taken by the disease in different individuals of the same race, and in their susceptibility to tubercular infection. These are, however, phenomena which reappear in a more or less marked manner with all infective diseases. It is usual to get out of the difficulty by supposing that there is a difference in the predisposition to the disease, both as regards taking it in the first place, and the

greater or less severity of the course it runs, but this specifies the fact rather than explains it. Some of the dissimilarities in the pictures presented by cases of tuberculosis may be ascribed to difference in the seat of infection. Then, too, the quantity of infective material coming into operation in the first place seems to be of essential importance. Single infective germs are, on account of their slow development, more easily and for a longer period kept within bounds and localized by the organism, than are many such germs poured into the system at once, when they support each other in their work of destruction. We may, further, form a clear conception of what is meant by individual predisposition in all cases in which, according to our previously enunciated hypothesis, certain auxiliary moments—such as defects in the epithelial lining of the respiratory tract, stagnating secretion, disturbances of respiration, &c.—favour the settling of the tubercle-bacilli.

But although a good many of the phenomena classed together under the head of "predisposition" may be referred to simple and easily explained conditions, some facts remain difficult or impossible to interpret, compelling us for the present to accept the view of a varying liability. Most important of all is the striking difference between the course of tuberculosis in children and in adults, and again the undeniable predisposition to tuberculosis that exists in some families. In the latter instance, many of the cases of illness where predisposition is supposed to be an important factor might rather be ascribed to increased opportunities of infection, and there are also peculiar predisposing influences connected with the family constitution to be thought of, such as a tendency to catarrhal affections of the respiratory organs, and imperfect development of the thorax; still, many carefully observed cases remain which cannot be explained in this manner. Further, individual cases of the disease have often shown that a person is not at all times an equally favourable subject for the development of the parasites, for it not infrequently happens that tubercular foci which had reached a fair size, contract, cicatrize and heal up. That means, however, that the same body which afforded a suitable soil for the tubercle-bacilli on their first invasion, so that they were able to multiply and spread, has by degrees lost the qualities favourable to them, and has changed into a bad soil, thus preventing further growth

of the bacilli. So that in the same person there was at one time a liability to tuberculosis, and again at another time none. Further investigation is required to show what occasions this difference, whether it is due to a change in the chemical composition of the juices of the tissues or to physical conditions. So much is certain—that these differences exist; and there is nothing against the view that conditions similarly favourable or unfavourable to the tubercle-bacilli are present in some human beings not only temporarily, but for the whole lifetime.

As to the vexed question of hereditary tuberculosis, after what has just been said I may express my opinion very briefly. There are no facts to prove the view that tubercle-bacilli may be present in the immature organism, either in the intra-uterine or extra-uterine state, without leading in a relatively short time to visible changes. Now tuberculosis is very rarely found in the fœtus and the newly-born, hence we must conclude that the infective material comes into operation only exceptionally during intra-uterine life. This view corresponds with the fact that those of the animals experimented on, particularly guinea-pigs, which were either pregnant before, or became so after, inoculation, in no case gave birth to young which already showed signs of tuberculosis. The young of highly tubercular mothers were free from tuberculosis, and remained healthy for months. In my opinion hereditary tuberculosis is explained most naturally by supposing that the infective germ itself is not inherited, but rather certain peculiarities favourable to the development of germs which may later on come into contact with the body—in fact, it is the predisposition to tuberculosis which is inherited.

The etiology of tuberculosis as here developed on the basis of our knowledge of the tubercle-bacillus offers in detail hardly anything new. Cohnheim had already recognized tuberculosis as an infective disease before the tubercle-bacillus was discovered, and had accordingly sketched out its etiology. Science has, therefore, made no material advance in this direction through my researches; but it must be regarded as a gain that on the highly important point of the infectious nature of tuberculosis, which up to that time was still disputed by most people, proof should have been brought forward which shuts out any further valid objections. Not less important is it that the tubercle-bacilli furnish a criterion as to what shall in future be considered to belong to the

domain of tuberculosis. In doubtful cases the diagnosis of tuberculosis will be made to depend on the demonstration of tubercle-bacilli. In practice this aid has already been extensively made use of, and that with perfect success, thus giving a valuable proof of the correctness of my opinion as to the significance of the tubercle-bacilli. In this way a material advantage has arisen from the discovery of the tubercle-bacillus. It is to be hoped, however, that it will be beneficial in yet another direction—namely, that of combating the disease. According to the experiments as yet made there does not appear any great prospect that we shall succeed in finding a therapeutic means of dealing with the parasites in the body. So much the more stress would I therefore lay on prophylactic measures, which should be directed partly towards the direct destruction of the tubercle-bacilli by suitable methods of disinfection, partly to the preservation of healthy persons from contact with tubercle-bacilli in all cases in which the parasites cannot be certainly destroyed.

It seems to me the time has now come to adopt prophylactic measures against tuberculosis. But owing to the wide distribution of the disease, all steps taken against it have to deal with social relations, and it must therefore be carefully considered in what way and how far we may proceed without neutralizing by unavoidable disturbances and other disadvantages the benefit obtained.

It would lead me too far to discuss the means of prophylaxis in detail, and I reserve the expression of my views on this subject for another opportunity.

ON THE

ETIOLOGY OF ENTERIC FEVER.

By Dr. GAFFKY.

(*Mittheilungen aus dem Gesundheitsamte*, Vol. ii., Berlin, 1884.)

TRANSLATED BY

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ON THE ETIOLOGY OF ENTERIC FEVER.

PREVIOUS PUBLICATIONS CONCERNING THE PRESENCE OF MICRO-ORGANISMS IN THE ORGANS OF FATAL CASES OF ENTERIC FEVER.

ALTHOUGH a considerable number of communications relating to the presence of micro-organisms in the organs of fatal cases of enteric fever has been published, there exists, nevertheless, among the various investigators no complete unanimity of opinion regarding the question as to which form of organism should be designated as characteristic of the disease, and as its probable cause.

As early as the year 1871 von Recklinghausen* observed colonies of micrococci in renal abscesses of typhoid cadavers which, indeed, he did not look upon as the cause of the disease, but still "as an indication where the localization of the problematical spore was especially to be sought."

This observation was corroborated in the following year by Eberth.† Eberth was also not of the opinion that the deposits of bacteria found in the internal organs stood in causal relation to the typhoid process. He found them exclusively in the stage of ulceration and cicatrization of the intestinal lesions, and thought, therefore, that they ought to be regarded as a secondary phenomenon, and indeed as diphtheritic aggregations. In this view he was strengthened by the circumstance that in their external characters the organisms could not be distinguished from those occurring in faucial diphtheria.

In 1875 Klein ‡ found in the intestinal mucous membrane of

* *Verhandlungen der physicalisch-medicin. Gesellsch. in Würtzburg.* Sitzung von 10 Juni, 1871.

† *Zur Kenntniss der bacteritischen mykosen.* Leipzig, 1872.

‡ Report on the intimate Anatomical Changes in Enteric or Typhoid Fever. Reports of the Medical Officer of the Privy Council and Local Government Board. London, 1875.

fatal cases of typhoid fever a fungus with distinct formation of mycelium and numerous makro- and micro-gonidia. According to Klein's views at that time, "zoogloea masses" (*i.e.*, accumulations of minute spherical bodies, held together by a gelatinous substance) were formed by the rapid subdivision of the gonidia. Klein* was inclined to consider this fungus as allied to the Crenothryx polyspora, described by Cohn. Afterwards Klein modified his view in so far as regards the *vegetable* nature of the larger bodies found by him, but maintained his previous statements as to the presence of aggregations of micrococci in the intestinal mucous membrane and lymph follicles. In his further researches he was also able to establish the presence of colonies of bacteria in the spleen. Klein did not think that a decision in regard to the meaning of the organisms could be arrived at, on the ground of an anatomical investigation; he was, however, inclined to consider them as specific.

According to a statement in Birch-Hirschfeld's *Handbook of Pathological Anatomy*, Browicz in the year 1875 found immobile, rod-shaped bacteria in the myocardium, kidneys, spleen, and intestinal canal of fatal cases of typhoid fever. I have unfortunately had no access to the original work in the report of the transactions of the Academy of Science of Cracow. Socoloff† in 1876, in connection with his investigations into the pathology of acute splenic tumour, found collections of micrococci in the spleen in three cases of typhoid fever, but in nine others he did not detect them. He expresses himself with great caution in regard to the significance of the organisms, but considers it probable that they stand in etiological relation to the typhoid process.

Further, Fischel‡ examined the spleen in 29 fatal cases of typhoid, and found accumulations of micrococci in 15 of the cases, but no organisms in the remaining 14 cases. The micrococci appearing as granules, some round, some ovoid, stained easily with hæmatoxylin, and never lay inside the vessels. Fischel leaves undecided the question whether they are to be regarded as the cause of the disease or as a secondary phenomenon.

Eberth's article, which appeared in the year 1880, denotes a

* Comp. Eberth, Virchow's *Archiv*, vol. 81, 1880.
† Virchow's *Archiv*, vol. 66, 1876.
‡ *Prager Med. Wochenschrift*, 1878, pages 33 and 45.

very real advance upon these earlier works, briefly referred to. This advance consists chiefly in the discovery by Eberth of a rod-shaped organism definitely characterized by its form, its arrangement in the diseased organs, and its conduct in relation to staining solutions, the confounding of which with putrefactive bacilli, or with the micrococci occurring in pyæmia and diphtheria, seemed excluded.

Eberth examined by preference the spleen and swollen lymphatic glands, then the typically altered portions of intestine and in some cases also, but with a negative result, the liver, kidneys and lungs. His communications upon the presence of bacilli are almost exclusively confined to the first-named organs—viz., spleen and lymphatic glands. The examination of the tissues was made on sections rendered translucent by concentrated acetic acid. In 23 cases Eberth found organisms 12 times—viz., 12 times in the lymphatic glands alone, and 6 times in the spleen as well. In one case the spleen was not examined. The lymphatic glands always contained a greater number of organisms than the spleen, which, on the whole, contained only sparse and small colonies. As Eberth states, one might be tempted to consider the organisms as micrococci in those places where they lie together in thick masses, but one easily recognizes that one is dealing with bacilli alone when they are arranged in a less clumpy manner, as in the outlying portions of the colonies. Eberth never saw indubitable spherococci in these aggregations. The bacilli, about the size of those occurring in decomposing blood, were short and often closely resembled shrunken ovoids; they had slightly rounded ends, very delicate outline, and often showed in their interior very small, feebly refracting, spore-like granules. Eberth draws prominent attention to the specially characteristic point that they, in contradistinction to putrefactive bacilli, stain very faintly with methyl violet, bismarck brown, and hæmatoxylin.

The bacilli were generally arranged in radiating or retiform groups, which appeared as if undergoing disintegration at the periphery. Eberth lays particular stress upon the point that in recent cases of typhoid the organisms were found more frequently and in greater numbers than in the older cases. Eberth thinks that the objection that merely bacilli of putrefaction were being dealt with, may be further invalidated by the fact that in two

instances in which the bodies (*Kranken*) had lain rather a long time the organisms were not discovered in increased numbers. He is much more inclined to the opinion that the bacilli really stand in etiological relation to the typhoid process.

In a later publication which appeared in 1881 Eberth* communicates the result of the investigation of 17 more typhoid cases. In six of these he found the same characteristic heaps of bacilli as described in his first work—viz., in three cases in both lymphatic glands and spleen, in three cases in the lymphatic glands only.

On the whole, therefore, in 40 cases of enteric fever Eberth found the organisms described by him as typhoid bacilli eighteen times in the lymphatic glands and spleen; in 22 cases he got a negative result. He did not meet with aggregations of micrococci in a single pure typhoid case; only quite on the surface on the superficial ulcers and cicatrices did he find other organisms, which he regarded as putrefactive bacilli, along with micrococci. As regards the relation of the typhoid bacilli to the length of duration of the disease—the average duration in the positive cases of the first series of observations was seventeen days, of those of the second series twenty-six days, whilst that of the cases which gave negative results in the two series amounted to twenty-three days. In one case bacilli were still found after a period of illness of about forty-three days, whilst they were not observed in three cases after fourteen days', and in one case after sixteen, seventeen, and twenty-one days' illness respectively. In the more recent cases Eberth did not succeed in staining the typhoid bacilli in sections; on the other hand they stained easily with methyl violet when the juice of the lymphatic glands was expressed and dried in a thin layer on the slide. In order to show that really specific typhoid bacilli were being dealt with, Eberth attempted to offer the indirect proof by establishing the absence of these organisms in other diseases. He examined the spleen and lymphatic glands of 11 cases, some of them of infective processes, as well as of 13 cases of tuberculosis and phthisis, but never found organisms which presented even a distant resemblance to the typhoid bacilli. In some of the cases there was very extensive ulceration of the intestinal mucous membrane.

* Virchow's *Archiv*, vol. 83, 1881.

Some months before Eberth's first work Klebs* stated that he and his assistants had succeeded in establishing the presence of a hitherto undescribed form of micro-organism—viz., bacilli—without an exception in 24 typhoid cases examined. He thought himself justified in regarding these organisms as the cause of the disease. Klebs regards the rod-shaped bodies found in laryngeal typhoid ulcers in the year 1878 by his then assistant, Professor Eppinger,† as identical with the more recently described organisms always met with by him in typhoid cadavers in the intestine, frequently also in the mesenteric glands, and further in the lungs, kidneys, heart, larynx, and in the pia mater (*Hohlräumen der Pia Mater*). Klebs also endeavoured, by means of controlling investigations, to bring forward indirect proof of the specific nature of these bacilli. They were never found in non-typhoid intestines.

This preliminary communication was afterwards completed by Klebs‡ in an exhaustive work, in which he brought forward also his experimental researches in regard to the cultivation and successful inoculation of the "bacillus typhosus" in animals. For the present we shall not consider these experiments on cultivation and communicability, as it is of the first importance to determine the existence of the organisms in the diseased organs, as well as their shape and arrangement. According to Klebs, "the bacillus typhosus, at the acme of its development, forms long, undivided, and unbranched threads more than 50 μ. in length and not quite 0·2 μ. in breadth, provided no development of spores takes place. In the latter case their diameter may increase up to 0·5 μ. The spores lie in a row, closely one behind the other. Before the bacillus typhosus attains this degree of development it forms shorter rods, which even already may contain spores, and these usually at the extremities; the transition to threads is led up to by a stage of small rods, arranged in rows, and not containing spores, which probably result from the transverse division of the larger rods, which are increasing in length (cartilages of larynx, intestine). The thread-like variety is found both in the form of thick mycelium in the tissues (intestine and larynx), and in that of parallel bundles

* Virchow's *Archiv*, vol. 83, 1881.

† Klebs' *Handbuch der Pathol. Anat.*, 7 Lieferung, bearbeitet von Prof. Eppinger.

‡ *Archiv f. exper. Pathol. und Pharmak.*, 1881, Bd. 13, Heft 5 u. 6.

or spirals in the blood-vessels." According to Klebs "this bacillus is of constant occurrence in the intestinal infiltration of typhoid patients and is also found in those anatomical changes which are regarded as secondary typhoid lesions (mesenteric glands, larynx, lungs, pia mater, kidneys)." It is further to be observed that Klebs' bacilli stain easily with hæmatoxylin.

Klebs found invasion with micrococci only in occasional cases of typhoid fever, and, in accordance with Eberth, considers them as a secondary phenomenon, in other words as a complication. How are the bacilli described by Klebs related to those described by Eberth? This question, indeed, imposes itself upon every one who compares the description, but especially the drawings, given by the two investigators. Klebs holds the view that Eberth's short bacilli represent the first stage of development of oval, free spores, and that they, therefore, are only a step in the development of the typhoid bacilli described by him. On the other hand Eberth regards the short forms found by Klebs as identical with his own; as regards the longer threads, which he has never seen, he leaves it undecided whether one is dealing with two different forms of "schizomycetes," or with two developmental stages of one and the same organism. The settlement of the point as to whether the typhoid process is always engendered by one and the same parasite, or whether perhaps other organisms play a part, must be reserved for further investigations.

R. Koch* took up a different position with regard to this question on the basis of his own researches. Quite independently of Eberth, and before his first publication, Koch found in about half the cases of typhoid examined by him exactly the same typical colonies, consisting of short bacilli, as were afterwards described by that author. Koch lays particular stress upon the fact that the short bacilli were found in extensive tracts in the deeper, non-necrotic portions of the intestinal mucous membrane beneath the ulcerations, and that, further, the characteristic groups composed of them occurred everywhere diffused throughout the internal organs, whereas the long delicate threads described and delineated by Klebs could only be observed in the superficial necrotic portions of the intestinal mucous membrane, where the nuclei were no longer stained.

* *Mittheilungen aus dem Kaiserlich. Gesundheitsamte*, Bd. i. Berlin, 1881.

From his own investigations Koch considers the presumption as justified, that the short bacilli described by Eberth stand in real causal connection to enteric fever, whilst he is inclined to regard the long forms figured by Klebs as a secondary phenomenon. A definite conclusion as to the signification of these different bacilli cannot, however, be arrived at from the foregoing facts. As has already been mentioned, Eberth had specially insisted in his first publication on the property of the short bacilli to take on staining materials feebly, as a characteristic of them. Koch, on the contrary, succeeded in staining these organisms deeply, even in sections, as his photographs of bacillary masses from the spleen, kidneys and liver show, to obtain which the essential element is that the preparations be stained with bismarck brown.

In passing, it may be further observed that these photographs were prepared before the appearance of Eberth's first publication. Koch is in complete accord with Klebs, as with Eberth, respecting the signification of the groups of micrococci found in some cases of typhoid, regarding them as something secondary.

We owe a further valuable contribution to our knowledge of typhoid bacilli to W. Meyer,* a young physician, since unfortunately deceased at the outset of his career, and a sacrifice to his profession. Under the direction of C. Friedländer he examined, in a considerable number of cases, the intestinal lesions in regard to the presence of micro-organisms, and for preference directed his attention to the *most intact*, swollen, Peyerian patches and solitary follicles. His examinations were made partly on cover-glass preparations, stained with gentian violet, but more frequently on sections, rendered transparent by 3 per cent. potash solution or concentrated acetic acid. Meyer was not completely successful in staining the bacilli in sections. In sixteen out of twenty cases examined the same short bacilli were found as had been described by Eberth and Koch. On the other hand, Meyer could only observe the fungus-like, thin, long threads lying in bundles as described by Klebs, in isolated, necrotic sloughs. They seemed to him to be "the highest type of schizomycetes occurring in the sloughs." Along with these he frequently found also in the necrosed parts colonies of micro-

* *Untersuchungen über den Bacillus des Abdominaltyphus*, Inaug. Dissert., Berlin, 1881.

cocci and closely packed putrefactive bacilli, staining deeply with hæmatoxylin and gentian violet, whilst in the deeper layers of the gut which had not yet necrosed, the short bacillar forms of Eberth alone occurred. Accordingly Meyer considers himself justified in declaring the latter to be characteristic of typhoid. In six cases, examined in order to control the former—viz., three cases of scarlatina, one of measles, and two of dysentery—he never found bacilli in the intestinal mucous membrane which had any resemblance whatever to the typhoid bacilli.

It is also noteworthy in the work of Meyer that he, in accordance with Eberth, generally found the bacilli in greater numbers the more recent the affection.

Coats* and Crooke† have lately, in one typhoid case respectively, found in the mesenteric glands bacilli, which exactly resembled those described by Eberth and Koch. Apparently Coats also succeeded without difficulty in staining the bacillar masses in sections with bismarck brown.

Although the foregoing summary has no pretension to absolute completeness, still all the important works concerning the determination of pathogenic organisms in the organs of fatal cases of typhoid fever have been taken notice of.

In the following pages I shall communicate the result of my own personal investigations conducted in the laboratory of the Imperial Office of Public Health. For the material I have to thank Dr. Guttmann, Dr. C. Friedländer, Dr. Bode, Dr. Herger, and Dr. Albrecht, Professor at the Obuchow Hospital in St. Petersburg. It is a pleasant duty for me to express my thanks to these various gentlemen in this place.

MICROSCOPIC EXAMINATION OF THE ORGANS OF TWENTY-EIGHT FATAL CASES OF TYPHOID FEVER.

The above-mentioned later works had rendered it highly probable that the short bacilli occurring in the internal organs in characteristic groups, first found by Eberth and Koch, stand in causal relation to the typhoid process. Very striking, nevertheless, remained the fact that these organisms could only be demonstrated in about one-half of the typhoid cases examined.

* *British Medical Journal*, March 18th, 1882. † *Ibid.*, July 1st, 1882.

Two explanations of this circumstance might obviously be given: either in the cases examined with a negative result, the whole of the bacilli had already perished before the diseased process set up by them had run its course; or the demonstration of bacilli still present had failed, owing to the technical difficulties in the way of finding scattered groups of them. The fact that the bacilli in many cases could be observed after a very long duration of the disease whilst they were absent in other cases of short duration, unquestionably might be advanced against the exclusive applicability of the former explanation.

On the other hand the correctness of the second assumption was rendered probable by various circumstances. Indeed in other maladies, proved to be produced by bacteria, the number of parasites present in the body is not always in direct proportion to the severity of the illness. As an example of this, the greatest differences are found even in splenic fever and in artificially induced traumatic infection in animals which are infected in the same way and killed within the same time after the inoculation. It must be added that in typhoid the examination in itself presents special difficulties, because the bacilli do not occur uniformly scattered throughout the body but in small, disseminated colonies in the organs. One will, however, regard the cases yielding a negative result with a certain reserve if one considers what a minute fraction of tissue is examined when even a hundred or more sections have been made. My own observations, at all events, completely justify such a standpoint. As regards the selection of organs to be examined it seemed to me judicious not to lay too much stress on the detection of bacilli in the diseased portions of intestine. If one examines swollen plaques and follicles as intact as possible, as Meyer preferred to do, one will certainly not easily feel in doubt as to the signification of the discovery of bacilli. But the conditions are different in those cases in which sloughing and ulceration have already set in. Here it is extremely difficult to differentiate with certainty the typhoid bacilli which may be present from other rod-like forms which, coming from the contents of the intestine, have migrated into the necrosed tissue, but evidently stand in no relation to the diseased process. In the mesenteric glands, however, in the spleen, liver and kidneys, the typhoid bacilli—by which I shall henceforward mean the bacilli

of Eberth and Koch—are found not only in perfect purity but also arranged in such a characteristic manner that any confusion with other organisms appears to be excluded. (Plate V., figs. 15 and 16.)

Accordingly I have only accepted a result as positive when I have found at least one characteristic group of bacilli in one of the organs named. Obviously it would be highly desirable to extend the observation in every single case as far as possible to all the organs, but I have had neither time nor material for this investigation. The sections of the organs, hardened in alcohol, were treated in the following manner. First, they remained from 20 to 24 hours in a deep blue, opaque staining fluid, which was freshly prepared for each examination by pouring a saturated alcoholic solution of methylene blue into distilled water. Then they were washed in distilled water—not acidulated—thoroughly dehydrated in absolute alcohol, cleared in oil of turpentine and mounted in Canada balsam. In preparations thus treated the larger bacillar groups especially stand out so markedly, even with a low power, that with a little practice one can scarcely overlook them. In searching for the groups I made use of the combination AA and ocular IV. and for the further investigation the homogeneous immersion lens $\frac{1}{12}$ ocular II. (Zeiss), of course along with the employment of Abbé's condenser.

I next give a *résumé* of the cases of typhoid examined :—

The sign — means "not examined."

A.—*Cases in which Typical Masses of Bacilli were found in the Mesenteric Glands, or in the Spleen, Liver or Kidneys.*

Number.	Description of Case.	Hospital.	Account of the duration of illness, and condition of Intestine.	Microscopic examination of Organs.				REMARKS.
				Mesenteric Glands.	Spleen.	Liver.	Kidneys.	
1.	Fusilier R.	Garrison Hospital, Wittenberg.	Death in the third week. Marrow-like (*markige*) swelling. Commencing ulceration.	In every section one or a few masses of bacilli.	In every section one or a few masses of bacilli.	In every section one or a few masses of bacilli.	In two sections a mass of bacilli.	Indubitable spore development in the bacilli.
2.	Musketeer R.	The same.	Death on the tenth day. Marrow-like swelling and ulceration.	Some masses of bacilli in every section.	Some masses of bacilli in every section.	Some masses of bacilli in every section.	Some masses of bacilli in every section.	Masses of micrococci also in the mesenteric glands.
3.	Fusilier G.	The same.	Death in the beginning of the third week. Numerous ulcers, some of them already cleaned.	—	A mass of bacilli in almost every section.	—	—	—
4.	Fusilier K.	The same.	Death in the fourth week. Numerous ulcers partially cleaned.	—	Out of ten sections two contained a bacillary mass each.	—	—	—
5.	Fusilier S.	The same.	Death on the 38th day of illness. Old and recent ulcers in intestine.	—	A few masses of bacilli in every section.	—	—	—
6.	Fusilier G.	The same.	Death in the beginning of the fifth week. Old and recent ulcers.	—	A few masses of bacilli in every section.	A few masses of bacilli in every section.	—	Death from hæmorrhage from the nose and bowel. The bacillar masses very large.

A.—*Cases in which Typical Masses of Bacilli were found in the Mesenteric Glands, or in the Spleen, Liver or Kidneys—continued.*

Number.	Description of Case.	Hospital.	Account of the duration of illness, and condition of Intestine.	Microscopic examination of Organs.				REMARKS.
				Mesenteric Glands.	Spleen.	Liver.	Kidneys.	
7.	Female, aged 37 years.	Obuchow Hospital, St. Petersburg.	Marrow-like infiltration.	Several masses of bacilli in every section.	—	—	—	—
8.	Female, aged 23 years.	The same.	Marrow-like infiltration and commencing ulceration.	—	One or a few masses in every section.	One or a few masses in every section.	—	—
9.	Male, aged 17 years.	The same.	Marrow-like infiltration and quite recent ulceration.	—	No bacillar masses in 18 sections.	In one of ten sections a typical mass of bacilli.	No bacillar masses in 13 sections.	—
10.	Female, aged 26 years.	The same.	Marrow-like infiltration.	—	Some bacillar masses in every section.	Some bacillar masses in every section.	—	—
11.	Female, aged 25 years.	The same.	Marrow-like infiltration.	—	*Ibid.*	*Ibid.*	In every section some bacillar masses.	—
12.	Girl, aged 15 years.	The same.	Marrow-like infiltration and recent ulceration.	—	—	A characteristic bacillar mass in one of three sections.	No bacillar masses in three sections.	—
13.	Girl, aged 11 years.	The same.	Marrow-like infiltration and commencing ulceration.	No bacillar masses in four sections.	One typical bacillar mass in one of nine sections.	A typical bacillar mass in one of ten sections.	—	—

14.	Female, aged 34 years.	The same.	Marrow-like infiltration and commencing sloughing.	—	—	A typical bacillar mass in one out of 12 sections.	—	Indubitable spore formation in the bacilli.
15.	?	Barrack Hospital, Berlin.	Marrow-like infiltration.	—	A few typical bacillar masses in several sections examined.	—	—	—
16.	Child, aged 12 years.	Städt. Krankenhaus, Friedrichsheim.	Smooth losses of substance, and a few recent swellings.	—	*Ibid.*	—	—	A relapse.
17.	Girl, aged 14 years.	Barrack Hospital, Berlin.	Death on the 16th day of illness. Marrow-like swelling and commencing ulceration.	—	A bacillar mass in almost every section.	—	—	—
18.	Male, aged 44 years.	Städt. Krankenhaus, Friedrichsheim.	Death on the 39th day. Ulcers cleaned.	—	One typical bacillar mass found after examining more than 100 sections.	—	—	—
19.	Male, aged 32 years.	The same.	Smooth losses of substance.	—	A typical bacillar mass in the first section.	—	—	—
20.	Girl, aged 15 years.	Barrack Hospital, Berlin.	Marrow-like swelling, and recent ulceration.	—	Two bacillar masses found in 12 sections.	—	—	—

A.—*Cases in which Typical Masses of Bacilli were found in the Mesenteric Glands, or in the Spleen, Liver or Kidneys—continued.*

Number.	Description of Case.	Hospital.	Account of the duration of illness, and condition of Intestine.	Microscopic examination of Organs.				REMARKS.
				Mesenteric Glands.	Spleen.	Liver.	Kidneys.	
12.	?	The same.	Recent swellings.	—	Two out of 50 sections contained one bacillar mass each.	—	—	—
22.	Male, aged 47 years.	The same.	Death in the 4th week. In part smooth losses of substance, in part ulcerations.	—	A few bacillar masses in every section.	—	—	—
23.	?	The same.	Recent marrow-like swelling. Commencing ulceration.	—	In almost every section a bacillar mass.	—	—	—
24.	Girl, aged 18 years.	Städtisches. Krankenhaus, Friedrichshain.	End of the first week. Marrow-like swelling.	—	*Ibid.*	A bacillar mass in almost every section.	No bacillar masses in 12 sections.	
25.	Male, aged 21 years.	The same.	Smooth losses of substance along with recent swellings and sloughs.	—	—	A bacillar mass in almost every section.	—	A relapse. Spore formation in the bacilli.
26.	Male, aged 24 years.	The same.	Beginning of the second week. Swelling and sloughing.	—	No bacillar masses in eight sections.	One out of seven sections showed a typical bacillar mass.	No masses in 12 sections.	

B.—*Cases in which no Bacillar Masses were found in either Mesenteric Glands, Spleen, Liver or Kidneys.*

Number.	Description of Case.	Hospital.	Account of the duration of illness, and condition of Intestine.	Microscopic examinations of Organs.				REMARKS.
				Mesenteric Glands.	Spleen.	Liver.	Kidneys.	
1.	Female, aged 35 years.	Obuchow Hospital, St. Petersburg.	Old and recent ulcers.	—	146 sections examined without finding a bacillar mass.			Numerous bacilli of Koch and Eberth found in the intestinal mucous membrane at the seat of a recent ulcer.
2.	Male, aged 42 years.	Städtisches Krankenhaus, Moabit.	End of the 4th week. Only smooth losses of substance.	—	No bacillar masses in 62 sections.	No bacillar masses in 21 sections.	—	Death from perforation and peritonitis.

Thus the typical bacillar masses were absent in only two of the 28 cases of typhoid fever examined. In the former of these two cases, besides old intestinal ulcerations, there existed also recent ones. On examining one of the latter, numerous organisms exactly corresponding to typhoid bacilli were found in every section, and not only superficially in the base of the ulcer but penetrating deeply into the intact tissue. Whether one had here to deal with the initial stage of a relapse must remain undecided. The negative result in the second case can scarcely surprise us. Death occurred towards the end of the fourth week of the disease, not owing to the typhoid process as such, but as the result of peritonitis from perforation, and at a period when only smooth scars could be observed in the gut. Possibly one might, even in those two cases, have found scattered groups after a more prolonged search. In this connection I would draw attention to the fact that in Case 18 (death on the 39th day of disease, with only clean ulcers in the intestine) a bacillar mass could be found only after the examination of more than a hundred sections of the spleen.

In order not to pass over in silence any of the cases of which the organs were placed at my disposal, I would mention the following:—Fusilier D., in Wittenberg, contracted typhoid fever at the time of the epidemic to be afterwards described,* and died on the 51st day of the disease as the result of pleuro-pneumonia of the right side. At the autopsy only a very few cicatrices in the intestine were found as the residue of his typhoid; ulcers were nowhere to be seen. In the organs no typhoid bacilli were found, as may be conceived. Finally, I have to mention the following case, not referred to in the above summary on another ground. The patient was a male, aged 19, treated in the Städtisches Krankenhaus, Friedrichsheim, who died suddenly at the end of the third week of the disease. The diagnosis during life as well as at the autopsy was that of typhoid fever. An extensive invasion of micrococci was observed upon microscopic examination by Dr. C. Friedländer, to whom I am indebted for the organs. In this case the attempts to search for typhoid bacilli among the very exceptionally numerous masses of micrococci in the organs had soon to be relinquished as hopeless. Moreover, it must be reserved for further investigations to decide

* The description of this epidemic has not been considered necessary.—J. J. P.

whether the clinical and pathological picture of enteric fever may not exceptionally be produced by a similar invasion by micrococci taking place from the intestine outwards, without any participation of typhoid bacilli in the process.

From the fact that in 26 out of 28 cases of enteric fever the presence of these characteristic bacillar masses was demonstrated, the view, that these organisms are in reality the cause of the typhoid process, has without question increased in probability to a high degree. It need scarcely be mentioned that no masses of bacilli, even at all similar, have ever been met with in the organs of persons dying from other diseases, in the exceptionally numerous investigations conducted at the Office of Public Health.

I can offer no judgment upon the relation which the number of masses found bears to the stage of the typhoid process, because information is wanting as to the duration of the disease in many of the cases. In looking through the summary one will find the experience of Eberth and Meyer generally confirmed, that the bacilli are the more numerous the more recent the process. When, as an exception, many bacillar masses were observable in old standing cases, possibly recrudescences were being dealt with.

My results agree entirely with the descriptions given by Eberth, Koch and Meyer in regard to the shape and appearance of the bacilli. (Plate V., fig. 16.) On the average they are about thrice as long as they are broad; their length corresponds to about the third part of the diameter of a red blood corpuscle. In isolated spots one may see somewhat longer threads, which on more thorough examination can, however, be seen to be made up of several members. Trifling differences in breadth occur even in different cases in the same epidemic, but this appearance is to be referred only to the greater or less intensity of the staining of the sections; at least I have never been able to observe it in preparations on cover-glasses, in which the staining is always deep. The extremities of the bacilli are distinctly rounded off. In several of the cases I examined, the bacilli found in the internal organs contained unmistakable spores which appeared as round portions, remaining unstained and occupying the whole breadth of the bacilli.

Attention has been drawn to a special structure in the stained

bacilli by Meyer,* and in greater detail by C. Friedländer.† These authors found in the otherwise uniformly stained substance of the bacilli circular or elliptical unstained portions, which occupied from one-half to three-fourths of the width of the bacilli and generally lay in the centre of the breadth of the organism or more rarely along the sides, where they presented themselves as almost semicircular defects. Dr. Friedländer had the kindness to show me the specimens in question.

I myself in my preparations have never found this appearance so clearly pronounced, although it sometimes has appeared to me as if the contents of the bacilli did not stain quite uniformly. At all events the spores which I have found in the bacilli, which always occupy the entire breadth of the bacilli, are not identical with these portions, which remain unstained and are limited to a part of the breadth of the bacilli. A positive opinion upon the position of the spores can scarcely be arrived at in sections, but they seemed to me to be at the extremities. I shall return to the point in greater detail in describing the cultivated bacilli. As regards the distribution of the bacilli in the separate organs I shall in the first place say a few words about the condition of the intestine. In almost all the cases examined in which the process had gone on to sloughing and ulceration, numerous organisms of different shapes, both bacilli and micrococci, were found in the necrosed tissue and in the base of the ulcers. In the deeper layers of the mucous membrane, which still took on the nuclear stain fairly well, there were sometimes observed, in addition to those which exactly resembled typhoid bacilli, other forms of bacilli, viz., longer, more delicate threads intermingled with exceptionally slender bacilli and broad rod-like bodies which stained deeply. It was not always possible to recognize typhoid bacilli with certainty among these various organisms. In such cases I sometimes found vessels quite filled with long bacillar threads, closely packed together and lying parallel to the long axis of the vessel, quite corresponding to the drawings given by Klebs. In those recent cases, however, in which there was no trace of loss of substance or necrosis in the marrow-like, swollen portions, and in which the cells still presented deep nuclear staining, only the one form was found in greater or less abundance, viz., short

* *Loc. cit.*

† *Verhandlungen der Physiolog. Gesellsch. zu Berlin*, 15th November, 1881.

bacilli entirely corresponding to those of which the groups in the other internal organs were exclusively composed.

Scarcely any doubt can exist with reference to this fact, already insisted upon by Koch and Meyer, that these other organisms have of themselves nothing to do with the typhoid process, and that their penetration into the tissue is entirely a secondary occurrence. I do not mean to say, however, that these forms may not also be pathogenic under certain conditions. Certainly the isolated cases of extensive invasion by micrococci in typhoid fever are most easily explained by the view that the necrosed and ulcerated intestinal lesions have been the point of entrance. It is extremely difficult to arrive at a decision as to the way in which the typhoid bacilli pass from the intestine into the mucous membrane. The solitary follicles and patches of Peyer may constitute by preference the seat of invasion. Probably the same thing occurs as in the spread of splenic fever infection from the intestine. In this connection I would refer to the results of the experiments conducted at the Office of Public Health, in which exactly similar conditions of intestine were observed, as regards invasion with bacilli, in sheep fed with anthrax spores.

With reference to the previous question, the circumstance is interesting that I found typhoid bacilli arranged in quite typical masses, as they otherwise only occur in other internal organs, in two cases in a greatly swollen solitary follicle upon which there was no sign of ulceration, and which took on the nuclear-stain perfectly well. Besides these masses the sections showed pretty numerous isolated bacilli of the same appearance near the surface of the follicle. At different spots one could also observe slender tracts of the same bacilli which extended into the follicle. It must be left undecided whether the glands of Lieberkühn in the neighbourhood of the follicles play any part in the invasion. In various specimens it seemed to me as if this were the case.

I may confine myself to the communications of Eberth and Koch as regards the appearance of the bacillar masses in the organs, and especially refer to the photographs of the latter. (Plate V., figs. 15 and 16.) They present irregularly limited masses, which can be easily resolved into separate bacilli, especially at the margins. According to Eberth's researches they exist in greatest number in the mesenteric glands and

in lesser number in the spleen, whilst they could not be demonstrated by him in the liver and kidneys. This latter circumstance might perhaps be alone due to the fact that Eberth did not succeed in staining the bacilli in sections sufficiently deeply. More recently also he seems to have had no better results in this respect.* Koch, on the contrary, found the masses in stained sections both of liver and kidneys without difficulty. As is manifest from the summary given above, I myself have examined in the great majority of cases the spleen, then the liver and then the kidneys and mesenteric glands. Among the 26 cases yielding a positive result the spleen was examined in 22, and in 20 of these bacillar masses were found; the liver was examined in 13 cases and bacillar masses observed in all without exception; the kidneys were examined in 7 cases, three times with a positive result; finally, the mesenteric glands were examined in 4 cases and the characteristic masses found in 3 of them.

On the whole, I have gained the impression that the masses in the spleen and liver are much more numerous than in the kidneys. I must leave it undecided whether this appearance may not have partly for basis the circumstance that single bacilli are frequently excreted through the kidneys, and so no opportunity is given for the formation of easily observed masses. I have, however, never found bacilli in the urinary tubules. Where it was possible to decide the matter, the masses always lay in the capillaries or smallest blood-vessels. (See Plate V., fig. 15.) In the liver also the bacilli always lay in the blood-vessels, often completely occluding small capillary areas. (See Plate V., fig. 16.) In a few cases in which the bacillar masses were specially numerous, I have also met with isolated rods, or small groups of them, between the blood corpuscles even in transverse sections of larger blood-vessels, in the liver and mesenteric glands. In nearly half of the cases examined I have also found in the liver roundish aggregations of irregularly shaped nuclei, as had already been seen by Friedrich† in 1857 in a case of typhoid fever, and had been regarded by him as lymphatic tumours. I cannot decide whether these growths, which occur in the liver in leukæmia also, have any relation whatever to the typhoid bacilli. They were first

* Compare Volkmann, *Sammlung klinischer Vorträge*, 1883, No. 226.
† Virchow's *Archiv*, Bd. xii.

observed by Virchow in a case of leukæmia, even before Friedrich's communication. At all events, I have only found scattered bacilli between the thickly packed nuclei in one case. In one other case I saw a small bacillar mass lying in immediate contact with such an accumulation of nuclei, without being able to recognize any relationship between them. In the lungs, which I have only examined in a few cases, the typhoid bacilli were never found in such characteristic masses as in the other organs. And wherever one meets with bacilli more or less scattered, any decision as to their signification is very much more difficult, especially when one is dealing with cases where the autopsy is not recent, when the presence of putrefactive bacilli may render a decision difficult. It must be reserved for further observations to decide more definitely the relations of the bacilli to the separate organs. It was not so much my aim, as one will easily understand, to investigate as thoroughly as possible individual cases, as to bring forward proof that the characteristic bacillar masses constitute in reality a regular occurrence in the internal organs of fatal cases of typhoid fever.

As has already been mentioned, Eberth and Meyer obtained only very unsatisfactory results in their attempts to stain the typhoid bacilli in sections, so that they preferred to examine their preparations unstained. On the other hand, Koch found that the bacilli stood only slightly behind other micro-organisms in regard to their staining power. Besides, very soon after the publication of Meyer's work, C. Friedländer,* under whose guidance that work was carried out, succeeded in staining the bacilli deeply in sections. In my researches methylene blue proved the most suitable stain, but the bacilli stained also very well with methyl violet, gentian violet, bismarck brown, fuchsin, and, though not quite so well, with hæmatoxylin. But one must not use too dilute staining solutions and must either allow them to act for a number of hours, or further the process by heating. Even when one stains in the above-mentioned way, it constantly happens that the depth of colour is not quite so intense as with many other bacteria. Preparations stained blue not infrequently fade rather quickly, therefore sections stained brown are in general more suitable for keeping. When subjected to the procedure recommended by Ehrlich for staining tubercle

* Compare *Fortschritte der Medicin.*, 1883, No. 2.

bacilli, typhoid bacilli behave in the same way as almost all other micro-organisms, *i.e.*, they are decolorized at once in nitric acid and take on the second staining material employed for the coloration of the tissue. Finally, I ought to say some words about the occurrence of groups of micrococci in the organs of typhoid patients. Leaving out of consideration the intestinal condition, I have found micrococci in only one out of twenty-eight cases, and these in the mesenteric glands. This was a case in which numerous characteristic groups of typhoid bacilli were observed in the spleen, liver and kidneys. The now pretty generally accepted view, that the micrococci have in themselves nothing to do with the typhoid process and that their presence is, on the contrary, a purely secondary phenomenon, receives therefore new support from my investigations.

THE CULTIVATION OF TYPHOID BACILLI OUTSIDE THE BODY.

If the attempts to cultivate the typhoid organism made within recent years have not afforded any satisfactory results, the reason has lain undoubtedly in part in faults in the method employed. For instance, cultivation experiments were organized without a sufficient understanding having been arrived at by anatomical examination as to which form of organism was to be regarded as the probable cause of the disease. Thus it happened that micrococci growing in the cultivations were regarded as typhoid germs without further inquiry, whilst, as we have seen, micrococci occur in the internal organs of typhoid cadavers only quite exceptionally and as something accidental, and on the contrary a definite form of bacillus is almost invariably found. A second fallacy was that in the cultivations quite impure material was made use of, such as the evacuations of patients, in regard to which one never knew whether they contained any typhoid germs or not.

As early as in the year 1870 a communication was made by Coze and Feltz that they had found in the blood of typhoid patients an organized ferment, the shape of which recalled that of "bacterium catenula." In the year 1879 this inquiry was further pursued by Feltz* in the following manner:—typhoid blood, taken with the greatest possible avoidance of accidental impurities, was poured into flasks free from organisms, and then

* Quoted in Virchow-Hirsch's *Jahresberichte* for 1879.

left alone for a considerable time; three months afterwards there were present in the flasks a great number of small oval cells, partly lying separate, partly arranged in rows of 3 to 5, without independent movements. In controlling experiments, blood taken from the crural vein of dogs and treated in the same manner, remained free from bacterial development. Further, Birch-Hirschfeld* performed some cultivation experiments in Pasteur's fluid with the blood of typhoid cadavers, in which he could not discover any bacteria on microscopic examination. The result was negative. In three cases no development of bacteria at all occurred, and in the others the cultivation fluid became putrescent. Letzerich,† in his first cultivation experiments, also employed blood from typhoid patients by transferring a small quantity of it into a jelly prepared from fresh veal. By this method, the details of which I cannot here further enter into, he obtained mobile (?) micrococci, which he regarded as the cause of the typhoid fever. Letzerich could institute no investigations as to whether the same micrococci were also to be found in the internal organs, because he had no opportunity of performing post-mortem examinations. In later experiments Letzerich‡ made use of the "hypostatic sputum"§ of typhoid patients, as the starting point for his cultivations, which, according to his account, "contains a great abundance of the cocci of the 'micrococcus typhi abdominalis.'" Freshly prepared fish jelly served him as material for cultivation. In this there appeared, starting from the portions in the sputum, numerous small, pure white, roundish masses, which, on subsequent microscopic examination, were recognized as colonies of the "micrococcus typhi abdominalis." These cultivations, when injected subcutaneously into rabbits, proved to be pathogenic. I must not enter into any criticism of Letzerich's work here, but in order to characterize it, would mention that Letzerich regarded his micrococci as identical with the bacilli described by Klebs, as appears from the following sentence at the end of his last publication :—"the fact is in the highest degree remarkable, that after infection with a jelly containing micrococcus typhi, the different forms of schizomycetes (micrococci and bacilli) were discovered in the intestine of the

* *Allgemeine Zeitschrift für Epidemiologie*, Bd. i., 1874.

† Virchow's *Archiv*, Bd. 68, Jahrgang 1876.

‡ *Archiv für experim. Pathol. und Pharmak.*, Bd. xiv. Heft 3.

§ This probably refers to the sputum in cases of hypostatic pneumonia.—J. J. P.

animals I experimented upon, these, of course, in the different stages of the typhoid process; hence the name which I gave to the fungus of 'micrococcus typhi abdominalis' has the same meaning as that of 'bacillus typhosus,' employed by Klebs."

In the beginning of this work I have already given an account of the investigations instituted by Klebs, as far as concerns the presence of the "bacillus typhosus" in the organs of fatal cases of typhoid fever. In the attempt to cultivate the organisms found, Klebs' procedure was as follows*: the substance of a typhoid mesenteric gland was triturated in distilled water, and of the fluid thus obtained—in which, besides cells and fat corpuscles, bacteria, some of them with spores, could be observed —very minute quantities were transferred into a 5 per cent. solution of isinglass. Twenty-four hours afterwards the cultivation fluid, which was maintained at a temperature of about 35° C., appeared cloudy. An examination carried out a few days later showed the cause of this cloudiness to be the presence of bacilli, for the most part containing spores. The cultivations of bacilli obtained were carried on through several generations, partly in a 5 per cent. isinglass solution, partly in meat infusion and were utilized for experiments on animals. One cannot gather from the work whether Klebs carried out further experiments beyond this single series of cultivations. Some control of such a sort as to extend over many cases had become meanwhile the more desirable, as the procedure of triturating the gland substance in distilled water before its transference into the cultivation fluid, very materially increased the danger of accidental impurities. It seems to me that the bacilli cultivated by Klebs were not identical with those cultivated by me from the internal organs of typhoid cadavers, because, apart from other circumstances, our experiments, carried out upon rabbits, yielded different results.

Brautlecht† has, in several instances, cultivated what he considers a specific form of bacillus from drinking water which lay under the suspicion of having caused typhoid epidemics. Rabbits infected with bacilli of this sort are said to have succumbed to a diseased process similar to enteric fever in man. Klebs,‡ however, states that his microscopical examinations in refer-

* *Archiv für experim. Pathol. und Pharmak.*, Bd. xiii., Heft 5 und 6.

† Virchow's *Archiv*, Bd. 84.

‡ *Loc. cit.*

ence to the presence of bacilli yielded only doubtful results. Besides, the description given by Brautlecht of the cultivated bacilli says nothing in favour of their being identical with the bacilli which occur in the internal organs of fatal cases of typhoid fever. Thus, they were described by Brautlecht as being only half as broad as "bacterium termo," and at least three times as slender as "bacillus subtilis," and therefore much more delicate than typhoid bacilli. Against the view that Brautlecht's bacilli stand in any relation whatever to typhoid fever is the circumstance, that he was able to obtain exactly similar cultivations from perfectly innocuous green algæ decomposing in the heat of summer.

Almquist* performed some cultivation experiments with the blood of typhoid patients. These, however, are open to many objections, as is also an attempt to transfer the disease which is communicated in the following words in the French note appended to the work: "From the second generation I succeeded in inoculating a dog with good results. The dog was only slightly ill, but on the fifteenth day I found the patches of Peyer very much swollen and containing the characteristic microbes." Maragliano† also made some cultivation experiments with blood taken from typhoid patients during life. The blood, which was sometimes taken from the parenchyma of the spleen by means of a Pravaz' syringe, and sometimes from the finger tip, contained micro-organisms both isolated and massed together. These consisted "almost exclusively of spherical corpuscles which appeared homogeneous, of delicate contour and analogous to micrococci." In the blood from the spleen besides these organisms, bacilli in smaller number are said to have been present, which were exactly similar to those described by Klebs and Eberth. By the method of fractional cultivation a large quantity of bacilli was obtained from the blood, similar to those which were found in the fresh blood. Maragliano makes no statement as to whether the spherical organisms had multiplied in the cultivations. It is obviously very difficult to come to any decision in regard to these bacilli, whether accidental impurities or typhoid bacilli were being dealt with. Up to this time there were no characteristics by means

* *Typhoid feberns Bakterie.* Stockholm, 1882.

† *Centralblatt für die medicinische Wissenschaften*, 1882, No. 41.

of which one was able to differentiate the latter organisms from others similar to them. In my efforts to discover such a means of recognition, it has again been shown what unusual advantages for pure cultivations the method recommended by Koch presents, viz., that of employing a solid cultivating medium in place of fluids. Indeed with the assistance of the above-mentioned method I have been able to distinguish the typhoid bacilli which I have cultivated from all organisms, pathogenic and non-pathogenic, hitherto known to me. My first attempt to cultivate the typhoid bacillus was carried out on October 16th, 1881, in the following manner, which almost certainly excluded accidental impurities. After the entire spleen from a typhoid cadaver, which showed no trace of decomposition, had been carefully washed with a 1 per thousand solution of corrosive sublimate, an extensive cut was made into it with a knife previously thoroughly sterilized by heating, dividing it almost completely in a longitudinal direction; then, with a second sterilized knife, from the clean surface of the section obtained another deeper incision was made, which, however, nowhere extended nearly as far as the capsule, and the same procedure was repeated with a third knife in a direction at right angles to the surface of the latter section. In this manner the danger of detaching impurities from the surface of the organ on to the surface of the section was entirely obviated. From the deeper part of the last section small quantities of blood along with little particles of spleen tissue were taken by means of platinum wires (which were heated before every fresh contact with the organ), and these wires thus charged were drawn across the surface of a solidified meat jelly containing peptone, spread out upon sterilized slides.* After inoculation the slides were preserved at the temperature of the room in a moist atmosphere under glass shades.

The inner portions of the spleen, from which the material for the cultivations was taken, were then microscopically examined by means of dried and stained cover-glass preparations. In every preparation some bacilli exactly corresponding to those of Eberth and Koch could be observed; on the contrary, no other organisms were present in the preparations. Pieces of spleen were also placed in absolute alcohol for further examination in

* In reference to the method employed compare:—*Mittheilungen aus dem Kaiserlichen Gesundheits-Amte*, Bd. i.

sections. It may at once be mentioned that in these also the bacilli in the form of the characteristic groups could be afterwards observed without difficulty. The case in point is referred to as No. 15 in the foregoing Table.

As soon as twenty-four hours afterwards a delicate, whitish cloudiness showed itself along the inoculated tracks on the gelatine, which after another twenty-four hours had increased in intensity, but was still confined to the inoculated tracks. At no point did liquefaction of the gelatine take place. Under a low power, and examined by transmitted light (Zeiss' AA, ocular 4), the cloudiness resolved itself into a large number of roundish, slightly granular colonies of a yellowish brown colour. No colonies of any other appearance were present.

Small morsels were next taken from different parts of these tracks with a platinum needle, and were examined in a drop of sterilized water under a higher power (Zeiss' homog. immers. $\frac{1}{12}$, ocular II.), and with the use of slides with a central cell. Thus it could be definitely determined that along all the very numerous tracks of inoculation, without exception, one and the same form of bacilli had developed. Corresponding as regards breadth with the bacilli found on microscopic examination of the spleen, they showed considerable differences as regards length; whilst the majority were three or four times as long as they were broad, longer threads were also present in smaller numbers.

A characteristic of the bacilli which at once attracted attention was a quite unmistakable spontaneous movement. They swam in the most various directions across the field, some slowly, some more rapidly. A serpentine movement could also be clearly recognized in the longer threads. Once that they had arrived at the margin of the drop they came to a standstill and collected, lying one against the other. It was easy to observe on the examination of stained cover-glass preparations, and especially of dry, unstained preparations, that the longer threads were composed of shorter members. The staining with various aniline dyes in the customary manner was successful without difficulty; it was, however, decidedly less intense than with the bacilli of anthrax, for instance. It is to be observed in regard to the further behaviour of the cultivations, that they had attained the acme of their development on the fourth day, and from that

day onwards remained pretty much unchanged to the naked eye. No extension from the point of inoculation into the depth of the gelatine occurred, and upon its surface they spread only to a limited degree. In the course of the following year and a half the spleens of twelve other cases were employed precisely in the manner described, in order to obtain cultivations. These cases are referred to in the foregoing Table as numbers 1 to 4 inclusive, and numbers 16 to 23 inclusive. Ten of these twelve cultivations were quite pure from the first, and consisted exclusively of the same bacilli as I have described above. In two cases only in which the spleen was not quite fresh, but an attempt at cultivation nevertheless undertaken, colonies of micrococci and a form of bacillus which liquefied the gelatine, were found here and there along the tracks of inoculation together with typhoid bacilli. In these two cases, however, one easily succeeded in the next cultivation in obtaining a quite pure growth of typhoid bacilli, as a mixture with the other organisms had not been able to occur at many parts of the tracks owing to the solid character of the cultivating medium. Therefore I have on the whole in 13 cases cultivated one and the same bacillus from the spleen. From 10 of these cases I have continued the cultivations up till the present time by means of repeated transplantations about every three weeks, without their having changed their properties in the slightest degree.

In one case a cultivation experiment was performed in an exactly analogous manner with the liver, and the same bacilli were obtained. The case referred to as No. 18 in the Table was specially interesting. No bacilli could be observed in the examination of cover-glass preparations of the spleen, and yet typhoid bacilli grew in the cultivations, free from all impurities and in small quantities, but still there were some colonies in every track. More than a hundred sections of the spleen had to be examined before one succeeded in harmonizing the result of the cultivation experiment with that of the microscopic examination, by the discovery of a characteristic mass of bacilli. One might therefore in certain circumstances prove the presence of bacilli by means of the method of cultivation when the microscopic examination of the organ in sections had failed, owing to the trifling number of organisms present.

The further cultivations were carried on in test tubes which

were filled to one-third with the solidified nutrient jelly, stopped with a plug of cotton wool and thoroughly sterilized. The plug having been carefully removed, a minute portion of the former cultivation was transferred into the gelatine by means of a puncture made with a platinum needle previously heated. In the course of the following day an opaque mass invariably formed, remaining limited to the point of inoculation, which generally attained the maximum of its growth in the course of six to eight days. On the other hand the bacilli extended outwards from the point of inoculation on the surface of the gelatine to the margin, in the form of a greyish white layer gradually increasing in intensity.

The typhoid bacilli could already be distinguished with certainty from many similar organisms by their manner of growth in the nutrient jelly as above described and unvarying in all cases. In this connection it is specially to be noted that no liquefaction of the gelatine ever occurred under the influence of their proliferation, whereas this phenomenon is observed with different mobile bacilli of similar shape, as, for example, with the hay bacillus. Of course I have endeavoured to discover further characteristics.

It was next attempted to cultivate the bacilli grown in gelatine on the cut surface of boiled potatoes, which according to experience form an excellent soil for numerous pathogenic and non-pathogenic organisms. For this purpose potatoes, which had been thoroughly cleaned and had lain for half an hour in a 5 per thousand solution of corrosive sublimate, were cooked in our steam sterilization apparatus, *i.e.*, in a current of steam at 100° C. After each potato had been cut in two with a freshly heated knife, a small quantity of the jelly cultivation was stroked over the surface of the section. The fertilized potatoes were preserved, either several together in glass vessels kept moist in the ordinary way, or, when the observation was to be more prolonged, singly in tall sterilized glasses plugged with cotton wool. Of course in the latter case care was taken to provide the necessary moisture. Sections of potato thus treated show only very slight changes to the naked eye in the course of the following day. The fertilized surfaces seem to take on a more uniform and moister appearance, but microscopically no growth can be seen. But if one attempts, after about forty-eight

hours, to detach from the surface a small quantity for microscopic examination with a platinum needle, one gets an impression as if the whole surface were changed into a coherent, resistent skin, without any trace of desiccation being perceptible. Everywhere, from whatever part of the surface one takes the most minute portion of potato, even from parts not inoculated, one finds on microscopic examination the inoculated mobile bacilli in surprising abundance, mostly of the usual length, but some also in the form of longer threads. The whole surface seems to consist almost entirely of bacilli, which stain only with moderate intensity with aniline dyes, like those cultivated in gelatine.

This sort of growth upon the potato is quite characteristic. It has up to the present time allowed of the typhoid bacilli being easily distinguished from all other similar organisms. The boiled potato forms a very favourable soil for typhoid bacilli, as for other pathogenic organisms; I would only instance here the bacilli of splenic fever and of glanders, and the micrococci of erysipelas.* The observation of Zöller† is interesting in regard to the explanation of this fact, according to which in the potato fibres no other modification of albumen, except a globulin substance, could be found after the starch and soluble constituents had been washed away. The behaviour of this substance was very like that of myosin. The juice of the potato also appears, according to Zöller, to contain globulin besides other albuminoids. Typhoid bacilli also grew luxuriantly on sterilized blood serum of sheep, which had been prepared according to Koch's directions and solidified in test tubes. Here they form a greyish white, somewhat translucent layer and render cloudy the water of condensation which collects in the lower part of the tube. In these cultivations I never observed the longer threads. The bacilli when cultivated on blood serum have somewhat smaller dimensions, whether examined fresh or in stained preparations. If one inoculates them on the surface of a potato, or back into gelatine, one finds the previously described, typical macroscopic characters and on microscopic examination, the larger bacilli.

The bacilli likewise grow luxuriantly when inoculated into the solidified blood serum in the form of punctures with the

* Fehleisen, *Die Ætiologie des Erysipels*, 1883.

† Zöller, *Globulinsubstanzen in den Kartoffelknollen. Bericht der deutsch. chemisch. Gesellschaft*, Bd. xiii., S. 1064.

platinum needle, but only in the immediate neighbourhood of the punctures. No extension throughout the whole serum, nor liquefaction of it, ever takes place. I further succeeded in cultivating the bacilli in fluid, sterilized blood serum and in meat infusion. In these conditions also the individual organisms seem less powerfully developed than those cultivated in jelly or on the surface of potatoes. An undoubted proliferation also took place in some vegetable solutions, as in a decoction of mallows, in carrot juice, in the juice of raw potatoes and in a mixture of water and bruised wheat.

SPORE-FORMATION.

I have never been able to observe the formation of spores with certainty when I have examined bacilli cultivated at the temperature of the room in nutrient jelly, even after several weeks growth. Nor did this occur in an indubitable manner on the surface of sections of potato at the temperature of the room. On the other hand, when the inoculated potatoes were maintained at a temperature of about 37° C., spores could be invariably observed in the bacilli on the third or fourth day, both in preparations examined unstained and in stained cover-glass preparations. The spores were always at the extremities, appearing as brightly refracting, round corpuscles which occupied the entire width of the bacilli. A well-developed spore was always present at one end only of each bacillus. At the opposite end one sometimes observed an indication of such, but I have never seen two completely developed spores in a bacillus which undoubtedly consisted of one member. When two members remained attached together the contiguous ends contained no spores, but each of the opposite ends contained one. The same appearance is present in each member of the longer threads, which are, however, not very numerous. The resolution of these into their single component members is best observed by allowing a small quantity of the cultivation to dry in a very thin layer on a cover-glass, and by examining it with a high power unstained and without the addition of fluid. When the cultivation has stood a longer time, besides the bacilli bearing spores there are also present free, round spores, which are characterized as such by their uniform size, their strong refracting power and their

inability to take on aniline stains. The question as to the temperature at which typhoid bacilli are able to produce spores being of great moment in regard to the etiology of the disease, I have attempted to clear up the conditions more fully. For this purpose I made use of Arsonval's excellent incubators, which enable one to have an uniform temperature at one's disposition for weeks at a time. The result of these experiments is that the formation and growth of spores takes place undoubtedly at 42° C., if not quite so abundantly as at the temperature of the body. The temperature most suitable for spore formation seems to be from 30° C. to 40° C. At 25° C. it occurs somewhat later, but still indubitably. The lowest limit seems to be 20° C., at least at this temperature after eight days growth I have only observed a very few and only moderately developed spores in the bacilli. After two more days the process was not much further advanced. Thus the bacilli always form undoubted spores at such a temperature as obtains in summer even in cold places.

At a temperature between 30° C. and 40° C. the formation of spores takes place both in cultivations in gelatine—which naturally becomes liquid under such circumstances—and in coagulated blood serum, just in the same way as in the bacilli cultivated on potatoes. As regards the duration of their vitality it may be mentioned that spores, cultivated in blood serum which was then dried in thin layers, sprouted when sown upon the nutrient jelly after more than three months and these cultivations consisted of the same mobile bacilli as had been obtained from the spleen of fatal cases of typhoid fever.

If we glance over the foregoing experiments, we shall find that we have already quite a series of characteristics enabling us to recognize the cultivated typhoid bacilli. I shall now shortly epitomize these.

(1) The bacilli possess spontaneous movement. This appearance is best observed when a small quantity of a recent cultivation, kept at the temperature of the room, is examined in distilled water and on a hollow slide. It is less marked in older cultivations and in such as have been kept until their examination at the temperature of the body. In the latter case the sudden lowering of temperature seems to have a restraining effect upon the spontaneous movement. It may be added here that in cover-glass preparations, examined dry and not stained, I have

often observed extremely fine threads at the extremities which might perhaps be considered as flagella. It is, however, extremely difficult to arrive at a definite conclusion on account of the great delicacy of these organs. (2) The bacilli take on aniline stains less deeply than most similar organisms. (3) Cultivated in solid nutrient jelly the bacilli remain limited to the points of inoculation and their immediate neighbourhood. They never liquefy the gelatine. Cultivated in the above mentioned material upon slides, they form at the deeper part compact colonies, which are composed of slightly granular, brownish yellow little heaps when seen under a low power and by transmitted light. They only spread slowly along the surface from the points of inoculation. In *test-tube cultivations** they remain limited to the track of the needle, and spread out only on the surface to the margin of the glass, and that very slowly. (4) Cultivated on the cut surface of boiled potatoes, and (5) on and in coagulated blood serum, the bacilli show the typical macroscopic manner of growth described. (6) The bacilli develop spores at their extremities. The development of spores occurs in three or four days at a temperature of 30° C. to 42° C., more slowly and incompletely at 20° C., and does not take place at all at a lower temperature.

After one succeeded in finding characteristics sufficient to distinguish the typhoid bacilli and to permit of their differentiation from similar organisms, the attempt could then be made to cultivate them from the dejecta of typhoid patients. For this purpose test-tube cultivations in solid nutrient jelly (see footnote) were made with small quantities of the diarrhœic evacuations of several severe typhoid cases, in the earlier weeks of illness. These attempts, however, have given no satisfactory result up to the present, in spite of the extremely small quantity used, because other organisms, which very rapidly permeate and liquefy the gelatine, have invariably rendered the separation of the numerous different forms from each other impossible. In two cases a cultivation experiment was made in the above-mentioned manner with the contents of a piece of ileum, several centimetres long, removed from the body after the

* These are carried out by the introduction of the bacteria into test tubes containing solid nutrient jelly, by means of a previously sterilized platinum wire, which is plunged down to the bottom of the tube and rapidly withdrawn.—J. J. P.

application of a feeble ligature. But in this case also liquefaction of the gelatine from putrefactive bacilli set in so soon that one failed in isolating the separate forms. Up to the present I have not been able from want of time to pursue these attempts further. I have also not succeeded in cultivating typhoid bacilli from the blood of typhoid patients. In two cases in the second week of the disease, blood was taken from the forearm—in one case from a recent roseolous spot—and sown in gelatine in the manner previously mentioned. Nothing at all grew along the inoculated tracks except a few colonies of micrococci, obviously referable to accidental impurities. In another severe case the attempt was made in the following manner towards the end of the second week of illness (compare the method of examining water practised in the Office of Public Health):—after the skin in the hepatic region had been disinfected with a one per cent. solution of corrosive sublimate and afterwards washed with absolute alcohol, blood was taken by means of two perfectly sterilized cupping-glasses, with every precaution against impurities. Each cubic centimetre of this blood, still fluid, was mixed with twenty cubic centimetres of nutrient jelly warmed to 40° C., and the whole while still fluid was poured upon a horizontally placed, sterilized glass plate, 15 cm. long by 13 cm. broad. The nutrient jelly employed contained 10 per cent. of gelatine. After the mixture had coagulated upon the plate it was placed under a glass shade, kept moist and maintained at the temperature of the room. Four glass plates were prepared in the manner indicated. At the expiry of four days from five to eight colonies of different aspects had grown on each plate in the substance of the gelatine, whilst besides these some showed themselves on the surface, the latter obviously spreading from germs which had fallen from the atmosphere on to the solid gelatine. A few more colonies developed in the gelatine in the course of the following days. On microscopic examination, however, these also very soon turned out to be accidental impurities, not one was composed of typhoid bacilli. As well as a few moulds there were present micrococci, yeast fungus and short rod-like forms, the first examination of which excluded all confusion with typhoid bacilli and the further cultivation of which rendered this certain. It was established by means of a controlling experiment, for which the same plates were utilized,

that the typhoid bacilli grew luxuriantly in the mixture of blood and nutrient jelly. According to these experiments, trifling in number as they are, it appears that typhoid bacilli occur at all events very sparsely in the circulating blood.

Probably the single organisms, detached from a group, become immediately fixed in a capillary of one of the internal organs. If the blood were taken directly from a vein and not through the skin, one might possibly arrive sooner at a positive result with this method of experiment. Besides, the possibility is not excluded that the bacilli were in reality very sparse in the few cases that I examined in this connection. At all events it can scarcely be doubted, as the result of the examinations of organs already mentioned (according to which the groups of bacilli always lie in the blood-vessels—in the liver, for instance), that cultivation experiments carried out with the blood of typhoid patients under favourable circumstances will yield a positive result.

EXPERIMENTS UPON ANIMALS.

The question, whether any species of animal can suffer from a diseased process identical with enteric fever in man, must even now be regarded as an open one, in spite of the numerous experiments already made on this subject.* Eberth† has with justice very recently pointed out that, considering the frequency of typhoid fever, one would expect that our domestic animals must occasionally be infected with the same virus, while as a matter of fact the occurrence of typhoid fever in these is still doubtful. Nor does the author consider as free from objections the observation recently published,‡ according to which four cows in a farm-yard adjoining the seat of a small typhoid epidemic suffered from typhoid fever. The autopsies were made by the district veterinary surgeon, Prümers. Without wishing to enter further upon the numerous opinions expressed in the same sense by earlier authors, I might, however, mention that Professor Schütz,

* The specimens recently shown at the Pathological Society of London by Mr. Bland Sutton demonstrate the fact that monkeys suffer from an affection which clinically and pathologically appears to be identical with typhoid fever in man; whether it be so etiologically was not investigated, the only fact bearing on this point being that typhoid fever was present in the neighbourhood of the Zoological Gardens. —J. J. P.

† Volkmann, *Sammlung klinischer Vorträge*, 1883, No. 226.

‡ *Mittheilungen aus der thierärzlichen Praxis im Preussischen Staate.* Neue Folge, VI., Jahrgang 1879-80, S. 19.

teacher of pathological anatomy at our school of veterinary medicine, has never (according to an oral communication) found changes in animals which he could regard as identical with enteric fever in the human subject, notwithstanding the unusually rich material at his disposal. But these conditions will be anew subjected to a thorough test, since we are now justified in considering a definite micro-organism as the cause of typhoid fever. As Eberth also emphasizes, the main question now is not in regard to the identity of the clinical and anatomical changes, but in regard to the identity of the cause of the disease, *i.e.*, in other words, whether the typhoid bacilli are capable of evoking any diseased process in animals. Before going into my own researches directed towards this point, I may shortly discuss the principal previous experiments.

As early as the year 1867 Murchison endeavoured to infect a pig by feeding it upon large quantities of typhoid stools for a prolonged period. The result was, however, totally negative. During the period of experimentation the animal became big and fat without showing any symptoms of illness. Klein,* in very numerous attempts, fed guinea-pigs, rabbits, dogs, cats, white mice and especially monkeys, with typhoid stools, without being able to bring about any infection. The experiments on monkeys were also without result, even though diarrhœa was set up by the administration of aloes or croton oil previous to feeding them.

At the suggestion of Küchenmeister, Birch-Hirschfeld instituted a large series of experiments on rabbits in the year 1874. Küchenmeister had observed a disease, described by him in 1850, occurring epidemically among these animals, which showed many points of resemblance with human enteric fever. After Birch-Hirschfeld had attempted in vain to produce this disease in rabbits by the subcutaneous injection of typhoid stools or typhoid blood, he succeeded by feeding them with large quantities of typhoid stools. The symptoms of the disease consisted of moderate fever, emaciation and, in many cases, of diarrhœa. The anatomical changes were enlargement of the spleen and swelling of the intestinal gland follicles. In two cases there was also incipient softening (*Verschwärung*) of a patch. In one rabbit, which had eaten the slough of a typhoid ulcer taken from

* Reports of the Medical Officer of the Privy Council and Local Government Board. London, 1875.

a cadaver and which died after an illness of five weeks accompanied by fever, a clean ulcer larger than a lentil was present on a *plaque* on the ileo-cæcal valve. After feeding with large quantities of non-typhoid diarrhœic evacuations, as a controlling experiment, the glands of the animals experimented upon were only slightly swollen and never ulcerated. Birch-Hirschfeld, nevertheless, expresses himself with great caution as regards the conclusions to be drawn from his experiments. Bahrdt* repeated Birch-Hirschfeld's feeding experiments upon ten rabbits with negative results. Four other rabbits also, which were kept in a large clay cylinder on sandy ground richly impregnated with typhoid dejections, remained perfectly healthy. The majority of the animals experimented upon gained in weight.

Inoculations with the blood of typhoid patients were carried out by Motschutkoffsky† both on men and on animals (monkeys, rabbits, dogs, cats), but without result in any case. Calves, dogs, cats, rabbits and fowls were fed upon typhoid motions—sometimes fresh, sometimes putrid—and upon blood from a typhoid cadaver by Walder,‡ without any tangible result being obtained. In one case, however, diarrhœa occurred in a calf after feeding it with putrid typhoid stools. When the animal was killed on the tenth day, besides some punctate injection present in the small intestine, there was marked narrow-like swelling of the Peyerian patches. It is quite obvious that such a condition cannot of itself be considered as a successful transference of typhoid fever.

More recently Klebs§ has communicated a number of attempts to transmit typhoid fever to animals. The cultivations previously described, as well as typhoid dejecta, were made use of in these experiments, which were carried out partly by Klebs himself, partly by Chomjakoff under his direction, the majority being upon rabbits. The infective matter was injected either subcutaneously or into the peritoneal cavity, or was mixed with the food. It would lead us too far if I were to enter into all these experiments in detail. In most cases the result, as Klebs himself admits, was doubtful. In one case only did Klebs, on microsco-

* *Archiv der Heilkunde*, 1876, p. 156.
† *Centralblatt für die medicinischen Wissenschaften*, 1876, No. 11.
‡ *Die Typhusepidemie von Kloten*, Inaug. Dissert., Zürich, 1879.
§ *Archiv für experimentelle Pathologie und Pharmakologie*, Bd. xiii.

pical examination of the intestine of a rabbit, observe conditions which seemed to him decidedly indicative of the infective process. Klebs does not hesitate to interpret the process as identical with that of ileo-typhus in man.

The subject was a rabbit, to whose food a small quantity of a cultivation had been added and about 0·5 c.cm. of the same had been injected subcutaneously. The animal died after 47 hours without any notable elevation of temperature having occurred. At the necropsy, performed six hours afterwards, the spleen was moderately enlarged; the lowest *plaques* in the small intestine were greatly swollen but not ulcerated. The mucous membrane of the vermiform appendage showed roundish and oval prominences of a whitish colour and smooth surface, along with brownish-red staining at various points, which here and there extended into the substance of the mucous membrane. The contents of the gut were rich in bacilli, for the most part short and with terminal spores. In the follicles there were present large numbers of short, rather thick bacilli, some containing spores, usually a central one. The extremities of the bacilli were rounded off but some were pointed. Tracts of extremely delicate, slightly sinuous threads, which entirely filled up the marginal sinus (*Randsinus*) of the follicles, were also present in the hemorrhagic, infiltrated parts at the periphery of isolated follicles. On the ground of this observation, Klebs regards it as a proven fact "that the bacillus typhosus under favourable circumstances, even in the mucous membrane of a rabbit, develops into that form of thread-like mycelium, which, in the human intestine, may permeate the whole thickness of the typhoid patch and fill the blood-vessels."

One can certainly only agree with Eberth when he says in reply,* "I should be very glad to subscribe to this dictum, were it not that one is dealing with a single isolated fact and one which admits of another interpretation. It is, however, quite possible that we have here to do with a mycosis not of a typhoid nature." I might add to this reflection of Eberth's another which concerns the morphology of the organisms found by Klebs in the intestinal mucous membrane of his rabbit. Palpably two quite different forms were present—viz., short, thick, rod-shaped bodies and long, extremely delicate threads—and this apart from

* *Loc. cit.*, p. 2043.

the possibility that the short bacilli with pointed ends represented a third form. The suggestion that these different forms were only different steps in the development of one and the same organism—the typhoid bacillus—is, with perfect justice, open to the same objections as I have brought forward in detail in respect to the condition of the intestine in fatal cases of typhoid fever in man. One can therefore understand that, for the reasons advanced, I also cannot adopt Klebs' view that the transference of human typhoid to rabbits has been proved by the experiments which he has communicated. If we look over the above-mentioned experimental researches, very numerous negative results stand out against a few extremely debateable results and the statement finds full justification that up to the present the production in animals of a diseased process identical with human enteric fever has not been successfully attained. Unfortunately my experiments, which I shall next communicate, in no way alter this fact.

In the first instance I made use of monkeys, exclusively of Java monkeys, of which I had altogether five at my disposal. The pure cultivations described above served me as material for infection. Of course I experimented with cultivations from different cases, some from Berlin, some from the typhoid epidemic at Wittenberg. The bacilli were cultivated on potatoes, or in coagulated blood serum, or in nutrient jelly. In most cases cultivations which undoubtedly contained spores were employed. The greatest importance was, of course, attached to those experiments which had for their object an infection from the intestine outwards. For a long time the monkeys were daily fed upon cultivations containing spores which were administered sometimes mixed with carrots, sometimes with boiled potatoes, with uncooked yolk or white of egg, or with bread, and were taken without resistance on the part of the animal. In two series of experiments boiled potatoes were given as almost the sole dietary and in two others repeated small doses of tincture of opium were administered along with the daily diet of bacilli. The general condition was ascertained by means of observations on the temperature made regularly twice a day during the continuation of the diet and also for a considerable time afterwards. The result of all these experiments was entirely negative. All the animals afterwards died from

general tuberculosis. At the autopsies no changes were found which could be held to indicate a bygone typhoid process. I must leave it undecided whether the tuberculous disease in its earlier stages did not already exist while the animals were being thus fed. I mention this point in reference to the view entertained by several authors, especially at a former period, that tuberculous subjects possess a certain immunity against enteric fever.

In one monkey a large quantity of typhoid bacilli, cultivated on solid blood serum, was injected into the brachial vein. The cultivation was mixed with distilled water until a whitish, cloudy liquid was obtained, of which a Pravaz' syringeful was introduced into the blood current. The operation, performed under chloroform and with the strictest observation of antiseptic precautions, passed off without any result apart from the after effects of the narcosis. In particular no rise of temperature occurred. In one case a cultivation was inoculated in the vicinity of the sternum without the occurrence of any reaction whatever. For the further experiments upon animals 16 rabbits, 18 guinea-pigs, 7 white rats, 11 white and grey house-mice, 4 field-mice, 2 pigeons, 1 fowl and 1 calf were employed. The infective matter was sometimes mixed with the food, sometimes injected subcutaneously into the abdominal cavity or into the blood current. In some rabbits the cultivations were also inoculated on the cornea or introduced into the anterior chamber of the eye. I desist from describing all these experiments in detail, and content myself by stating that almost all the animals were unaffected by the operation, and that in the very few which afterwards died no changes could be found which stood in any certain connection with the infection. In particular, no bacilli could be found in these cases.

The calf mentioned above, a powerful, healthy animal, was repeatedly fed upon great quantities of cultivations containing spores. When it was killed, a month and a half after the last infection experiment, there was nowhere any indication present either of an existing or of a past typhoid process. The Peyer's patches were pale and without any trace whatever of ulceration; the spleen and mesenteric glands were not swollen.

Although the prospect of arriving at a full understanding of the etiology of typhoid fever by means of experiments upon animals appears a slight one on a survey of the results of

former and of my own experiments, still the possibility is not excluded that this object may be successfully attained by altered methods of experimentation or by the employment of other animals.* Till then we must content ourselves with the careful anatomical examination of the organs of fatal cases of typhoid fever and extend further the results thus obtained by accurate cultivation experiments. At all events we are now in a position to be able to examine suspected drinking water, air, or soil for the presence of these organisms and their spores, which we must regard with the greatest probability as the cause of enteric fever.

DISCUSSION OF THE ETIOLOGY OF TYPHOID FEVER FROM THE STANDPOINT OF THE KNOWLEDGE OF TYPHOID BACILLI NOW OBTAINED.

If I make the attempt in the following pages to discuss briefly the principal questions in the etiology of typhoid from the standpoint of the results obtained by research, I do so really in reference to the epidemic to be described in the appendix to this work. I have thought it well to give my views in their entirety here, and to allow the description of the epidemic itself, and of the researches with regard to its origin, to follow in the appendix.†

ETIOLOGICAL SIGNIFICATION OF THE TYPHOID BACILLI.

The question which first comes under consideration and at the same time is the most weighty, is, whether we are now justified in regarding the bacilli, described in detail in the first part of this work, as specific for enteric fever and as its cause. As has already been mentioned, Eberth thinks himself in a position to answer this question in the affirmative, on the ground of his own researches and of those of R. Koch and W. Meyer. Eberth lays particular weight, and with justice, upon the point that the typical typhoid bacilli could never yet be observed in any other morbid process. He himself had examined numerous cases of different diseases, amongst them being twelve cases of

* See the remarks made by Dr. Koch in his second paper on Cholera, which follows later in this book.

† This epidemic occurred in the Military Barracks in Wittenberg, and was distinctly traced to infection of drinking water from latrines. The description of the epidemic is not included in this translation.—J. J. P.

intestinal tuberculosis, without meeting with the characteristic bacillar masses which occur in typhoid, even in a single case. Meyer also, in similar controlling investigations, never encountered bacilli which could be mistaken for typhoid bacilli. I have already stated that the bacilli never have been found in organs of cases not dying of typhoid, in the numerous examinations made at the Office of Public Health.

On the other hand, the bacilli have not been observed in all the cases of enteric fever up to the present. Eberth found them in 18 out of 40 cases examined, Koch in about the half of the cases examined, and Meyer in 16 cases out of 20. A part of these negative results is undoubtedly explained, as Eberth has specially insisted, by the bacilli being generally present in smaller number the older the diseased process. But the circumstance that the bacilli are particularly numerous, and consequently much easier to find, in recent cases is justly considered by Eberth as of value as regards their etiological signification.

A further explanation of the fact that the bacilli have not been observed in a great number of typhoid cases lies without doubt, as I have previously pointed out, in the difficulty of the examination. I would here remind the reader that neither Eberth nor Meyer succeeded in staining the bacilli in sections; and further, that in many of the cases which I investigated the first bacillar groups were encountered only after the examination of a very large number of sections. If one considers these circumstances and further, that the bacilli were observed in 26 out of 28 cases of enteric fever which I examined, one must admit that now we have every justification in assuming the presence of these organisms in enteric fever as constant. We are the more justified in adopting this view, seeing that a positive result has been noted in the cases which I examined only when one of the characteristic bacillar masses had been found in a section of the spleen, liver, kidneys, or of a mesenteric gland, quite apart from the condition of the intestine. But, in regarding this exclusive occurrence of one and the same form of micro-organism in a definite infectious disease we are not dealing with merely a single isolated fact. We already know other different processes of this sort in which the conditions are entirely analogous. Thus quite characteristic, delicate bacilli are always found in leprosy, regarding which it has not

yet been absolutely proved that they are the cause of the disease. And yet no one now-a-days doubts that we have here to do with specific organisms representing the real cause of the disease. The facts are similar in regard to our knowledge of relapsing fever. We only know that in this disease characteristic spirochætæ can invariably be observed in the blood during the attacks, that these have never been observed in any other disease and that the blood when it contains these organisms is capable of transmitting the disease. The exact proof has not yet been brought forward that the spirochætæ are in reality the *causa morbi*. Nevertheless, it is universally considered that they are so. One is the more justified in adopting such a view as, in a considerable number of other infective diseases in which a definite form of micro-organism can always be found, the etiological signification of these has been proved in an exact and incontestable manner. I need only mention splenic fever, tuberculosis, erysipelas and glanders.

As soon as we admit that a typical form of micro-organism is invariably present in enteric fever, which is never present in other diseased processes—and one can hardly doubt it after the foregoing investigations—we may regard these organisms as the cause of the disease with quite as much justice as is now the case with spirochætæ for relapsing fever and leprosy bacilli for leprosy.

ARE THE TYPHOID BACILLI SPECIFIC PATHOGENIC ORGANISMS?

After the question whether typhoid bacilli constitute the cause of the disease, the most important point is undoubtedly whether we have here to do with specific pathogenic organisms, or with such as may be derived, under favourable external circumstances, from similar organisms, devoid of pathogenic properties, more especially from putrefactive bacilli. It is obviously without importance as regards the essential nature of the process whether this progressive development—this attainment of virulence—in an organism harmless in itself, occurs in the sewage, in the ground or in the intestinal canal. Nor is the question at all a new one. Formerly it was put only in this general manner, not prejudging the nature of the morbid process: given the existence of typhoid epidemics, has one always to deal with a virus originating

from a typhoid patient, or can the virus originate spontaneously? For some decades past endeavours have been made to advance reasons and observations, sometimes for the one, sometimes for the other view, but no agreement has been arrived at up to the present. If I range myself upon the side of those who consider the typhoid bacilli as specific organisms and their origin from putrefactive bacilli as at least very improbable, it is upon the following grounds: in almost all the cases of typhoid fever which I have examined, whether they came from Berlin hospitals, from St. Petersburg or from Wittenberg, there was always present a definite form of rod-shaped organism in the internal organs, arranged in quite a characteristic manner and undoubtedly the same as had previously been observed at various times and in various places by Eberth, Koch, Meyer and C. Friedländer. Anatomical examination gives us not the slightest ground for thinking that these bacilli had anything whatever to do with putrefaction. To all appearance they did not proliferate after death, as one does not find that the masses are at all more numerous or larger in cases in which putrefaction has already set in, than in those examined as soon after death as possible. Further, in an organ removed as early as possible from the body, although it may contain ever so numerous masses of bacilli, all coarse signs of commencing putrefaction perceptible to the senses are awanting. In the same way the microscopic appearance of the tissue presents nothing suggestive of that process. The nuclei in the vicinity of the masses stain rather exceptionally deeply with aniline stains, whilst the bacilli themselves, (apart from the conditions found in the intestine,) always form limited masses and never permeate the organ in all directions, as we are accustomed to see in the case of putrefactive bacilli.

I would also adduce as a very weighty argument against the view that typhoid bacilli have any connection with putrefaction, the fact that, when cultivated outside the body, they never become causes of putrefaction, as far as my researches permit of a judgment. I have carried out a great number of cultivations in succession with those derived from thirteen different cases and the bacilli never produced putrefaction in substances extremely liable to putrefaction, in spite of their luxuriant growth. Even when cultivated outside the body for more than a year, nothing

of the sort has occurred. As the bacilli always form the same masses in the internal organs, whether one examines fatal cases of typhoid fever in Russia, Germany, Switzerland or England, so in the same way, cultivated outside the body on the same nutrient matter they have hitherto always displayed the same manner of growth and the same mode of spore development, whether I obtained the cultivations from typhoid organs in Berlin or from the Wittenberg epidemic. I might compare the relationship of typhoid bacilli to putrefaction with that which exists between putrefaction and the exciting cause of another infective disease, viz., septicæmia. For a long time putrefaction and septicæmia were considered as two synonymous conceptions. We are only beginning to separate these ideas since we have been placed in such a position as to recognize the nature of the pathogenic organisms in question by the improvements in our apparatus and methods. The specific exciters of septicæmia certainly often migrate into putrefying fluids and render them infective, as has been shown by numerous experiments on animals, but in themselves they have nothing to do with putrefaction and putrefactive bacilli, as far as our present knowledge extends. When they are not present one may inject subcutaneously into animals putrefying fluids of the most different sorts, in quantities which would amply suffice for the production of infection, without succeeding in producing infective septicæmia. The relationship of typhoid fever to putrefaction is quite similar.

For decades past one has been in the habit of considering the contents of cesspools and of drains as the chief sources of the typhoid virus. There was no unanimity of opinion as to whether the typhoid germs could, under certain circumstances, arise spontaneously in such a putrefying medium or whether the previous infection by means of the evacuations of a typhoid patient was necessary. As the typhoid evacuations, the virulence of which is not contested by an overwhelming majority of physicians, always represent a mass in a condition of putrefaction, the notion lay very near at hand that the typhoid germs stood in casual connection with the putrefaction or, as we should now say, that under certain circumstances typhoid bacilli could originate from putrefactive organisms. As we have seen, the study of the typhoid bacilli presents up to the present just as little ground for such a view as has been found for the origin of

septicæmic bacteria from putrefactive organisms. The argument most frequently advanced against the specific properties of the typhoid virus and in favour of its spontaneous origin, is that in very many instances, even after the most strict investigations, a source of infection has not been discovered in localities previously free from the disease and in which the malady has broken out suddenly and unexpectedly. But obviously this fact is only of doubtful value, when one considers the extraordinary vitality of the spores and the difficulty of excluding with certainty the previous occurrence of cases of typhoid fever. Besides, our experience in regard to other infective diseases is not at all opposed to the view of the specific nature of the typhoid bacilli as exposed in the foregoing. On the contrary, the further our knowledge of the etiology of these diseases has been advanced, the better grounds have been obtained for the view of the specific nature of pathogenic micro-organisms. The sole observation which is totally irreconcilable with such an idea—I refer to that of Buchner, according to which mobile, non-pathogenic hay bacilli were transformed by cultivation into motionless pathogenic anthrax bacilli—has been combated by competent persons and with weighty arguments. A disease, which perhaps affords the best example in connection with this question, is gonorrhœa. Hardly any one now-a-days doubts that the characteristic micrococci, first described by Neisser, represent in reality the virus of the disease, even if one leaves out of consideration the well-known experiment, lately carried out by Bokhard, as being an isolated fact. No one would wish to assert that these micrococci stand in any relation whatever to other similar, but not pathogenic, organisms or that they are developed from them. For although micrococci have been unintentionally introduced innumerable times with bougies and catheters into a urethra, not always intact, they have never provoked that virulent urethritis which we call gonorrhœa. We have also no ground for the view that the bacilli of glanders can be developed in any conceivable space of time from similar but non-pathogenic organisms. The extension of this disease exclusively by contagion is now pretty generally accepted. In the same manner nothing in regard to tuberculosis indicates that the virus of the disease, the tubercle-bacilli, can originate from other organisms. In this disease one has to deal with the same organisms, recognized

by extremely characteristic properties, in whatever part of the world one examines phthisical sputum or tubercular organs. These bacilli have been cultivated by their discoverer through several generations outside the body for more than a year without altering in the slightest as regards their specific properties, or being deprived of their pathogenic nature. Transmitted to animals they always produce the same specific morbid process, while the leprosy bacilli, for example, which morphologically are very similar, in spite of their having been successively cultivated for many centuries on the same soil in man, may be inoculated upon animals without doing harm to them, yet they always produce leprosy in man, and never tuberculosis. I will not multiply these instances here. No agreement will probably be arrived at as yet in regard to the question whether pathogenic micro-organisms in general, and typhoid bacilli in particular, are to be regarded as specific. At all events it can only be an advantage for investigators who study these lower forms of life and for the determination of their relationship to infective diseases, if we regard them as specific, until such time as we shall have more substantial grounds than is the case at present for holding an opposite opinion.

A question, which must be entirely separated from that just discussed, is whether, under certain circumstances, a diminution in virulence may not take place in the case of the typhoid bacilli, in a manner similar to that which can be demonstrated experimentally with regard to the bacilli of anthrax. It is self-evident that I have not been able to carry out researches on this point, as I have not succeeded in producing the disease in animals with my cultivations. Undoubtedly, however, many facts may be adduced from the relative behaviour of individual epidemics which support the idea that the virulent power of the typhoid bacilli is not always the same. The malignancy of some epidemics and the relative benignness of others are, for example, most simply explained by such an assumption.

Finally, I would refer in a few words to the so-called poisoning with meat, as has been observed in a number of epidemics (Andelfingen, Kloten, and others). Obviously I am considering here only these cases in which one was dealing with a really infective, a transmittable disease. I do not refer to such general diseases as are most probably traceable to the action of

chemical compounds like the alkaloids of putrefaction, recently investigated in a thorough manner by Brieger. The dispute, whether or not these were epidemics of enteric fever, cannot now be decided; weighty arguments may be advanced both for and against it. We should, however, find ourselves in a much more favourable position as regards diagnosis if another epidemic of the sort were to break out and an opportunity were afforded for making a post-mortem examination of some of the sufferers. The examination of the organs in sections as well as appropriate cultivation experiments would, beyond question, decide whether an invasion of typhoid bacilli lay in reality at the root of the disease, or whether we were dealing with a process which is produced by other, perhaps also rod-shaped, organisms. Should the latter prove to be the case we must consider the two processes from an etiological standpoint as different, however closely they might agree in their clinical phenomena.

BEHAVIOUR OF THE TYPHOID ORGANISMS OUTSIDE THE HUMAN BODY.

Typhoid bacilli, as we have seen, form spores inside the organs of the patient, especially in those portions of the intestinal mucous membrane which are permeated by them. There can be no doubt that this occurs in the typhoid bacilli present in the intestinal contents. The typhoid organisms are thus evacuated along with the dejections of the patient in their most resistent condition, *i.e.*, as resting spores, and thus pass into cesspools, &c., or into the ground. We must in this wise imagine the further behaviour of these permanent spores outside the economy, that they remain in a quiescent condition for a long time for want of suitable nutriment until they arrive by chance in a body capable of being infected and there developing into bacilli, begin anew their cycle of existence. The experiments we have just communicated make it, however, in the highest degree probable that their development does not occur only in the human organism, but that, like the bacilli of splenic fever, they may sprout and form bacilli in favourable circumstances, even outside the animal economy, may increase enormously in numbers and in the warmer part of the year may form spores afresh.

PATHS OF INFECTION.

After the statement of my researches and of the views based upon them as to the behaviour of the typhoid germs, it is pretty easy to see what position I take up in regard to the theories, still warmly disputed at the present day, viz., the so-called drinking water and ground air theories. According to my opinion the most different paths stand open to the infecting germs for admission into the human organism and it would be one-sided to fix one's attention solely on one or another of these. Besides the air which we breathe and the water which we drink, our food may evidently, under certain circumstances, be the carrier of the typhoid virus. These cases are particularly striking in connection with the occurrence of infection through the medium of the atmosphere, in which articles of clothing, linen, &c., soiled with typhoid dejections have excited the disease in other individuals. Such cases can scarcely be explained otherwise than that portions of the desiccated fæcal masses had been transferred to the atmosphere in the form of dust and conveyed through the air to the individuals in question. As regards the relative frequency of the occurrence of infection by the air and by drinking water, I entirely concur in the arguments developed by Virchow,* more than ten years ago, in which he maintained that its accomplishment by means of drinking water is by far the most frequent. Virchow said, "I further pointed out that in typhoid epidemics the area of the disease was no such widespread one as in cholera—in which the cases frequently occur scattered throughout a whole town—but that almost always small tracts, as I called them 'typhoid-islets,' are present, isolated houses or groups of houses, so that a much more accurate specialisation of the cases was necessary. That also seemed to me to be an argument that in typhoid it is much more frequently the drinking water which conveys the virus of the disease than the air and the ground water, and that if the ground water becomes infected the virus probably gets to the nearest springs much sooner, and is there carried off to a certain extent. At least one ought

* "Canalisation oder Abfuhr, eine hygienische Studie," Virchow's *Archiv*, Band 45, Heft II. S. 294.

to expect a much greater dissemination of the disease in the event of an infection which essentially affects the ground water. I further added that it is altogether very much more simple to think of the direct reception of the 'materies morbi' into the digestive tract than to invoke the agency of the respiratory process. The striking limitation of the anatomical changes to a deeply situated portion of the intestine, the lower end of the ileum and cæcum, seems to indicate that a local action of the morbific matter takes place, as these are exactly the situations where the contents of the intestine are relatively most frequently retarded, where therefore the longest contact with the mucous membrane occurs. This consideration harmonizes best with the inception of the typhoid matter in drinking water."

I should like to prove by means of an example that many of the facts which are considered as eminently in favour of the occurrence of infection by means of the ground air, are certainly capable of another construction on objective examination. Particular importance is laid by the adherents of the ground air theory upon the frequently observed occurrence of epidemics of typhoid fever in connection with a fall in the ground water level. It is thought that those strata of the earth which came in contact with the ground air in a moist state, as the result of the recession of the water, must be regarded as the situations in which, besides many other decompositions, the formation or reproduction of the typhoid virus takes place and that the infection of the air breathed is thence brought about. The fact that the rise and fall of the ground water may alter the conditions of wells as regards the possibility of infection has not received the attention it deserves according to my opinion. I think that for a considerable number of cases, at least, the following explanation seems the most likely; the more the ground water sinks, *i.e.*, the less water is available for the feeding of the springs, the more quickly, more surely and in more concentrated form must (supposing the ground to be permeable) all the impurities saturating the ground in the vicinity of the springs be mixed with the drinking water, as the spring sucks up all the liquid in its neighbourhood. To take the simplest case: the contents of a leaky cesspool known to be infected with typhoid stools soak into the permeable ground, and must naturally reach the ground water. When the ground

water stands at a high level this material is not only incomparably more diluted, but is carried away by more powerful currents necessarily existing under these circumstances. On the other hand, the lower the level of the ground water and the greater its consequent stagnation, the more certainly must we expect that a greater or smaller proportion of the typhoid germs which obtain entrance into the ground would be sucked into the springs and, obtaining entrance into the body along with the drinking water, would give rise to new cases of disease. It must be added that in most cases a low level of the ground water occurs along with a great consumption of water, a condition which must be taken into consideration especially in barracks and other crowded institutions. The epidemic described at the end of this work* seems to me to afford some grounds for the correctness of this view.

I need scarcely point out that in this detailed exposition it has not been my wish to add a new theory to those which already exist. The question in my opinion has only to do with the determination of conditions really existing and very simple, which undoubtedly may render comprehensible the striking co-existence of epidemics with a low ground water level, at least in a number of instances.

The views upon the question as to the organ in which the typhoid germs which have once entered the body first settle, and from this their primary seat of invasion extend and bring about the general disease, have lately become more and more clear. An overwhelming majority of physicians now search for the point of invasion in the intestine. With regard to these matters I can fully accept the statements made by Eberth† quite recently. Eberth lays particular and justifiable stress upon the case observed by Meyer‡ (No. 13 in his table), in which death ensued on the second day of illness. In this case there were found at the post-mortem examination hyperæmia of the lungs, spleen and kidneys, in the lower portion of the ileum marked swelling of the solitary follicles and patches of Peyer, but nowhere any trace whatever of necrosis or of loss of substance. None of the mesenteric glands were swollen. In

* *Vide* foot-note, page 245.

† "Der Typhus-Bacillus und die intestinelle Infection," Volkmann, *klinische Vorträge*, 1883, No. 220.

‡ *Loc. cit.*

this quite recent case microscopical examination revealed a very exceptionally large deposit of the bacilli of Eberth and Koch in the cells of the submucosa and in the intermediate muscular layers. Several hundred bacilli lay in each field. Eberth thinks that after the discovery of such a condition scarcely any doubt can exist as to the path which the infection takes, viz., that the bacilli are first localized in the intestinal mucous membrane, that thence they pass into the mesenteric glands, thence into the blood-stream and accumulate again in the spleen and, as the author would add, in the other organs. Eberth further points out that anatomical investigations have afforded no evidence of the admission of typhoid germs through the lungs.

It is without doubt necessary that many more cases of typhoid fever which have proved fatal at a very early period should be examined, as in the case communicated by Meyer, before we can decide with certainty whether that path of infection forms the rule. Even now we must consider it as highly probable, or at least the possibility cannot be contested, that the lungs may occasionally represent the seat of invasion.

The feeding experiments carried out in the Office of Public Health with anthrax spores, given in detail in this volume, seem to me of paramount importance for an understanding of the process. Anthrax spores, in some cases in large quantities, in some cases in very small quantities, were administered to sheep along with their food, in a manner which excluded all injury to the cavity of the mouth. In the former case all the animals, without exception, died of splenic fever in a few days. On the other hand, of ten sheep, each of which received daily a small silk thread, impregnated one year previously with anthrax spores and kept in a dry state, one succumbed on the fifth, sixth, eleventh and nineteenth day, respectively, after the commencement of the dietary which was not continued for a further period. In all the cases which terminated fatally, the autopsy left no doubt that the infection had taken place from the intestine outwards and the microscopical examination rendered it probable that the solitary follicles and Peyerian patches formed the seat of invasion. And, indeed, these researches seem to me to present a complete analogy for the path of infection, such as we must suppose to be the rule in enteric fever. Whether it be

that the spores of the typhoid bacilli are taken up in drinking water, or, in rare cases, along with articles of food; whether it be that they are inspired with the air breathed, remain attached to the mucous membrane of the mouth and throat and are afterwards swallowed, in all probability they pass without damage through the stomach, sprout to form bacilli in the alkaline contents of the intestine, multiply there and penetrate into the intestinal mucous membrane at those spots which are most adapted for their reception, viz., the Peyer's patches and solitary follicles. Afterwards they arrive in the mesenteric glands, where they form the very numerous characteristic masses, and are then carried away in the blood current into the other organs. Becoming fixed here and there in these, they multiply to form those groups, the almost constant presence of which in the spleen, liver and kidneys has been described in detail in the earlier part of this work. Obviously the infection will be more certain to ensue the greater the number of spores taken into the body. In regard to this point also the conditions may be quite analogous in typhoid fever to those briefly sketched in connection with feeding experiments carried out with anthrax spores.

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ON ERYSIPELAS.

By Dr. FEHLEISEN.

TRANSLATED BY

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ON ERYSIPELAS.

THE word ἐρυσίπελας is used as early as Hippocrates, but its derivation cannot with certainty be determined. The simplest derivation would be from ἐρυθρός, red, and πέλλα, skin, and this is the nearest approach to the meaning which we attach to the word at the present time.

It is very tempting to translate phrases such as φλεγμονή καὶ ἐρυσίπελας* as inflammation and redness of the skin. An argument against the derivation from πελλα, however, is the fact that Hippocrates does not designate diseases of the skin only by the word ἐρυσίπελας, but on the contrary speaks much more frequently of an ἐρυσίπελας ἐν τῷ πλεύμονι and ἐν τῇ ὑστερῃ than of an erysipelas of the skin.

A more likely derivation would be from ἐρυθρός and πέλας—tumour or swelling—which Billroth has suggested. I would prefer to trace the word from ἐρυθρός and πελός—*pallidus, lividus.* In support of this may be cited particularly a passage from Galen, where it is said of phlegmon and erysipelas: "dissident primum et maxime colore. Quum enim is ruber sit, phlegmone affectum appellant, quum pallidus flavusve vel ut ex pallido flavoque colore mixtus, erysipelas."

As already mentioned, Hippocrates comprises under the name ἐρυσίπελας not only almost all acute inflammatory affections of the skin and the subcutaneous connective tissue, but also various kinds of inflammation of the internal organs. Nevertheless there can be no doubt that he was well acquainted with the true rose or genuine erysipelas of modern authors. He describes it so minutely in the third book on epidemics that no mistake is possible. He says that at certain seasons of the year erysipelas becomes epidemic. He mentions that it often originates from small inconspicuous wounds, sometimes even arising without

* Hippocrates, ἐπιδημίων τὸ τρίτον, τμῆμα τρίτον.

any visible injury, that it preferably attacks the head, and that it does not originate from neglected wounds only, for even the most careful treatment does not prevent it, an opinion which to-day is shared by many surgeons. As regards its ætiology, Hippocrates mentions only certain meteorological conditions, and he believes that the "rose" arises by a determination of blood to the affected part, but he does not give any further explanation of the cause of this.

Galen did not, like Hippocrates, use the expressions ἐρυσίπελας and φλεγμονή as synonymous, but distinguishes clearly between "rose" and phlegmon, concerning the last of which he says: "alte magis in corpus demittitur"; whilst of erysipelas "in cute vero potius constitit"—"affectio cutis solius est." On the other hand, Galen often confused erysipelas with different kinds of skin diseases, of which he describes herpes only as a special form. Galen considered that erysipelas was caused by an abnormal condition of the blood. He says: "A biliario sanguine generationem obtinet." This view of the matter was for a long time accepted, and it was reproduced by nearly all the authors of the last century, and even of the first half of this century. For instance, Heister* thinks that it is produced not only by cold, &c., but also by a "thick, heated, and acid state of the blood, which readily produces stagnation and inflammation."

Callisen† among other things gives as a common cause of rose, "suppressed sweat and excited bile." He was the first, moreover, who gave a definition of erysipelas approximately coinciding with the modern clinical conception, and in which it is characterized as an inflammatory swelling "which involves the surface of the skin, and does not invade the underlying parts, but rather spreads on the surface and changes its position." Further on he says, "Seldom or never is a true suppuration to be expected in rose, unless in some accidental manner it becomes a common inflammation."

Richter‡ says, "Rose is a spurious and commonly a biliary inflammation. It generally arises from two causes, namely, bilious acidity and suppressed perspiration. The bilious acidity

* L. Heister's *Chirurgie*. Nürnberg, 1770.

† Callisen, *Einleitungssätze in die Chirurgie*, Deutsche Ausgabe. Frankfurt and Leipzig, 1783.

‡ August Gottlieb Richter, *Anfangsgründe der Chirurgie*. Göttingen, 1787.

is for the most part found in the prima via, but a part of it appears to be absorbed into the circulation, and deposited at the seat of the external inflammation."

Rust* viewed the matter from a similar standpoint, and moreover did good service with regard to the definition and differential diagnosis of erysipelas, by separating not only all deep-seated phlegmonous processes, under the name of pseudo-erysipelas, from true rose, but also by distinguishing it from the acute exanthemata, miliaria (*Friesel*), and such like, with which it was formerly often confused. In reference to the ætiology he mentions, besides the individual predisposition, the *constitutio annua* as of the greatest importance, but says further on, "Not less important are emotions, fright, annoyances, anger, which can be easily explained, because from the advantage derived from the employment of purgatives one obtains the clearest indication that the 'rose' is to some extent connected with the bowels, especially with the secretion of bile."

We can see, therefore, that the old theory of Galen, according to which erysipelas was a natural consequence of a disturbance of the bile secretion, governed even then the views of physicians to such an extent that they practically overlooked its contagiousness. Owing to this also the able paper of Henle "On Contagion and Miasma and Miasmatic Contagious Diseases,"† remained at first without influence on the ideas in vogue as to the nature of erysipelas. Henle concluded from theoretical grounds that contagious diseases must be caused by organized contagia, which he considered were probably of the nature of low vegetable organisms. He further added that these parasites need not necessarily be so small that the magnifying power of our microscopes was not sufficient to demonstrate them, but perhaps they escaped observation only because of the difficulty of distinguishing them from the surrounding tissues, a supposition which has recently been brilliantly confirmed by the discovery of the tubercle-bacillus.

Henle's ingenious and able theory quickly met with great approval among physicians, but still no one thought of extending it to erysipelas, which even then was regarded, as before said, as not contagious. At least we do not find in the best known German works on surgery up to the beginning of the

* Rust, *Handbuch der Chirurgie*, 1832. † Berlin, 1840.

sixth decade of this century any reference to the infectious nature of erysipelas.

A. G. Richter, C. J. M. Langenbeck, Rust, and others, do not seem aware of it. Even M. J. Chelins says in his Handbook, which appeared in 1851, "The real cause of this (the true) 'rose' is irritation of the bile, disturbance of the function of the liver, accumulation of gastric impurities, obstruction of the portal system, and a peculiar prevailing wind and condition of the weather."

In England, however, by this time the contagious character of erysipelas had long been known, while in Germany it had been overlooked in an astonishing manner. Wernher* in the latter country was one of the first who disputed the bilious nature of erysipelas, which latter theory had still a warm defender in Schönlein. Wernher says in connection with the theory of the gastric bilious origin: "It appears to me, on the one hand, that too much importance has been attached to an old-established systematic theory, and on the other that effects and complications have been taken for causes, symptoms interpreted wrongly, and the actions of medicines misconstrued." Furthermore, he adopts the opinion of the English physicians concerning the contagiousness of erysipelas which was then much disputed by many German authors.

In France also about that time it was not universally accepted, although Velpeau had acquired great credit for his ideas on the ætiology of erysipelas. In opposition to the prevailing opinion he sought the cause not so much in individual predisposition and constitutional anomalies, but rather accepted as *causa efficiens* a material coming either from the outside or originating in the diseased tissues. Like Trousseau he laid great stress upon the fact that there may almost always be observed a solution of continuity of the skin, often insignificant, which forms the starting point of the "rose." This last fact was very emphatically pointed out by Volkmann,† and erysipelas was characterized by him as a true traumatic infective disease; that is to say, as "a local disturbance caused by the action of a special poisonous material." As regards the nature of this poison, Hüter started the hypothesis that here also we have to do with a living virus, with one

* Wernher *Handbuch der allgemeinen und speciellen Chirurgie.* Giessen, 1862.
† Pitha-Billroth, 1869.

of those low organisms of the class of so-called schizomycetes (*Spaltpilze*).* He thought also that he had found the germ of erysipelas in the form of "little actively moving cocci" in the blood, and in the little drops of clear fluid† which could be squeezed out of the erysipelatous skin when pricked with a needle. Later on Lukomsky, Billroth and Ehrlich, Tillmanns, M. Wolff, and others published the discovery of bacteria in erysipelas. Lukomsky‡ found, in the post-mortem examination of those who had been affected with erysipelas, micrococci in the blood and lymph-vessels of the skin and subcutaneous tissue, as well as in the capillaries of the kidneys and muscular substance of the heart. In phlegmonous erysipelas Billroth and Ehrlich§ noticed bacteria in the blood and lymph-vessels of the skin, in the subcutaneous tissue, in the capillaries of the liver and kidneys, as well as in the urinary tubules. Tillmanns‖ observed the connective tissue spaces, the lymph-vessels and smaller blood-vessels of the skin filled with a growth of micrococci. He remarks, however, particularly that they were chiefly found in connection with "pyæmic erysipelas," and he thinks that in the pure form, as a rule, micro-organisms were not present. Consequently, like Billroth and M. Wolff,¶ he denies that erysipelas depends upon an invasion of bacteria. The last-named author had also found micrococci as well as bacilli in the blood of erysipelas patients, but he did not consider them to be the true cause of the disease. He only attributed to them the roll of carriers of an unorganized poison, which, however, might occur independently of bacteria and by itself cause erysipelas.

Finally, a number of observers have discovered bacteria of different kinds in the contents of erysipelatous vesicles. But all these discoveries were not sufficient to give a definite explanation of the nature of erysipelas. In order to establish the parasitic nature of a disease it is not sufficient to demonstrate the existence of bacteria in the organism, nor is it enough, as R. Koch rightly points out, to find them in every case of the disease in question; but what appears to me the principal thing, there must also be

* Berlin, *Klin. Wochenschrift*, 1869, No. 33, s. 357.
† Hüter, *Grundriss der Chirurgie*, 1880.
‡ Virchow's *Archiv.*, Bd. 60.
§ Billroth und Ehrlich, Langenbeck's *Archiv.*, Bd. 20.
‖ Tillmanns, *Erysipelas*, *Deutsche Chirurgie*, 5 Lieferung, 1880.
¶ M. Wolff, Virchow's *Archiv.*, Bd. 81.

proved to exist, for every individual infective disease, a specific and morphologically distinct micro-organism. None of the investigators who have published researches on this question have given a minute description of the bacterial forms observed by them, owing partly to the defectiveness at that time of the method of investigation, and partly to the botanical theories held by the authors in question. The great majority of scientific men at that time agreed with Nägeli in considering all the different forms of schizophytes as modifications of one or of a few species, which in the course of generations assumed alternately various forms differing morphologically and physiologically. On account of this it is easy to understand that they took no special trouble to determine accurately the morphological characters of the bacteria which they found. Nägeli says "schizophytes change into one another; the malarial bacilli originate under favourable conditions from putrefactive bacilli, or from some other widely disseminated schizophyte, and return to the latter under opposite conditions." Thus the existence of a specific disease germ was denied, and it was concluded that one and the same schizophyte can produce erysipelas when vegetating in the superficial layers of the cutis, phlegmon when introduced into the subcutaneous tissue, and again in other cases can even cause a septicæmia and pyæmia. If one disputes the specific nature of the virus, one must consequently deny the ætiological difference between the morbid processes in question, and so it happens that various authors, especially Tillmanns, have of late associated erysipelas with progressive phlegmon and acute purulent œdema, as well as with pyæmic and septicæmic processes, in other words, with diseases which, as Volkmann has pointed out, are entirely different as regards their external appearances, progress, significance, and therapeutic indications.

Erysipelas is distinguished in uncomplicated cases by a characteristic morbid appearance and by a definite clinical progress, and it was characterized in the same manner at the time of Hippocrates and Galen as in our own day, and I think that a contagium which has existed unchanged for centuries must at least have attained a certain stability of form. Henle says in his pathological researches:* "I have classed the miasmatic contagious diseases as the naturalist his species; as

* Berlin, 1840.

something, at least in their present nature, constant and unchangeable; and really the more characteristic a miasmatic contagious disease is, the more certainly has it existed in essentially the same form since the time to which historical research extends. When a disease appears with such specific characters we are justified in considering it a distinct species, and its origin as something constant and unchangeable." The pathogenic bacteria are certainly not quite so unchangeable as Henle thinks, or at least not all of them. Toussaint and Pasteur have found that the bacillus of anthrax can lose its poisonous properties under certain conditions, and that it is possible to cultivate a physiological variety which is no longer pathogenic. The bacillus of anthrax does not however in any way change its morphological characters during this process, but on the contrary remains constant in form when subjected to Pasteur's new method of cultivation; and this is only one more reason for regarding it for the present as a constant morphologically unchangeable species.

The task which I set myself in these investigations was in the first place to determine whether the same special kind of bacterium could always be demonstrated in erysipelas; and in the second place, if this were the case, to investigate if these micro-organisms stood in an ætiological connection to erysipelas.

I have already published *in extenso* the results of my investigations with regard to the first part of this task.* I have altogether examined thirteen cases, two of which terminated fatally, and in the remaining eleven small pieces of skin were excised, which were quite sufficient for the investigation and did no harm to the patients. The results agreed in all the cases. The lymph-vessels of the skin as well as those of the subcutaneous cellular tissue, but more especially those of most superficial layers of the corium, were found filled with micrococci growing in chains. In those places in which a particularly great development of the micrococci had taken place they were found lying in the lymph spaces and channels of the skin. (Fig. 12, Plate IV.) They never entered the blood-vessels however, and this fact I would like to emphasize as being directly opposed to the statements of Lukomsky, Billroth, Ehrlich, and Tillmanns.

In the quite recently affected parts of the skin, which do

* *Verhandlungen der Würzburger medicinisch-physikalischen Gesellschaft*, 1881, *und deutsche Zeitschrift für Chirurgie*, B. xvi.

not as yet present any change to the naked eye, no alteration is discernible in the tissues. In the next zone, however, near the sharp edge of an erysipelas marginatum, the inflammatory changes begin. The tissue of the cutis appears swollen, and along the lymph-vessels, which are filled with micrococci, there is a more or less extensive small-celled infiltration. In still older parts of the affected skin there is only a small-celled infiltration and micrococci can no longer be demonstrated. With regard to the methods of investigation, as well as to the histological details, I must refer to my earlier works. R. Koch* has published quite confirmatory results, and his excellent photographs make further description unnecessary. (See Plate IV., figs. 11 and 12.)

On the strength of these investigations I even then believed that I was justified in declaring that these micrococci were specific pathogenic schizophytes. But in order to determine this with absolute certainty it was necessary to cultivate them external to the human body. In the first place, Koch's method of cultivation on solid culture media is especially indispensable for the morphological determination of micrococci, because the minute spherical individuals of the different species, which are just visible in many cases, are not easily distinguished from one another, whilst the mode of their growth, the difference in form and the peculiar formation of their colonies become especially clearly visible on the transparent nutrient jelly, and generally give very marked distinguishing characters for each species. Moreover, the experimental investigation of the question whether the above-mentioned bacteria were capable of producing by themselves a true erysipelas could not be determined without pure cultivation.

At first I endeavoured to obtain the micrococci from the contents of freshly opened erysipelas vesicles, but always without result. Some of the vesicles examined contained only clear serum without any admixture of micro-organisms. In other cases erysipelas bacteria were indeed present, but so many different kinds of micrococci and bacilli were mixed with them that I failed to obtain a pure cultivation, because the non-pathogenic bacteria, which were present in excess and were capable of multiplying much more rapidly, grew to the exclusion

* [illegible], vol. ii. 1881.

of the others, and perhaps also because the erysipelas micrococci had already died out. Moreover I have convinced myself, by the examination of the contents of the vesicles of burns, that a chain-forming micrococcus is sometimes present in them which is very difficult to distinguish from the micrococcus of erysipelas. In order to avoid any possible mistake I used for my further researches only small pieces of skin cut out with heated scissors after the surface had been thoroughly cleansed and disinfected. I placed them on nutrient jellies of various composition and on coagulated blood serum. After many failures I finally succeeded by placing the small pieces of skin in the nutrient jelly, liquefied at 40° C. in order to bring them into better contact with the nourishing medium than is possible by merely laying them on gelatine or on coagulated blood serum.

The test tubes were kept for two hours at the temperature of the body, then, allowing the gelatine to become solid, I preserved them at a temperature of about 20° C. After two days there appeared on the cut surface of the tissue small white points, which grew slowly, and finally formed a delicate white layer. Then I inoculated a number of tubes containing gelatine with this culture, which had been first examined microscopically as to its purity. After 24 to 30 hours small white granules were observed forming along the needle track, which soon ran together and formed an opaque white layer.

At the end of about six days the growth came to a standstill and did not further increase. During the space of two months I cultivated 14 generations in this way. Dr. Koch and Dr. Gaffky, who were kind enough after my departure from Würzburg to undertake the preservation of the pure cultivation, have continued to cultivate it through many generations, and found that it grows particularly well at the temperature of the body on coagulated blood serum. On the latter the erysipelas micrococci form a white layer easily removable from the surface, and the cultivations cover a larger area than on the nutrient jelly, and do not cease growing so soon. The mode of growth on the artificial cultivation media is quite characteristic for the micrococci of erysipelas. In the pus of wounds, in pyæmia, in phlegmonous processes and such like, chain-forming micrococci occur which as single individuals I readily admit cannot be distinguished, and even as chains are very difficult to distinguish from the micro-

cocci of erysipelas, but when placed on a suitable artificial culture medium they show such different conditions of growth that they cannot be confounded with them.

After it had been established that the micrococcus which is always present in erysipelas differs morphologically from similar micrococci, there still remained the need of proving that when inoculated on healthy individuals it would be capable of producing genuine erysipelas. I will first give the results of some experiments on animals, for which rabbits only were used. In all, nine rabbits were inoculated. One of them did not take, the only effect after repeated inoculations being a slight reddening in the neighbourhood of the points of inoculation, together with a hardly perceptible rise of temperature. The other eight took a typical erysipelas. Six of the animals were inoculated on the tip of the left ear in four different places. After 36 to 48 hours the temperature rose 1·0° to 1·5° C., and a sharply defined reddening spread from the points of inoculation, especially in the direction of the course of the veins, and quickly advanced as far as the root of the ear. The reddened part felt warmer to the touch, but there was no appearance of an œdematous swelling, such as occurs, for example, in "erysipelatous processes in rabbits."*

On holding the ear to the sunlight the affected zone appeared of a beautiful light red colour; its vessels were distinctly visible, and appeared dilated in contrast to those of the healthy ear. After two or three days the process reached the root of the ear, and, as the ear became pale again, spread to the head and neck, where, however, the margin of the redness was not so sharply defined and distinct as on the ear. Another rabbit was inoculated on both ears, and on each side erysipelas set in, which spread to the back of the neck and there united with that of the other side. In other respects the course was the same as in the first six animals. In the last animal experimented upon, on the third day after inoculation, when the erysipelas had spread from the tip of the ear to a little over the middle, the whole ear was amputated with a Pacquelin thermocautery. After 12 hours the animal's temperature became normal, and it remained perfectly well. The duration of the whole process, with the exception of the last case, was 6 to 10 days, and in every case had a favourable termination, none of the animals dying.

* R. Koch, *Untersuchungen über die Aetiologie der Wundinfectionskrankheiten*, 1878.

Opportunity for anatomical investigation was afforded in the last case, in which the ear was amputated. It became paler at once, the redness being no more apparent after the amputation than is the case after death in human beings. On section the lymph-vessels appeared filled with micrococci. The microscopic appearances coincided completely with the results obtained in the case of man, and the identity of the process induced in rabbits with that of human erysipelas is the less open to dispute as the course of the disease was quite analogous in the two cases.

In contradistinction to the pseudo-erysipelas produced by the application of putrid fluids by Lukomsky, Orth, and others, true erysipelas of the rabbit is characterized by a sharply defined fugitive redness, by the healing of the inoculation punctures *per primam*, by the absence of suppuration and by the quick and complete *restitutio ad integrum*. With regard to the statement of Ziegler, that "a fatal termination was the rule with rabbits,"* I can only repeat that none of my rabbits died; and the suspicion that Ziegler had not to do with a true erysipelas is all the more probable in view of his statement that "the micrococci act so as to produce necrosis of the tissue." This is decidedly not correct, for the tissue of the skin shows, on the contrary, a decided capability of resistance to the erysipelas micrococci. A necrotic disintegration of the skin, or of some parts of its tissue, does not occur as a rule, and the occurrence of suppuration, or even of gangrene, is one of the rarest exceptions.

One must guard especially against a confusion with erysipelas-like diseases in rabbits, as these animals have shown themselves to be very susceptible to bacterial diseases of the most diverse kinds. Thus, two diseases in rabbits caused by bacilli have been observed by Koch and Löffler, which present so thorough a resemblance to erysipelas that Koch described one of them without hesitation as an erysipelatous process. This erysipelatous process in rabbits differs, however, from true erysipelas not only in its specific virus, but also by its more unfavourable prognosis, and in many respects by its different clinical appearance. Thus, for instance, the ear in rabbits in true erysipelas remains unchanged in its shape, but in the erysipelatous process it becomes thicker and flabbier, and the tip hangs down. By transmitted light the affected part appears dark red, and no vessels are seen shining through, while

* Ziegler, *Lehrbuch der patholog. Anatomie*, 2 Aufl., 1882.

the ear of a rabbit inoculated with erysipelas, when held against the light of the sun, appears bright red, and shows dilated vessels.

I think I may omit the full details of the various experiments on animals, and give instead, all the more exactly, the clinical history of the cases in which man has been inoculated with erysipelas, this being more important. Erysipelas is in truth the only severe disease which may be accompanied by therapeutic advantages, notwithstanding the dangers associated with it; and it is, along with vaccinia, probably the only infective disease the artificial production of which has up till now been suggested as a means of cure.

The first accounts of the curative effects of erysipelas date from the 17th century. I pass over the communications which were made concerning its favourable effect on mental diseases, neuralgia, typhus, and acute rheumatism, because they are too few in number, and therefore might rest on chance. I shall also omit the descriptions of its curative effects on chronic joint affections, and different forms of syphilis, although, particularly in connection with the latter, many reliable accounts exist. The curative effects of erysipelas on lupus are, however, well guaranteed by a number of trustworthy and experienced observers, of whom I need only mention Hebra; and also it is undeniable that many tumours have entirely disappeared by the action of erysipelas. Mistake is impossible as to this, because such a degeneration of new formations does not otherwise occur. Many swellings of the skin, epithelioma, keloid, carcinoma of the mamma, and lymphatic gland enlargements of various kinds have been partially or entirely absorbed as a result of an attack of erysipelas. The first attempt to employ erysipelas as a curative agent was made by Ricord and Després, who endeavoured to produce artificial erysipelas in phagedenic chancres. With great zeal W. Busch followed up the idea of healing, by means of erysipelas, malignant new formations of the lymphatic glands which were unsuitable for operation. He was successful in infecting a patient by placing her in a bed in which patients with open wounds usually became attacked by erysipelas. The desired result took place, and the swelling, which was an extensive lympho-sarcoma of the neck, disappeared all but a small portion, which, however, again enlarged. The result was

thus only a partial one, but at the same time it encouraged further trials.

The first case* in which I had the opportunity of producing a so-called *erysipèle salutaire* by means of the pure cultivation was in the case of a patient 58 years of age, under the care of Professor von Rinecker. She was suffering from multiple fibro-sarcoma of the skin. There was a large number of nodules in the left gluteal region, which formed a sack-like dependent tumour of considerable size and weight, so that the patient was much inconvenienced in standing upright, and especially in walking. On the 21st of August, 1882, at 3 P.M., a fresh cultivation of erysipelas micrococci of the fourth generation was inoculated on this tumour by making five superficial scarcely bleeding scarifications with the lancet. As indicated by the temperature chart the temperature was slightly increased next morning (37·5° C.), and about 10 A.M. a slight rigor took place. During the day the patient complained of loss of appetite and headache, and in the evening the temperature rose to 38·8° C. The punctures showed no change beyond a slight reddening. On the third day the patient was without fever, and felt bodily well. At 4 A.M. on the fourth day, 61 hours after the inoculation, a rigor occurred. The morning temperature rose to 40·5°, and at the morning visit there was apparent a slightly elevated sharply defined redness about half the size of the hand, a typical erysipelas marginatum. The erysipelas was separated from the punctures by an apparently normal piece of skin about 3 ctm. broad. Perhaps this erysipelatous eruption close to and not at the punctures is connected with the exceptionally long incubation stage, to which I shall presently refer. Moreover, the commencement of erysipelas a few centimetres from the wound is not very uncommon, and has been mentioned by others, especially by Roser. In the course of the fourth day the erysipelas spread decidedly, and the evening temperature was 40·6° C. During the next two days the temperature curve indicated constant high fever. On the evening of the sixth day there was a threatening of collapse which made the administration of stimulants necessary. On the evening of the seventh day the temperature rose to its highest point, 41·6°. The

* *Veröffentlicht in den Sitzungsberichten der Würzburger medicinisch-physikalischen Gesellschaft*, Sept., 1882.

erysipelas spread over a surface of more than one square foot, around which there appeared numerous large red patches of an irregular and serrated form. In its further progress the erysipelas spread slowly, and the temperature remained high. On the eleventh day the edge was less sharply defined and the redness began to decrease, but it was still distinctly visible on the thirteenth day, when the temperature again rose in the evening to 40·0° C. On the fourteenth day the evening temperature marked 38·4°, and from the fifteenth day it remained normal. Even during the first days the superficial-lying nodules became somewhat softer, then a shrinking set in, and finally some of them disappeared. On the other hand the principal mass of the tumour in the gluteal region swelled considerably and became distinctly heavier while the erysipelas lasted. After it had run its course there occurred, it is true, a partial degeneration, but the therapeutic result in this case was not so evident as to make it advisable to repeat the inoculation, especially as the collapse which occurred during the erysipelas made caution necessary.

The second case was a patient 49 years of age, who had already been operated on three times for carcinoma of the mamma, the last occasion being on the 29th of December, 1880. In the spring of 1881 she again noticed a small lump in the cicatrix, but she would not again consent to an operation, notwithstanding the surgeon's advice. When I was in possession of the pure cultivation the proposition was made to her that she should allow herself to be inoculated, and to this she consented without hesitation. There was found in the old scar a tumour of about 5 to 6 ctm. in diameter, firmly adherent to the reddened skin, and downwards and outwards from the same several small nodules about the size of a hazel-nut could be felt. On the 15th September, 1882, at 11 A.M., the inoculation was made in five places with a cultivation of the ninth generation. Next morning the temperature was somewhat elevated (38·0° C.). Three of the inoculation punctures were each surrounded by a pale redness about the size of half a crown, which disappeared on pressure and was slightly painful. The general health remained undisturbed until 5 P.M., when, thirty hours after the inoculation, a rigor occurred. When I saw the patient about half an hour later the erysipelas had already spread over

the whole swelling, and had reached the size of about twice the palm of the hand. The temperature was 40·5° C., and there were several attacks of vomiting.

17th September. Extension of the erysipelas over the whole of the right half of the thorax as far back as the posterior axillary line. Pulse frequent, sometimes intermittent. Headache severe.

18th September. Sleep disturbed during the night, and at times slight delirium.

19th September. The erysipelas had extended in front as far as the left edge of the sternum, and behind as far as the vertebral column. The patient complained of a sharp pain during deep inspiration, and an examination demonstrated a slight pleuritic effusion on the affected side. The tumours had distinctly lessened in size.

20th September. A restless night, and continuous headache. The larger lump only measured about 3·5 ctm. in diameter, and the smaller ones could no longer be felt.

21st September. Difficult respiration, and the pleuritic effusion reached to the lower angle of the scapula. The erysipelas extended behind beyond the spine, and in front invaded the left breast. In some places vesicles formed. The pulse was small and intermittent. Camphor was ordered internally.

23rd September. The patient was free from fever, and felt well. The redness was somewhat less marked. During the evening the temperature rose again to 38·5° C. The tumours had entirely disappeared, and the skin lay perfectly flat on the thorax where formerly there was a hemispherical swelling. At one point only, in the scar of the old operation, a hardness could be felt of about the size of a pea.

24th September. The erysipelas spread over the left breast, as well as to the left side of the spine.

25th September. The pleuritic effusion was entirely reabsorbed. On the other hand, the erysipelas again broke out in the region of the right scapula, where the redness had previously completely disappeared.

27th September. The erysipelas had still further extended as far as the umbilicus. The evening pulse was very frequent and intermittent. Camphor was ordered internally.

28th September. The erysipelas had spread no further.

29th September. The skin had become pale, and the temperature returned to the normal, and the patient remained henceforward free from fever.

The therapeutic result of this inoculation is up till now perfect, but whether it will remain so can of course only be determined by further observation.

The third case was a girl of 8 years, whose right eye had been enucleated during the summer session on account of an intraocular sarcoma. In a few weeks she had a relapse, and, on admission to the clinic, the whole of the orbital cavity was filled by a swelling which at the inner canthus spread somewhat in the direction of the forehead. In the right masseteric and submaxillary regions several lymphatic glands could be felt from the size of a hazel-nut to that of a walnut. On the 7th October, at 6 P.M., the child was inoculated from a cultivation of the fourteenth generation, the skin in the neighbourhood of the swelling being lightly scratched in six places with a knife and some of the inoculation material rubbed in. The temperature rose on the evening of the next day to 39·6° C., a rigor having occurred at 5.30. If this is considered to be the commencement of the illness the incubation would be 23½ hours. On October 9th, at 2 A.M., the temperature sank 0·2°, and shortly after 3 A.M. a rigor was observed which lasted three minutes, and at 4 o'clock the thermometer registered 40·3° C. This was the highest temperature, and the curve showed from this time until the morning of 11th October a steady fall to 36·3° C., but on the evening of that day it rose again to 39·8° C., and afterwards remained normal.

The erysipelas commenced at the punctures on the upper and inner periphery of the tumour, and from these spread over the swelling and the right half of the forehead. The inoculation punctures situated on the lower half of the swelling, namely those on the cheek, developed somewhat later, and from this point the erysipelas extended as far as the submaxillary region. Possibly the two rigors and the interruption in the rise of the fever by the slight remission of 0·2° C. may be explained by the difference in the time of taking of the inoculations. The tumour became intensely red under the action of the erysipelas and covered with innumerable little vesicles, but a diminution in its size could not be demonstrated, although the

previously described glandular swellings shrank to more than half their original size.

The fourth inoculation (culture 15th generation) was performed on the 14th October at 5 o'clock in the evening, on a woman in her fifty-second year suffering from disseminated mammary carcinoma. The right breast had become an ulcerating tumour about the size of two fists, and in the axilla a mass of glands of considerable size could be felt. There were numerous small disseminated nodules, about the size of a bean, in the skin in the neighbourhood of the tumour. They extended backwards as far as the lower angle of the scapula. Nitrate of silver was applied in a circle round these nodules in order to see if the spread of erysipelas could be controlled. On the 15th October, at 12 o'clock, 19 hours after the inoculation, the patient had a severe rigor and the temperature then registered 40·3° C., two hours later it was 40·5° C., and in the evening it fell to 39·7° C. The erysipelas spread on the first day over the whole mamma, and as far back as the inferior angle of the scapula. In this, as in the previous case, six small scarifications of the skin were made and the cultivation rubbed in. The inoculated points lay in a semicircle along the lower and outer periphery of the diseased mamma, and the inoculated material appears to have adhered to all, so that immediately after the rigor the erysipelas spread over a large area. This method of inoculation seems to be the most suitable, and it was therefore afterwards regularly adopted. Inoculation by means of puncture only succeeded in about half the first series of cases, but by the last method the result was more certain. Nevertheless if one wishes to be certain of the result it is well to inoculate several places, because at times, especially if some blood appears, the result may be negative at one place. On the following day the temperature curve was very irregular. The highest temperature, 40·0° C., was in the morning hours, the lowest 38·1° C. was noted at 4 P.M., and in the evening it rose again to 39·1° C. During the next three days the fever showed a nearly complete morning remission, while the evening temperature oscillated between 40·0° C. and 39·5° C. The erysipelas spread in the meantime in front to the middle of the left breast, and behind it extended about 10 centimetres beyond the middle line. On the evening of October 20th the reddening on the back became paler, but on the other hand the erysipelas

spread over the shoulder to the middle of the right upper ... where several vesicles formed. On October 21st it became ...here also, but in the afternoon dyspnœa was felt which ... increased, and there was found a fairly large right-sided ... effusion, which during the night had to be aspirated ...g to the increasing difficulty of breathing. Eight of the ...les which had been surrounded with nitrate of silver dis- ...red altogether, but five others remained, although the ...as had spread over them. The swelling itself showed a ... diminution on the fourth day, and it was still less at the ... of the first week after the cessation of the erysipelas, so that on ...vember 1st the tumour was scarcely half its original size. ... skin, which at the time of inoculation was tense over the ...mour, now exhibited little folds and was easily movable. The pleuritic effusion had to be aspirated once again and on November 1st was not completely absorbed, although the patient remained free from fever and quickly recovered. A further diminution of the tumour did not take place.

On the 24th of October, at 6 P.M., the girl who had been successfully inoculated on October 7th (3rd case) was again inoculated (16th cultivation), in order to ascertain whether, after the disappearance of an erysipelas, an immunity is conferred for some time. No erysipelas was produced. In order to exclude the objection that the cultivation was dead, a fifth patient, a girl 29 years old, who was suffering from an extensive lupus of the face, was at the same time inoculated with the rest of the same cultivation. She had also recovered from an erysipelas of the face in December, 1881. The rigor appeared 47 hours after the inoculation; the temperature rose at once to 39·5° C., and reached on the same day 40·1° C. On the evening of the next day the temperature was only 39·5° C., and on October 28th it fell to normal. The disturbance of the general health was in this case very marked. After the rigor several violent vomitings occurred, and the patient complained during the whole time of a severe headache. The short duration may be accounted for by the fact that the patient had 10 months before an attack of erysipelas at the same place. The lupus healed as a result of the erysipelas, with the exception of a few nodules in the neighbourhood of ...strils. These were afterwards scooped out, and up till ... relapse has occurred.

The sixth patient, who was inoculated on November 9th at 5 P.M. (culture 17th generation), was a woman of 40 years of age, who six years previously had been operated upon for the first time for mammary carcinoma. During the interval she had two returns of the disease, which was removed by operation. She had now enlargements of the glands of the axilla and neck which could not be operated upon. On November 10th, at 8 A.M., 15 hours after inoculation, she had a rigor and the temperature rose to 39·8°. As the temperature curve showed, the fever remained high for 11 days and ended by a sudden fall. On the 18th November one of the tumours was found to be soft and fluctuating, and on being opened discharged about 10 cub. cent. of a yellowish white pus-like fluid, and in 3 days the incision had closed.

The erysipelas had spread over the whole anterior surface of the thorax, and over the shoulder as far as the spine, and had extended down the arm as far as the hand. With the exception of this nodule, which shrunk after the incision, there was not on the whole a general diminution of the swelling. With the same cultivation patients Nos. 3 and 5 were inoculated for the third and second times respectively. In the case of the first patient (No. 3) the result of the inoculation was entirely negative, but in the last (No. 5) a slight redness developed, which disappeared after two days without fever.

The seventh patient was a man 20 years of age who had suffered from lupus for twelve years, and had recovered from many attacks of erysipelas, the last occasion being during the summer. He was inoculated on November 22nd and 28th, and on each occasion without any result.

It would be too soon, on the basis of these few observations, to form a decided opinion as to the therapeutic value of erysipelas. Naturally the inoculation of erysipelas can only be thought of in tumours for which operative measures are no longer available. In such cases, however, I think the expedient is undoubtedly justifiable, especially as the cases observed by Busch * and Volkmann † seem to prove at least the possibility of a permanent cure. The frequency of such a result can only be determined by further experience. Probably all tumours would not be

* W. Busch, *Berl. klin. Wochenschrift*, 1866.
† R. Volkmann, *Erysipelas*, Billroth und Pitha.

affected in the same manner by erysipelas. In any case it is worthy of mention that in each of the three cases of carcinoma inoculated in the clinic of Professor von Bergmann there occurred at least a diminution of the swelling, and in one case even complete absorption. As regards malignant enlargements of the lymphatic glands, in the treatment of which erysipelas has been specially recommended, I have unfortunately no personal experience.

There is still little known concerning the manner in which absorption of the tumour takes place through the action of erysipelas. As far as I know only one case has been examined as regards this point. This was the case of a patient 28 years of age, under the care of W. Busch, who was suffering from a lympho-sarcoma of the neck, and who succumbed to an erysipelas caught by accident, and after a diminution of the tumour had taken place.

On examination by Rindfleisch, the cells of the tumour appeared to have undergone fatty degeneration, and they were found, as in our case No. 6, to have degenerated into "a yellow-white emulsion (*emulsiven gelblichweissen Flüssigkeit*)." Concerning the action of the erysipelas micrococci on the cells of the growth we have as yet no observations. We can only suppose that they multiply inside the growth and perhaps penetrate the cells, and so cause them to disintegrate. This is the more probable when we remember that many tumour cells appear to be differently affected, even by chemical agencies, from the normal tissues.*

Apart, however, from their therapeutic effect, these inoculations seem to me to be valuable as deciding the question as to the cause of erysipelas. There can be no doubt that the erysipelas of our patients was a genuine one, and not merely a pseudo-erysipelas. Each individual case was repeatedly examined by Professor Bergmann and several experienced colleagues. The initial shivering, the characteristic sharply defined redness, the more or less rapid spreading on the surface—the so-called migration of the rose, the progress of the fever, and finally the termination in resolution and healing without suppuration and abscess, all confirmed the diagnosis. They were cases of true erysipelas caused by cultivations of the above-described micro-

* Delbastaille, *Centralblatt für Chirurgie*, 1882, No. 48.

coccus. In opposition to the assertions of Hüter, who, as already mentioned, thought he had discovered the germ of erysipelas in little rapidly moving micrococci, I must point out that the erysipelas micrococci are quite immobile. A spontaneous movement, such as occurs in many bacilli, has not yet been demonstrated in the case of micrococci.

As regards the discovery by Lukomsky, Billroth and Ehrlich, Tillmanns, and others, of micrococci not only in the lymph-vessels of the skin and the subcutaneous tissue, but also in the blood-vessels, the liver, kidneys, and the heart, it was apparent, and in fact was in some instances expressly stated, that the cases were complicated with pyæmia, or lymphangitis and phlegmon. In spite of careful investigation I have never been able to discover micrococci in the blood-vessels in true erysipelas. The spread of the micrococcus of erysipelas exclusively along the lymph-vessels is a peculiarity of that organism. It is true that in cases of lymphangitis micro-organisms are found in the lymph-vessels, but apart from the fact that these various forms of bacteria, which I have found and cultivated, essentially differ morphologically from the micrococcus of erysipelas, their mode of spreading, and possibly also their signification is quite distinct. Their point of origin is, as a rule, a pustule which generally contains bacteria in large quantities. From this focus of bacterial inflammation the inflammatory products are absorbed and carried away by the lymph-vessels, in which they excite an inflammation. In many cases simply a coagulation of the contents of the lymphatic vessels—a thrombosis—occurs, which is very rapidly again absorbed. On examining a section of such a vessel only a few bacteria are usually found, and one has the impression that these have been brought by the lymph stream from the pustule, and that no growth of them has taken place within the lymph-vessel itself. In other cases, it is true, one meets with a larger number of bacteria in the affected lymph-vessel. This happens especially in those cases in which there is suppuration in the lymphatic glands. There is always, however, in these cases a secondary affection of the lymph-vessel through absorption of substances capable of producing inflammation from a primary inflammatory or suppurative source.

In erysipelas, on the other hand, the development of the bacteria in the lymph-vessels is primary. The spread of the

disease also does not take place, as in cases of lymphangitis, by micrococci being carried along in the lymph stream, but rather by their active growth in all directions, often spreading even in a direction opposed to that of the lymph stream. The micrococcus of erysipelas is distinguished so completely from the different kinds of micro-organisms of lymphangitis, both morphologically and by its physiological conditions of growth inside the body, that the separation of erysipelas from other bacterial inflammations of the lymph-vessels seems perfectly justified from the practical as well as from the ætiological point of view. The same may be said regarding phlegmon. I have examined, in the clinic of Professor von Bergmann, during more than a year, all suitable cases and have never found a micrococcus which was not essentially different from that of erysipelas.

A crushing proof that progressive phlegmonous inflammations are not to be regarded as deep-seated erysipelas, as Tillmanns tried recently to prove, and that they must be distinguished from it not only clinically and anatomically, but also ætiologically, lies in his own admission that he had never succeeded in producing erysipelas by inoculation with so-called putrid matter, whilst phlegmon can be produced with certainty by such means. Conversely, I have injected erysipelas micrococci repeatedly into the subcutaneous tissue and muscles of rabbits without causing inflammation.

Regarding the spread of erysipelas, after what has been said there can be no doubt that it is contagious, *i.e.*, that it can be propagated by direct contact of one individual with another, or by means of instruments, and so forth. I do not of course assert that this is the only or even the common mode of infection; on the contrary, there is no doubt that the micrococci develop outside the human or animal body. The frequent epidemic occurrence of facial erysipelas, and the observed increase of the disease in many places at certain periods of the year, can hardly be otherwise explained than by an "ekanthropic" spread of the infective material. Again, the dependence of many hospital endemics upon foci of bacteria, as also their cessation after the removal of the same, is too well known to make it necessary to mention individual examples here. On the other hand, many authors agree in stating, and I have learned the same from many unsuccessful experiments, that it is not easy to produce an

artificial erysipelas without a pure cultivation. Numerous trials of direct infection from man to man, carried out in the most varied manner, always had a negative result. This shows at least that the danger of infection from erysipelas patients is not very great. The bacteria which have entered the body perish as fast as they are produced. No opportunity is given them of reaching the surface again and thus infecting other individuals, because erysipelas, as a rule, does not produce any secretion which contains the infective germs except in the case where vesicles are formed. But even the contents of the vesicles are not very infectious, as may be seen from the numerous unsuccessful attempts at inoculation which are recorded in the literature of the subject, and to which I could add several instances. For the vesicles of erysipelas often contain, as mentioned above, either cocci already dead or none at all. The erysipelas micrococci would thus soon die out if they had not an ekanthropic as well as an enanthropic method of propagation. A proof of the possibility of this is shown by the circumstance that I have found that they may be cultivated not only on coagulated blood serum and nutrient jelly, but also on potatoes and at the ordinary temperature of the air, that is to say, under conditions similar to those which are found in the outer world.

Regarding the incubation stage, the shortest observed (case No. 6) lasted 15 hours, the longest 61 hours (case No. 1), if we reckon from the moment of inoculation to the appearance of the first rigor, which period coincides fairly well with the time of the appearance of the redness. Disturbance of the general health, such as loss of appetite, headache, &c., was hardly ever absent during the incubation stage; a transitory increase of the temperature was, however, only observed in one case (case No. 1).

Finally, the results of inoculation are interesting with regard to the question of immunity. There were seven persons inoculated, of whom six took erysipelas. The seventh patient, who was twice unsuccessfully inoculated, had previously suffered from frequent attacks of erysipelas, the last being a facial erysipelas from which he had recovered two or three months before. Of the other six individuals, two were inoculated on several occasions. Case No. 3 was a girl 8 years old, who was successfully inoculated on the 7th of October, and unsuccessfully

on October 24th and November 9th. Patient No. 5, who had recovered from erysipelas in December, 1881, deserves special consideration. The first successful inoculation was on the 7th of October, 1882, whilst the second inoculation on November 9th, 33 days later, had no result. It appears, therefore, that an attack of erysipelas only confers a short period of immunity.

Finally, I should like to state shortly the results of some experiments I have made concerning the behaviour of the erysipelas micrococci towards certain antiseptics. R. Koch* has shown that in testing the action of antiseptics on micro-organisms the presence or absence of spores in the material employed is not the only point to which attention must be directed, but that apart altogether from the spore stage different species of bacilli and micrococci show marked differences with regard to their power of resisting the action of antiseptic substances. Since, however, the behaviour of the pathogenic micro-organisms is much more important for the practitioner than that of the mixture of bacteria which until now has been chiefly used for the experimental testing of antiseptics, it appears desirable to examine with this object more minutely those micro-organisms which play a part in traumatic infective diseases of man. This is all the more advisable with the micrococcus of erysipelas since the views of surgeons still differ considerably concerning the protection which the antiseptic dressing affords against erysipelas. Unfortunately I was not able to test a large series of antiseptics, and at present I must confine myself to stating the results which were obtained by the two solutions chiefly employed in the clinic of Professor von Bergmann, viz., a 3 per cent. carbolic acid solution, and 1 per cent. corrosive sublimate solution. I proceeded as follows:—I inserted a previously heated platinum wire, the point of which was roughened, into a well-developed cultivation, and then dipped it for a definite time into the antiseptic solution, and afterwards inoculated the nutrient jelly. After the 3 per cent. carbolic acid solution had acted for twenty seconds the cultivations developed as well as in the control vessels. After thirty seconds there was observed in only a certain number of the glasses a retarded and defective development; forty-five seconds sufficed to check it entirely. The

* *Mittheilungen aus dem Kaiserlichen Gesundheitsamte*, 1 Band, 1881.

action of the 1 per cent. solution of corrosive sublimate was much quicker, for ten or fifteen seconds were sufficient to prevent the growth in the nutrient jelly. Of course these results only apply to the above described method of investigation, where the material to be disinfected adheres in a thin layer to the platinum wire and in a somewhat moist condition, so that the antiseptic solution can penetrate easily.

If the gelatine cultivation had been dry, or if some porous material such as linen, between the fibres of which micrococci can penetrate, had been impregnated with it, of course a longer action of the antiseptic would have been necessary to obtain the same result. Nevertheless it appears to me that the experiments show that by thoroughly disinfecting the hands and instruments with corrosive sublimate, and by repeated and thorough washing of the wound with carbolic acid we can prevent infection during an operation; and as regards an antiseptic dressing it is absolutely necessary to have one which shall effectually prevent the occurrence of erysipelas, and numerous clinical observations have shown this to be the case with the Lister dressing. In the clinic of Professor von Bergmann there occurred, for instance, only two cases of erysipelas under the antiseptic dressing during the last nine terms, a number which might be easily explained by some error during the changing of the dressing, and which is all the more insignificant since erysipelas occurs frequently in Würzburg, and since several patients were attacked with erysipelas after operations on the face (where no effective antiseptic dressing could be applied) without its being possible to isolate them.

Antiseptics such as iodoform, which according to experience do not protect against erysipelas, cannot, as the so-called iodoform phlegmon proves, afford sufficient guarantee against the entrance even of other excitors of inflammation, and should therefore only be employed when the antiseptic dressing cannot be used, or in combination with other antiseptics. Of course even the best dressing can only fulfil its purpose if a perfect disinfection of the wound and its surroundings has preceded its application.

For this purpose a strong solution of carbolic acid is the best, because it is the only one of the reagents which has as yet been tested which does not undergo chemical change in the wound, forms no coagulum, penetrates to some extent into the tissues,

and can at the same time destroy sufficiently quickly all the micro-organisms which are not in the spore stage.* For the destruction of the latter, however, we are at present chiefly dependent upon corrosive sublimate, for the exclusive application of which as a disinfectant for wounds there is only one objection, viz., that if brought into contact with alkaline and albuminous fluids it decomposes and forms insoluble albuminous compounds which are no longer antiseptic.

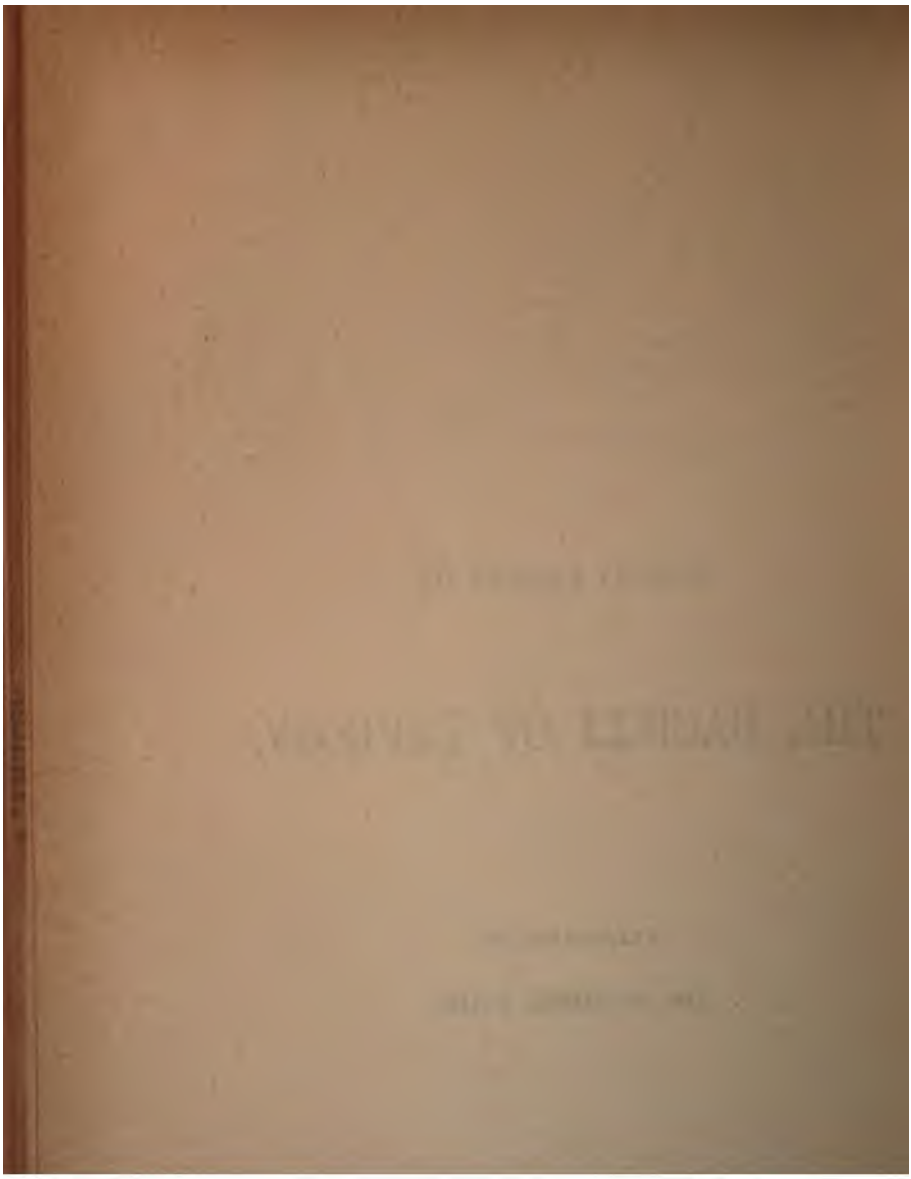
* *Mittheilungen aus dem Kaiserlichen Gesundheitsamte*, 1 Bd., 1881. R. Koch *über Desinfection.*

RECENT PAPERS ON

THE BACILLI OF LEPROSY.

TRANSLATED BY

DR. GEORGE THIN.



I.—EXTRACTS FROM A PAPER BY DR. ALBERT NEISSER ON THE ETIOLOGY OF LEPROSY.*

(Virchow's *Archiv*, vol. 84, 1881.)

PRESENCE OF BACILLI (LEPRÆ) IN THE ORGANS.

ALL the pathological products which are found in the progress of leprosy show with certainty in all the cases which I have examined the presence of a single and, so far as is known, a specific form of bacillus. The bacilli are found in the neoplasms of the skin, of the mucous membrane of the mouth, of the pharynx and the larynx; in the interstitial deposits in the peripheral nerves and in those of the cornea, cartilage, and testicle; also in the lymphatic glands, spleen, and liver. So far as we yet know they are not present in the spinal cord or in the muscles, and are not concerned in the bullous skin eruptions and the affections of the bones and joints, processes which cannot be considered as primary, but as secondary, and depending on nerve lesions.

The bacilli in the skin are present in the circumscribed nodular formations as well as in the more diffuse infiltrations, which are found, for example, somewhat evenly enveloping the whole of the face.

The material examined was taken partly from the dead body, and was partly excised from patients and hardened in absolute alcohol.

The bacilli are found almost entirely in the interior of the large round cells described by Virchow as lepra cells. These cells, which often are five times larger than a pus corpuscle,

* The first notice of the bacillus of leprosy is contained in a report made to the Medical Society of Christiania in 1874, by Armauer Hansen. A paper by Hansen on the subject will be found in the *Quarterly Journal of Microscopical Science*, New Series, vol. xx., 1880.

contain one or several (3 to 12) large clear nuclei, which are exceedingly like those of epithelial cells, and often appear unsymmetrically situated close to the wall of the cell.

The bacilli and their products either fill the whole protoplasm of the cells—being equally diffused through them—or more frequently they are found as several small circumscribed collections of rods, which consist of six or seven bacilli lying parallel to each other. Sometimes two or three are found lying immediately one behind the other, so that there is an appearance of a long, although not quite straight, thread, or there exists a compact mass composed of an accumulation of bacilli, the fact that it is composed of a mass of individual organisms being only ascertained by careful examination. In such cells we find, in addition to the slender bacilli, smaller rods and minute particles.

In proportion to the mass and form of bacilli which are deposited in the cells, the cell itself becomes altered both in size and in chemical constitution.

We have already mentioned that the lepra cells exceed in size the white blood corpuscles, or lymph cells, to which we can now with greater certainty than before attribute the origin of the lepra cells.

Bacilli lying free between the cells in connective tissue spaces are not often found.

In the blood-vessels I could not demonstrate them with certainty.*

Sweat and sebaceous glands are not primarily involved, but become involved subsequently to the affection of the blood-vessels and the peri-glandular connective tissue.

The description of the bacilli as they are found in the skin holds good in all essential points for the leprous affections of the mouth, pharynx, and larynx.

An examination of the mucous membrane of the larynx showed that bacilli invade the epiglottis and the thyroid cartilage. An examination of the cornea showed that an infiltration of lymph cells took place from the border towards the centre. All these cells contained bacilli, although some organisms were found isolated between the laminæ of the cornea. Bacilli were found abundantly in the testicle in the intratubular tissue as well as in

* Their presence in the blood-vessels has now been definitely ascertained.—G. T.

the epididymis, also in the intra-acinous connective tissue of the liver, in the spleen, and in the peripheral zones of the lymphatic glands.

Especially important is the fact that bacilli were found in the peripheral nerves, showing that the tubercular and anæsthetic forms of leprosy depend upon one pathogenic cause.

I was able in nerves which had recently become diseased to establish the identity of the interstitial deposit of the peripheral nerves with the leprous neoplasm in the skin, and to demonstrate the bacilli in the cells which were found between the nerve fibres and bundles.

In unstained preparations hardened in alcohol, bacilli are not visible. Through the addition of acetic acid they become to a certain extent visible, but so faintly so that if I had not known they were present their presence would have escaped me. Far better results were obtained by the application of a solution of caustic potash, 1 to 12. On the other hand the bacilli are stained by gentian and methyl violet, and best in fuchsine. Faintly acid solutions of the dyes, or subsequent decoloration in acid alcohol, especially if the section before it was stained had been subjected to the action of a weak solution of potash, gave the best stained preparations. Bismarck brown, and all the other brown and yellow dyes which I tried, did not stain the bacilli. Koch obtained a slight staining with vesuvin; dahlia in acid solutions also afforded available preparations. Methylene blue did not stain them, nor did nigrosin, aurantia, or methyl green. Eosin does not stain the bacilli; on the other hand the acid mixture of eosin and hæmatoxylin gives good results.

I. Eosin, 0·5.
Aq. destil., 100·0.
Alumin., 2·5.
Glycerine, 2·5.

II. Hæmatoxyl., 0·5.
Alcohol abs., 100·0.

Mix I. and II. After three days, during which time the mixture has been exposed to light, add

Acid. acetic. glac., 2 per cent.

The sections after being stained were washed in water and decolorized in alcohol. The nuclei were then found to be blue, the ordinary cell protoplasm a rose-eosin; whilst the protoplasm of the cells which contain bacilli was coloured a bright orange,

and even with weak magnifying powers the presence of bacilli can be ascertained.

The micro-organisms which are made visible by these methods are extremely faint slender rods, sometimes pointed at both ends, of from $\frac{1}{4}$ to $\frac{2}{3}$ the length of a human red blood corpuscle. The breadth is about $\frac{1}{4}$, or less, of the length. They are either straight or slightly bent, and are very similar to the small bacilli which Koch has described in septicæmia of the mouse, although they are not quite so delicate as the latter.*

That the bacilli and spores are the cause of leprous growths is shown by the absolute constancy with which the bacilli are found, and by the immense number of organisms which are nearly always present—a fact which has been observed in material obtained from Norway, Spain, Guiana, East Indies, Roumania, Brazil, and Palestine.†

It can also be proved that with the presence of the bacillus lepræ the typical processes of development from a migratory cell to the form of a connective tissue cell lead to the result which Virchow described as a lepra cell. If this is found to be stained, then we can say the specific form and qualities of the lepra cell are etiologically caused by the specific bacillus.

[Neisser sums up his paper by the following propositions:—]

1. Leprosy is a purely bacterial disease, produced by a specific bacillus. For this hypothesis there testifies the constancy of the characteristic condition: the peculiarities of the bacilli; their existence in all the diseased organs in masses which are proportioned to the progress of the disease. Further, it has been proved that the specific properties of lepra cells due to the invasion of bacilli can be experimentally produced.‡

2. These bacilli are introduced into the organism either as such, or more probably as spores, and remain during a period of incubation of varying duration. They are deposited probably in the lymphatic glands. The incubation and course of the disease appear to be more rapid in tropical countries than in those parts of Europe which are affected with leprosy.

3. From the parts of the body in which the spores have been

* On this point compare Mr. Thurston's careful drawings in Plates 12 and 13, vol. xvi. of the *Medico-Chirurgical Transactions*.

† Also in leprous tissue sent to England from China and Australia.—G. T.

‡ There is no confirmation of this statement.—G. T.

deposited invasion of the system takes place, and chiefly (*a*) In the skin, as in variola, syphilis, &c. Those parts of the body which are otherwise specially exposed to injuries are affected by predilection—the face, the hands, the elbows, the knees. (*b*) In the peripheral nerves (anæsthetic leprosy). The effects in the muscles, as well as the other disturbances of nutrition, correspond to symptoms which are well known as accompanying other diseases of the peripheral nerves. (*c*) The other organs, testicle, spleen, cornea, cartilage, liver, were less affected.

4. Through the bacilli or their spores inflammatory processes are set up in vascular organs, or, where there are no blood-vessels, by cell emigration. These lymph cells thus form materials for the leprous neoplasm. Through the specific effects of the bacilli the emigration cells become lepra cells characterized by their special form, course, and process of decay.

5. From these propositions it will be seen that we regard leprosy as being probably an infective disease, and that its products are specifically contagious. These products are cells from the tubercles, and serum and pus containing spores which are capable of development. But all pus that comes from a leper is not infective, because it does not always contain bacilli. For the same reason the contents of the pemphigous bullæ of leprosy are not infective. The disease can only thus be considered contagious indirectly if the bacilli or spores are conveyed by various objects. In leprosy, more than in any other bacterial diseases, the susceptibility of the individual appears to be of importance.

I do not consider leprosy to be an hereditary disease.

II.—NOTE ON THE SITE OF THE PARASITE IN LEPROSY.

By MM. V. CORNIL ET SUCHARD.

(*Annales de dermatologie et de syphiligraphie*, 2nd series, vol. II.)

According to Hansen's observations — confirmed by several Norwegian observers, MM. Heiberg, Bidenap, and Winge, and by a German observer, M. Neisser—there is no longer any room for doubting that leprosy, or elephantiasis of the Greeks, ranks among parasitic diseases.

We have been able to examine tubercular leprosy in preparations, of which some obtained from the Leper Asylum of Grenada were kindly placed at our disposal by Dr. Bernito Hernando, and others were obtained from a patient of Dr. Labbé, Physician to the Municipal Hospital.

The leprous tubercles of the skin are formed by the infiltration of the "corpus papillare" and the cutaneous derma by great numbers of large globular, spheroid, or slightly flattened cells, which are situated between the fibres of the connective tissue.

At the centre of the tubercle the papillæ are no longer distinct, and the glands and hair follicles are shrivelled and destroyed.

The layers of the epidermis at this level are so thinned that the surface of the non-ulcerated tubercles is smooth. It is easy to convince oneself of the correctness of these details by examining under the microscope sections of leprous tubercles coloured with picro-carmine.

In fig. 24, Plate VII., which is drawn under a magnifying power of 100 diameters and represents the projecting part of a tubercle, the layers of the epidermis from *a* to *d* are thin, and the projections of the papillæ do not exist at *f*, at the surface of the derma. The latter is, throughout its whole extent, infiltrated with cells, *e e*, which are nothing else but migratory cells interposed among the fibrillæ. These cells are less numerous in the superficial layer of the derma than deeper down. In these sections of the derma nothing but blood-vessels are to be seen, *v*.

These vessels are represented highly magnified in figs. 27 and 28, in longitudinal and transverse sections. They show a noticeable thickening in their wall, especially in that of their inner coat, so that, in a transverse section, they resemble spheres composed of concentric layers. It was probably this appearance which made Neumann say that colloid spheres are found in leprous tissue.

In preparations obtained after the action of hardening fluids, such as alcohol, and coloured with carmine, it is impossible to see the parasites of leprosy. Nevertheless with a strong magnifying power (500 to 1,000 diameters) there may be seen in the protoplasm of the large cells small ovoid or elongated bodies with no clearly defined shapes, which refract light and are of a rose colour. But with this treatment it could not be affirmed that we have to do with rods so characteristic as those obtained when the following methods are employed :—

1. In fresh tissue from a tubercle taken from the living body and teased out with needles in water spherical granules and rods may be seen undergoing spontaneous movement. These rods move so as to be seen as points or rods, and they show twisting movements.

2. In order to obtain sections in which the rods were distinctly visible we used small pieces of skin cut from the living body and immediately placed, first in alcohol at 40, and then in absolute alcohol. Subsequently the sections were coloured by being kept some time in a solution of methylaniline violet, 5 B, (manufactured by M. Poirier), of 1 to 5 per cent., then successively washed in 1 to 4 per cent. solution of carbonate of soda, and in absolute alcohol, and then treated with oil of cloves and Canada balsam. These preparations are not to be obtained without a series of trials, for the amount of necessary decoloration obtained by the soaking in absolute alcohol has to be found out by experience. The latter agent takes away a part of the colouring matter which impregnates the cells, and its action must be arrested before the rods themselves become discoloured.

In successful preparations the cells above-mentioned all show in their protoplasm a large number of rods intensely coloured with blue, whereas the protoplasm itself is scarcely tinted with blue, and the fibres of the connective tissues are colourless. With a magnifying power of 300 diameters, such as may be obtained for instance with Vérick's objective No. 8,

the blue coloured rods may be very well seen as represented in fig. 25, Plate VII.

Each cell encloses a variable number of rods. The protoplasm of the cell is slightly tinted blue, and since the fibres which separate them are entirely colourless, they are represented by clear spaces between the cells.

In fig. 25 we have shown, at *v*, the lumen of a blood-vessel.

The flat cells which form the wall of the vessel show, as do all the cells in the connective tissue, numerous bacteria. But in order to see these rods well, the use of immersion lenses is necessary. If, together with these lenses, the more intense light of the condenser be employed, the diffused light which veils the cells brings out to perfection the blue-tinted rods.

In this manner a very instructive general view is obtained, in which all the cells, whether rounded or flattened, appear full of very numerous rods, irregularly arranged in groups (see B, C, fig. 26). These rods are straight, a result due apparently to the action of the alcohol; sometimes they lie quite separately one from another, at others they are close together in a lengthened bundle, at the extremities of which the ends of each rod may be seen (see F, fig. 26). Together with the rods, little oblong granules are found. Very few rods are found outside the cells.

One remarkable peculiarity that we have noticed about these preparations is that the various layers of epidermis contain not a single microbe.

From this it would appear that the epidermal covering forms a varnish impenetrable to the special parasite of leprosy.*

We shall return to this point when we study the conditions of the contagium of this malady compared with those of the contagium of other cutaneous affections.

By the latter method we have examined many specimens of ulcerated and non-ulcerated leprous tubercle, obtained from the pathological collection in Granada. These specimens gave us for the most part similar results, and bacteria could easily be distinguished in them; but owing to their having been obtained at post-mortem examinations made at least twenty-four hours after death, the phenomena of post-mortem decomposition had

* Compare Plate XII. vol. lxiv. of the *Medico-Chirurgical Transactions*.—G. T.

rendered the preparations less clear than in specimens obtained during life.*

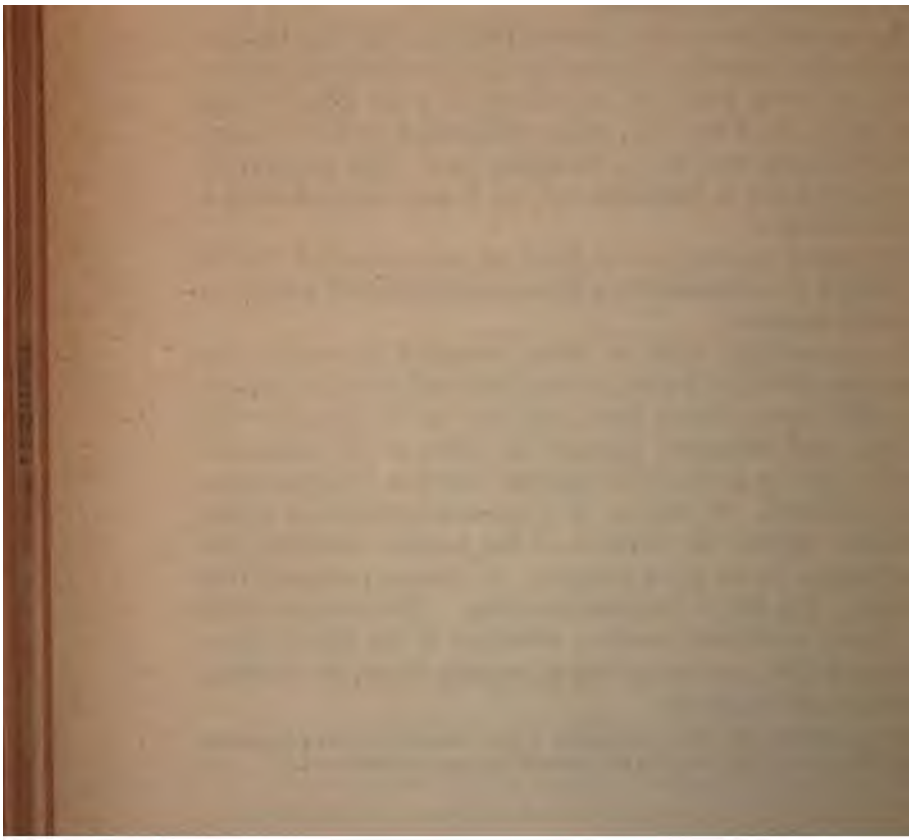
We have also found bacteria in organs obtained at post-mortem examinations, notably so in a liver which presented all the lesions of hypertrophic cirrhosis with the considerable fibrous thickening and the increase in number of the biliary radicles which characterize this disease.

The favourite seat of the parasitic bacilli in this liver was the cells of recent formation, situated in the interlobular connective tissue, but some were also to be found in a few hepatic cells. Thus in fig. 29, Plate VII., which represents a section of liver, hepatic cells are seen at *c c*, containing rods. The round cell *b* is without doubt a lymphatic cell, as is also the isolated cell represented at A.

The nerves (median nerve) which we have examined showed a condition of sclerosis with a fibrous thickening and atrophy of the nerve elements.

In summarizing what we before remarked concerning the cutaneous tubercle of leprosy, we see that these tubercles, formed by a solid, dense, dermic tissue, are made up of cells filled with bacteria, and interposed amongst the fibres of the connective tissue; that the layers of the epidermis are free from parasites, but are thinned. So long as it is preserved, this layer of the epidermis opposes the diffusion of the parasite outwards, and constitutes a barrier to its progress. It renders contagion very difficult. The seat of the parasite is deep. The usual case with the highly contagious parasitic affections of the skin is quite contrary to this, such as in that of eruptive fevers, for example, small-pox and erysipelas.

* By the fuchsine and nitric acid method I have obtained excellent preparations from organs obtained post-mortem, and preserved for years in alcohol.—G. T.



THE

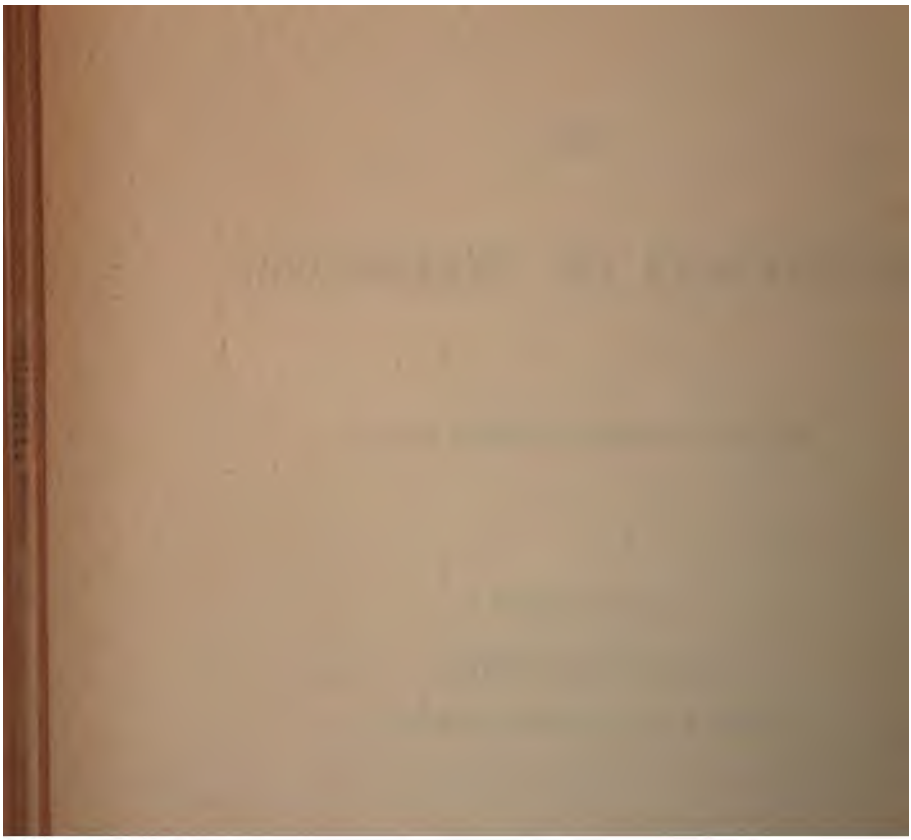
MICROCOCCI OF PNEUMONIA.

By Dr. C. FRIEDLÄNDER, Berlin.

TRANSLATED BY

EDGAR THURSTON,

Curator of the Central Museum, Madras.



THE MICROCOCCI OF PNEUMONIA.*

(*Fortschritte der Medicin.*, vol. i., No. 22, Nov. 15, 1883.)

INTRODUCTION.

THE first observations on the micrococci of pneumonia are those of Klebs,† of which it is not easy to estimate the value. Eberth‡ found in a case of pneumonia, complicated with purulent meningitis, ellipsoid cocci, mostly in pairs, in the lung infiltration, in the inflamed pleura and in the pia mater. Further, Koch§ has demonstrated the presence of, and photographed, micrococci in the alveolar exudation, in the pulmonary capillaries and in the capillaries of the kidney in a case of acute pneumonia after recurrent fever.

Soon afterwards we succeeded,‖ by positive investigations, which were undertaken in eight consecutive cases of acute pneumonia, in furnishing a proof that the appearance of micrococci in the alveolar exudation is a constant factor, that they are found in recent cases in enormous quantities and that they are further found in very large numbers in the lymphatics of the affected parts of the lungs.

These facts we were able to confirm by the examination of a large number of other cases. In all, more than fifty positive cases came under our observation; only in a few cases were the micrococci missed, and these should not be taken into consideration, as the cases were in a late stage of the disease, as a

* These investigations were mostly carried on along with Dr. Frobenius, Assistant at the Pathological Institute in Munich; Dr. Frobenius has taken a very important part in them.

Originally we intended to publish them in our joint names; as, however, Dr. Frobenius, on account of his departure from Berlin, was not able to take part in the experiments after the middle of October nor in the conclusion of the work, he expressed the wish that his name should not appear on the title page of this publication.

† *Arch. f. exper. Pathol.*, Bd. IV.

‡ *Dtsch. Arch. f. klin. Med.*, Bd. 28.

§ *Mittheil. aus d. kais. Gesundheitsamt.* Berlin, 1881.

‖ C. Friedländer. Virch. *Arch.*, Bd. 87.

rule from the 9th to the 13th day. It is no wonder that in these cases the micrococci no longer existed.

This experience was also confirmed by several other investigators. That many fellow-workers have not succeeded, as we know from private communications, in verifying our observations, depends for the most part on the difficulty of the demonstration. As there are generally a very large number of nuclei at the spots where the micrococci are present, it is sometimes not very easy to distinguish the micrococci among the deeply stained nuclei in stained preparations, especially as the fibrin is also usually faintly stained, and the micrococci are hidden from view. One of our co-workers, Dr. Gram, of Copenhagen, has recently discovered a method by which the nuclei as well as the fibrin are almost or entirely unstained, whereas the micrococci take on an intense staining. The method consists in placing the sections (from preparations hardened in alcohol), which have been deeply stained in Ehrlich's solution of gentian violet in anilin water, in a weak watery solution of iodine and iodide of potassium for a short time. The sections, which were previously of a deep blue colour, then become almost colourless in oil of cloves; on microscopical examination one sees the nuclei, which are almost or entirely decolorized, while the micrococci stand out of an intensely blue colour on the faintly yellow ground substance. The stain is to a great extent removed in the oil of cloves. In this way the demonstration of the micrococci always succeeds, even in those cases in which they are present in small numbers; one can conveniently use thick sections.

Dr. Günther, at that time assistant physician at one of the hospitals in Berlin, and also Herr Leyden, first succeeded in demonstrating the presence of micrococci in the pneumonic juice which was taken from the living subject by puncture of the lungs. Both observations are published in the Report of the Meeting of the Society of Internal Medicine on Nov. 20, 1882. Dr. Günther made at that time the observation that the micrococci were surrounded by an unstained sheath. The preparations were stained with gentian violet. In the preparations of Herr Leyden no trace of a sheath was to be seen, but the micrococci appeared large and plump; they were stained with methylene blue. We shall shortly see that this behaviour is characteristic of the sheath or capsule of the pneumococcus.

I.—THE CAPSULE OF THE PNEUMOCOCCUS.

In consequence of these interesting discoveries we have made a regular series of investigations of the pneumonic juices from the bodies of persons who had died of the disease. They were mounted and dried on cover-glasses in the usual way, and stained with various aniline dyes, as well as treated with other re-agents. The pleural and pericardial exudations which are found so frequently in acute pneumonia were also examined in this way; and in them, as well as in the juices, the micrococci were found in very great number. The turbidity of these exudations chiefly depends in many cases on micrococci which are contained therein in large numbers, and are frequently many times more numerous than the lymphoid cells of the exudation; they are also often contained in the interior of the latter. (Fig. 13*a*.)

We found in these investigations that in most cases of pneumonia the greater number or all of the micrococci were surrounded by a more or less broad band of a substance which is faintly stained by gentian violet or fuchsin, and invests the organisms like a capsule. This capsule is seldom of less breadth than the micrococcus itself, and is frequently twice or four times as broad. Its outer contour is generally sharp. If the micrococcus is lying isolated the form of the capsule is seen to correspond geometrically with that of the micrococcus. If the micrococcus is round (or spherical) the margin of the capsule is likewise spherical; if the micrococcus is elliptical the margin of the capsule is elliptical. If, as frequently happens, the micrococci are in pairs, the capsule forms an elongated ellipse round the diplococcus. One frequently finds longer chains made up of three, four or more cocci, invested by a nearly cylindrical capsule rounded at both ends. Sometimes one sees in the interior of the long capsules, in place of a diplococcus or a chain of cocci, a rod-shaped structure with not quite regular boundaries, so that the appearance is as if the cocci were fused together into a rod-shaped structure. Further, capsules are met with in which the central coccus or cocci are absent. In this case one sometimes sees, in place of the cocci,

one or more clear spots in the middle of the capsule, or the capsule may appear perfectly homogeneous (probably dead cocci). We never saw the pneumococci aggregated into zoogloea masses. (See fig. 13, Plate IV.)

If one examines the micrococci in an unstained condition floating in fluid* no trace of the capsule can usually be detected, but sometimes one observes a faint shimmer around the cocci as if their contour was not quite sharp.

In preparations dried on the cover-glass the capsules are fairly distinct, especially if one examines them in air. The contour naturally always appears somewhat hard. If one allows distilled water to run in beneath the cover-glass the contour of the capsules entirely disappears, one sees nothing except the micrococci which are contained within them; the same result is obtained when one allows a strongly diluted solution of potash to operate. The behaviour is different under the influence of acetic and mineral acids of various strengths. With these the external contour of the capsule persists, but the boundaries of the central micrococci are lost; the micrococcus plus the capsule presents itself as a nearly uniform, round, or ellipsoid structure. If one stains the dried preparations after previous treatment with distilled water or weak alkali, no trace of the capsule can be demonstrated; one sees the micrococci perfectly naked. In the preparations treated with acid a distinct staining of both the micrococci and the capsule can, on the contrary, be very easily obtained. Alcohol, ether, and chloroform have no apparent influence on the capsules.

From these reactions one was able to prove that the capsules of the pneumococcus are composed essentially of mucin, or an allied substance. They are soluble in water and weak alkali, insoluble in acids. The staining reactions with aniline dyes are in unison with this interpretation.

As to the results of different staining re-agents, we may cite the following:—

If one treats a large number of identical cover-glass preparations with different aniline dyes, one at once learns that with con-

* We may remark here that we have never been able to discover a trace of motion in the micrococci. The investigations were carried out on drops of fluid depending from the lower surface of the cover-glass. The cover-glass was placed on a hollow ground slide, and its edges sealed by fluid vaseline, so that all evaporation was prevented.

centrated solutions of methylene blue and bismarck brown the micrococcus and its capsule are uniformly stained. By the use of dilute solutions of the same dyes for a longer time one can produce a differentiation, though slight, between the micrococcus and its capsule by the deeper staining of the former. By this method one also finds that the margin of the capsule is generally not circular or elliptical, but slightly serrated. As we have mentioned, one obtains very distinct staining of the capsule by treatment with gentian violet or fuchsin for a short time, but the picture is often confused, because the ground substance is also deeply stained. If one subsequently treats the stained cover-glasses with alcohol, it frequently happens that the stain is completely removed from the capsule, while the ground substance (coagulated albumen of the fluid) retains a faint colour. The still intensely stained micrococci then appear surrounded by a colourless space,* which corresponds to the capsule.

In this form Dr. Günther first saw and figured them. If one uses the gentian violet or fuchsin, not as a watery solution, but dissolved in aniline water, one obtains in a very short time a staining of the capsule, which resists subsequent treatment with alcohol for a long time. We consequently recommend staining with gentian violet dissolved in aniline water as the most convenient and quickest method of demonstrating the capsule; the cover-glass is afterwards placed for half a minute in alcohol in which the colour of the ground substance is quickly, while that of the micrococci and the capsules is much more slowly, abstracted. The preparation is then treated with distilled water, and can be examined directly in water, or mounted in Canada balsam or dammar. The capsule can also be stained with eosin. We recommend that a weak eosin solution should be allowed to act for 24 hours. The contour of the capsule is also sharply brought out by osmic acid; there is, however, no blackening.

A like structure—a capsule formation—has hitherto been described in hardly any of the schizomycetes.† One certainly speaks of a gelatinous ground substance between micrococci

* It is to be noted that not every clear space around micrococci, in dried preparations, is to be put down to a capsule.

† As is known, broad mucous envelopes occur in various Algæ (Beggiatoa, Oscillaria, &c.). By the kindness of Dr. Frobenius my attention was directed to a passage in Zopf's *Morphologie der Spaltpflanzen*, 1882; on Plate V., figs. 11 and 12 in that work cocci with a gelatinous membrane are figured.

which adhere together to form zooglœa masses, but it is almost unknown for the individual micrococcus to be invested by a quite characteristic gelatinous capsule. Is, then, this gelatinous capsule present in other schizomycetes besides the pneumococcus? In the most various kinds of pus, in the different forms of lobular pneumonia, in the contents of the bronchi, in the intestinal canal, in diphtheritic masses, &c., we have never been able to point out, in the abundance of micro-organisms which were present, more than a trace of a capsule formation, and in most cases generally none at all. The examination of sputa was also nearly always negative in relation to this point, as we found in only a very few cases an indication of quite a narrow capsule. Even in the not very numerous cases in which we have examined the sputum of pneumonia (*Fortschr. d. Med.*, S. 471) for the presence of capsules,* the results were always negative. We do not doubt, however, that they are to be found in pneumonic sputum, as we have repeatedly identified them in the bronchial contents in the bodies of persons who have died of pneumonia. Possibly the action of the parotid scretion has produced a partial solution of the capsule; besides, it is clear that as the sputum of patients is usually expectorated into a vessel containing water, the capsule would be dissolved. In only one case where pus was examined was a distinct capsule formation noted in some of the numerous micrococci which were present; this was in a case of peritonitis, which resulted from the perforation of a duodenal ulcer.

We see, then, that the capsule formation is a highly characteristic mark of the pneumococcus, which is only found in extremely few cases in other kinds of micrococci.

As already mentioned, we have up to the present time only found micrococci without capsules in other forms of pneumonia. In recent cases of genuine acute fibrinous pneumonia, on the contrary, the capsule was hardly ever missed.

That in our earlier observations the capsules of the pneumococci remained invisible depended on this, that they were made almost exclusively on sections of specimens hardened in alcohol. In these the capsules are in fact only rendered visible with extreme difficulty, as every investigator will testify. Naturally, the

* We first directed our attention to the question of the constant occurrence of capsules at the beginning of this year.

section to be stained should not be placed in distilled water; also the subsequent treatment with alcohol should not be continued too long, so as not to abstract too much of the stain from the capsule. By using this precaution, we have succeeded in finding the capsule of the pneumococcus in human pneumonia, even in specimens hardened in alcohol; but this is no easy task for the histologist.

We found the capsule-bearing micrococci not only in the pneumonic lung tissue, but also in great quantities in the pleuritic and pericardial exudations, further also in pleuritic adhesions, in those cases in which the pneumonia affected a part of the lung which was united to the chest-wall by preceding pleurisy. We also found them in the uninfiltrated simply œdematous parts of both lungs in several cases of pneumonia of one lobe, in which death occurred at the height of the disease. We have not as yet found them in the blood and other organs in man, but we have made very few investigations in this direction. In several cases of genuine croupous pneumonia we certainly found micrococci in large numbers, but none with capsules. All these cases were more than six days old; also in the other positive cases, which form by far the greater number, all the micrococci were not provided with capsules, but only a certain proportion of them. However, in the recent cases in which death ensued during the progress of the disease, we found that practically all the micrococci had capsules.

We are accordingly inclined to assume that the capsules belong to the highest stage of development of the micrococci. That the capsules should not be considered as passive precipitates round the micrococci from the surrounding fluid follows from this: that other corpuscles suspended in the same fluid do not show capsules, and further from the circumstance that they are found round the pneumococci in exactly the same manner in whatever substratum they exist. We shall have to mention later that in animals, even in the blood, pneumococci with the characteristic capsules are regularly found. We have no doubt, moreover, that they are also to be found in human blood in grave cases of pneumonia. There is in fact already a corresponding statement by Koch (*l. c.*).

We must necessarily look on the capsule as a product of the vital action of the micrococcus.

II.—CULTIVATION OF THE PNEUMOCOCCUS.

Our problem was now to cultivate the pneumococcus outside the body. Its growth must, under favourable conditions, be eminently rapid, as a colossal development of the micrococci is found in pneumonic lungs in the course of a very few days. We employed, of course, in our cultivation experiments Koch's method of cultivating on a solid nutrient soil, by the introduction of which a great deal of the difficulty has been removed which up to that time stood in the way of the application of pure cultivation. The method has its control in itself, errors which are at any time committed being at once detected; we find ourselves by its application on sure ground.

We tried first to cultivate the micrococci from pneumonic lungs on coagulated blood serum which was prepared by Koch's well-known process. Very small portions of several pneumonic lungs,* which contained large quantities of micrococci with capsules, were placed on the surface of or pushed into the blood serum by means of a platinum wire sterilized by heat.

The test-tubes containing the blood serum, of which the mouths were plugged with sterilized cotton wool, were placed in an incubator which was kept at the temperature of the body. A growth of micrococci always developed in a short time on the upper surface of the serum in the form of faint grey spots, and of an opaque cylinder in the interior of the serum along the needle track.

By microscopical examination round and elliptical micrococci of the size of the pneumococcus were found.

These experiments, which were made in the early months of 1883, had to be broken off, and were continued in September of the same year.

Dr. Frobenius had the good fortune to work in the laboratory

* In taking the material from the lung we had to see—1, that the lungs were quite fresh, i.e., free from putrefaction; and 2, that external impurities were excluded. The latter point was attended to by following Koch's directions. A broad piece of the pleural surface or of the cut surface of the lung was cut off with a heated knife. From this cut surface fresh sections were made with a heated knife in different directions till one had got a part which was quite free from external impurities. Those parts of the lung were especially employed where the bronchi were completely blocked by fibrinous clots, so that the fluid bronchial contents, which always contain various micro-organisms, were avoided as much as possible.

of the Sanitary Institute under the direction of Dr. Koch and his colleagues, so that we were in a position to apply his methods to our purposes.

Dr. Frobenius prepared besides blood serum a nutrient jelly composed of meat infusion, peptone, and sodium chloride, according to the receipt which is given in the supplement to the 13th number of our journal.*

The first experiment with this jelly was successful; we obtained from a case of acute pneumonia of the right upper lobe in the stage of grey hepatization (a case complicated with hypertrophic cirrhosis of the liver, but without other complications; even enlargement of the spleen was absent) a large number of precisely similar cultivations; the material was taken from three different spots of hepatization. At the temperature of the room prominent knobs of the size of a pin's head rose above the level of the surface of the jelly, and the needle track was surrounded by a cloudy turbidity. The knob grew very quickly into a hemispherical faint white pearly elevation, and, besides the cloudy turbidity, a large number of very fine pure white granules (fig. 14, Plate IV.) developed round the needle track.

The generations obtained by inoculation of jelly with these cultivations had just the same appearances; we have up to the present time bred eight generations from this case. Microscopical examination of these cultivations showed that they were almost entirely composed of elliptical micrococci. We have never been able to discover any impurity either by cultivation on glass slides or by pouring them out on glass plates. The cultivations are in a high degree characteristic, and are different from all other jelly cultivations which we know of; they resemble a nail with a hemispherical head, and for this reason we call them the nail-shaped cultivations.

The cultivations consist entirely of dense clusters of micrococci, mostly of elliptical form; we have not been able to discover a capsule round them. Also in the micrococci cultivated on serum the capsule was found in only a few cases, but was generally missed. We shall state directly that by introducing the cultivation into a suitable animal body the capsules reappear in the rapidly multiplying micrococci.

* The composition of this material is given in [illegible] volume.—Ed.

When the cultivations are very old, *e.g.*, more than five weeks, a light brown colouration of the adjacent parts of the jelly commences, and the granules in the substance of the cultivation also become gradually more thick and plump. If cracks form in the jelly, the cultivation grows mostly in the form of a cloudiness on their surface. The jelly never becomes liquid; on the other hand we have by using a very thin, less than 4 per cent. jelly, seen a development of gas from the cultivation contained in the gelatine. It is to be noted that the cultivations in thin jelly develop less marked prominences, and grow more flat; a central depression, however, never developed.

From the jelly cultivations blood serum was inoculated. The form of the serum cultivation was then the same as that which we have described above. The cultivations grow well on the cut surface of potatoes in the form of grey drops; if jelly is inoculated with these they develop into the characteristic nail shape.

In several other cases of pneumonia our cultivation experiments failed, as we obtained either no result or micro-organisms developed, which by their slow growth, by sinking beneath the surface, or by liquefying and colouring the jelly, were proved to be foreign bodies. In these cases micrococci, but without capsules, were found on microscopical examination of the lungs. Lastly, in two cases of pneumonia,* in which micrococci both with and without capsules were found, we made cultivations which were not pure nail-shaped, but were distinguished by a flatter head,† and in part also by a central depression. In later generations we succeeded in obtaining typical nail growths from the originally impure cultivation. In the infective experiments which will now be mentioned we have chiefly employed the cultivations from the first case, but we have also obtained positive results on mice with cultivations of the fourth generation, which were obtained from the two cases last mentioned.

* One of these was a somewhat rare case of true fibrinous pneumonia as a sequela of typhoid fever; five weeks had already elapsed since the beginning of the typhoid fever, which had quite come to an end.

† It must be noted that a typical semi-globular prominent head very soon becomes flat when the gelatine commences to liquefy under the influence of heat, and this may readily happen if the tubes are too near the fire or exposed to the sun's rays.

III.—EFFECTS OF THE PNEUMOCOCCUS ON ANIMALS.

A.—INJECTION EXPERIMENTS.

By means of a platinum needle sterilized by heat one can easily pick out the heads from gelatine cultivations contained in test-tubes, after removal of the cotton wool plug. If we put these into a vessel which has been sterilized by heat, and is filled with sterilized distilled water, the cultivation is distributed through the water in a short time; we obtain a uniform, very slightly milky, turbid fluid. This fluid is introduced into a Pravaz syringe, made according to Koch's suggestion, without any caoutchouc connections, which has also been sterilized in a hot box, and then (after previously cutting the hair and disinfecting the skin with 1 per cent. solution of corrosive sublimate) injected into the right lung of the animal through the chest wall. We used rabbits, guinea-pigs, mice, and dogs.

1. *Rabbits.*

Nine rabbits were injected with about ½ to 1 ccm. of fluid. In not a single case was there the slightest disturbance of health; respiration and temperature remained perfectly normal, and the animals went about as if they were quite well. Six of these rabbits were killed on the second to the fourth day after injection (the other three were allowed to live). In four cases the lungs were quite unaltered, and even the point of puncture, which was seen on the costal pleura as a red point, was not visible on the lung. Once we found a blood infiltration the size of a pea, at another time one the size of a bean, in the right upper lobe, corresponding to the point of puncture. In both these cases several drops of reddish fluid were present in the right pleural cavity, but in the other cases it was quite free or contained several drops of yellowish fluid in which on microscopical examination a few lymphoid cells, but not a single micrococcus, were found.

Also by inoculation in jelly the fluid proved to be perfectly sterile (the pleural cavity was naturally opened with heated instruments under antiseptic precautions).

Rabbits are then perfectly refractory to our cultivations, and the organisms, which are introduced, die in a short time.

2. *Mice.*

In contrast to these results on rabbits, all the mice into which living cultivations were injected (32 in number) died in from 18 to 24 hours as the result of the injection; only one mouse lived as long as 40 hours.

All these animals showed, even a few hours after injection, distinct dyspnœa, and they sank with increasing weakness, laboured respiration, and lowering of the body temperature to 30° C. and lower.

In not a single one of these cases did we fail; the result was always identical.

On post-mortem examination one finds a perfectly typical picture; in both pleural cavities a relatively large mass of red, slimy, turbid fluid; both lungs of a deep red colour, almost entirely airless, and scattered through them ill-defined foci of red infiltration. The spleen is swollen to nearly three times its natural size, dark red; the other organs unchanged.

On microscopical examination one finds that an extraordinary quantity of micrococci are present in the slimy contents of the pleural cavities.

The micrococci lie partly free in the fluid, partly in the interior of lymphoid cells. They have all the characters of the pneumococcus, and show a well-marked capsule formation. We also found numbers of micrococci with capsules in the lungs, especially in the red infiltration, and also in the spleen and blood.

One can, in the blood of the living mouse, demonstrate the existence of abundant capsule-bearing micrococci, often in the proportion of several micrococci to every 100 red corpuscles; on these facts we would lay special stress as they show that, contrary to the usual doctrine, one is not dealing with a purely local growth of micro-organisms, but with a general invasion of the system.

As it might be assumed *a priori* that the injection of the fluid into the lungs might lead to the production of a grave inflammation, to which the growth of micrococci was only secondary, we made control experiments by the injection of

heated fluid containing the micrococci floating in it. The injection fluid was divided into two parts, of which one was kept at the temperature of the room, the other placed for 15 to 20 minutes in an incubator, regulated at 65° to 80° C.

Both fluids were then used for injection. Of the nine mice injected with the heated cultivation fluid (0·3 ccm., a relatively large amount for such small animals), one died very soon after the injection from double hæmothorax,* in consequence of puncture of the heart; the other eight remained perfectly well. Two of them were killed on the second day, and showed a small quantity of perfectly clear fluid in the pleural cavity without a trace of micrococci. The mice, which were injected at the same time with the portion of the same fluid which was kept at the temperature of the room, and which only received one drop of the fluid, died, as usual, in from 20 to 26 hours, with the typical appearances during life, as also post-mortem.

It is consequently demonstrated with great precision that the pathogenic agent in the injection is dependent on the vital properties of the organisms contained in the injection fluid.

We need only remark, in passing, that injection of distilled water was followed by similar negative results.

We are consequently led to the conclusion that pure cultivations of the micrococci of human acute croupous pneumonia, introduced into mice, produce pleurisy and pneumonia, with copious growth of the fungus elements in the pleuritic fluid, lungs and blood, whereas it produces no effect on rabbits.

From most of the mice, which died after the injection of active cultivations, fresh jelly cultivations were made from the pleural contents, the lung secretion and the spleen. (The left pleural cavity was generally used for this purpose, as the injection was made on the right side.)

In every case we obtained perfectly typical nail growths, which were further bred through several generations. These cultivations also, when injected into mice, acted in precisely the same manner as those obtained from human croupous pneumonia.

We must add that we have seen pleurisy also produced in

* In the fluid contained in the thorax of this animal there were pretty numerous micrococci without capsules. These were the organisms in the fluid injected. They had, however, been completely killed by the heat, as was at once evident from the fact that the inoculation of jelly with the fluid from the thorax was not followed by any growth.

mice by the injection of cultivations of other micro-organisms; but the micrococci in the pleuritic fluid in these cases never possessed a capsule.

3. *Guinea-pigs.*

Of eleven old and young guinea-pigs into whose right thoracic cavities cultivations floating in distilled water were injected (about 0·1 to 0·3 ccm.), six became very ill. After twenty-four hours they had urgent dyspnœa and exhaustion; the temperature was either normal or subnormal. Three of these animals were killed after 24 to 48 hours; the three others died in the course of the second day. In all these animals we found, on anatomical examination, severe double pleurisy, with more or less turbid, purulent fluid to the amount of several ccm., also turbid, purulent fluid in the pericardium. The lungs were deep red and œdematous. In three cases there were no further changes, but in three others there was lobular, red, grey or reddish-grey infiltration at the parts of the lobes removed from the hilus.

The five other guinea-pigs showed no definite evidence of disease. Two of them were killed on the third day after injection, and showed only a few clear drops of fluid, without micrococci, in the pleural cavity; on inoculation of this fluid into jelly no growth occurred. On the contrary, the pleuritic fluid of the animals which became ill or died contained numbers of micrococci with capsules; on inoculation of jelly with them a perfectly typical nail-growth developed. In the infiltrated parts of the lungs only very few or even no micrococci were found.

It results from these experiments that guinea-pigs stand midway between mice and rabbits as regards their relation to our cultivations. About half the animals were refractory; the other half became affected with severe double pleurisy with abundant growth of micrococci with capsules, some also with pneumonia. Micrococci with capsules were only found in small numbers in the blood of two of the guinea-pigs which died. Swelling of the spleen was not made out.

EXAMPLE OF EXPERIMENTS.

15th Sept. Material—cultivation of croupous pneumonia of 7th Sept. 3rd generation.

4 guinea-pigs.

a. This animal is already very weak, 24 hours after injection, and has urgent dyspnœa; killed 27 hours after injection. Double pleurisy and pericarditis; about 5 ccm. of turbid, flocculent, purulent fluid on each side, containing micrococci with capsules; the fluid gave nail-growth cultivations. Masses of red and greyish-red infiltration in the lungs, which are almost airless.

b. Killed in death agony 48 hours after injection.

Result of autopsy.—Double pleurisy, slimy purulent exudation containing micrococci with capsules. Typical nail-growth when cultivated in jelly. Scattered masses of grey infiltration, the size of a bean, in both lungs.

c. *d*. Remained well.

2 rabbits, both absolutely unaffected by injection.

a. Killed 17th Sept. Quite healthy.

b. Continued well.

5 mice. All dead 20 to 25 hours after injection.

Red and greyish-red infiltration in the lungs. Greyish-red greasy fluid in the pleural cavities, with abundance of micrococci with capsules, from which typical nail-growths were cultivated. Swelling of spleen.

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4. *Dogs.*

Five dogs (all full-grown animals, weighing 10 to 15 kilogr.) were treated with injection of fluid, with cultivations of the micrococci floating in it, into the right thoracic cavity. One of these animals became very ill with dyspnœa after injection, and sank within forty hours. At the autopsy a typical red and greyish-red infiltration of the whole of the right upper lobe was found with a thin layer of lymph on the pleura; a very few drops of fluid in the right pleural cavity. Swelling of the spleen. In the red and grey hepatisation great abundance of micrococci with capsules was found. In the contents of the right pleural cavity and in the blood micrococci with capsules were also found, but in small numbers. The pneumonia of this animal forcibly reminded one, in its clinical course as well as in its anatomical characters, of the typical lobar pneumonia of man. The cultivations which were made from the red and grey hepatization, from the blood and the contents of the right pleural cavity, were again typical nail-growths which produced the characteristic effects when injected into mice.

The four other dogs, on the contrary, showed, after injection, in part only a transient illness, with slight rise of temperature (to 40·5° C., which is, as is known, very slight for dogs), in part

even this was absent; in every case the normal condition was restored on the day, or at latest on the second day, after injection. At the end of several days or a week all these animals were treated with a second injection, applied nearly at the same spot. In this case, too, a transient illness at most with speedy and complete recovery, was produced. A positive result was obtained by simultaneous injection of mice with the same fluid.

EXAMPLE OF EXPERIMENTS.

22nd Oct., 1883, 2 o'clock (with the kind assistance of Drs. Gram, (Copenhagen) and Brosin (Halle).) Material—cultivation of croupous pneumonia of Sept. 7. 4th generation. Head of nail cultivation, about 2 ctm. big, distributed through 3 ccm. of distilled water.

1. Mouse—one drop in right thoracic cavity.

The mouse sank on the 23rd Oct. at mid-day, with the usual appearances. In the blood of the tail before death abundance of micrococci with capsules; copious tough, slimy contents in both pleural cavities; lungs much compressed, airless, red, also dark red areas therein. In the pleural exudation, and in the lungs, abundance of micrococci with capsules. Swelling of spleen.

2. Black dog (*Teckel*) about 0·8 ccm. in the right side of chest.

22nd Oct., 7.30 A.M. Appears languid; slight dyspnœa; T. 39·8° C.

23rd Oct., 11 A.M., T. 39.5°. 2 P.M., T. 40·5°. 7.30 P.M.. T. 39·5: quiet breathing; from that time it was quite lively. A second injection on the 24th Oct. of the same generation in the right side of chest. No result followed.

3. Dog (*Affenpinscher*) (43 cm. long) nearly 1 ccm. in right side of chest.

22nd Oct., 7.30 P.M. Languid, slight dyspnœa: T. 40·5° C.

23rd Oct., 11 A.M. Great languor: dyspnœa; R. 80. T. 40·8°. Coarse breathing on both sides of chest.

2 P.M. Dyspnœa, frequent cough, and pain indicated in the side by raising of the fore paw: T. 41°.

7 P.M. T. 39·4°. The animal is very weak, cannot support itself on its legs; coarse breathing marked over lower part of right lung; on left side bronchial breathing.

Midnight. Extremely weak.

24th Oct., 6 A.M. Found dead and stiff.

Autopsy.—Nothing special at seat of injection; no fluid in abdominal cavity. Spleen enlarged, 10 ctm. long, dark red; other abdominal organs unaffected.

Left pleural cavity and pericardium without change.

In the right pleural cavity only a few drops of a red, slightly turbid fluid (with considerable numbers of micrococci with capsules).

The right lung shows a considerable increase in bulk in the lower lobes only, whereas the upper and middle lobes are nearly unaltered. The right lower lobe has in itself a greater bulk than the rest of the right lung and the left lung together.

Left lower lobe	42 mm. broad.	Right lower lobe	75 mm. broad.
„	60 „ high.	„	75 „ high.
„	17 „ thick.	„	35 „ thick.

The pleural membrane covered with a thin fibrinous coagulum. The consistence is universally firm. The lower lobe is quite airless, and for the most part grey or greyish-red, only in its lower part is there a small area of red infiltration, which is of softer consistence, and depressed a little below the level of the greyish-red mass. The cut surface is smooth, mostly greyish-red, except at the small red foci, which occupy about a fifth of the lobe. Turbid secretion in the bronchi.

The left lung, and remainder of the right lung, are faintly pigmented, only slightly œdematous, with small diffused areas of reddening; bronchial secretion red.

The exudation in the alveoli shows, in the red infiltration, abundance of lymphoid cells, red blood corpuscles, only small masses of fibrin, and abundance of micrococci with capsules. In the greyish-red infiltration, besides lymphoid cells, large collections of micrococci with capsules, and a little fibrin, are present. The sections made from preparations hardened in alcohol swell up in distilled water and become slimy, the slime consisting, in every case, in great measure of the capsules of the micrococci.

Typical nail-growths were obtained from the greyish-red and red hepatization, as well as from the contents of the right pleural cavity, and lastly from the blood (from the inferior vena cava).

B.—Inhalation experiments.

Though it was demonstrated by the above-mentioned control experiments with the heated organisms that one can propagate the disease only by introducing the living organism, still the objection was possible that besides the introduction of the organism, a slight wound of the lung, such as is undoubtedly made during the injection, is necessary.

As, however, a traumatic origin can only quite exceptionally be pointed out for human pneumonia (contusion pneumonia) we tried to introduce the fungus elements into animals by inhalation.

Three elaborate inhalation experiments in all were performed, the cultivations being suspended in distilled water in the manner already described. We usually took a large quantity of distilled water (10 to 50 ccm.) and a correspondingly large mass of cultivation—several large heads about the size of several ctm.; the water was only rendered slightly turbid by them. The fluid, with the cultivation floating in it, was scattered by a hand-spray. So as not to place ourselves in danger we inserted a long caoutchouc tube between the hand bellows and the spray. The spray was then directed towards the opening of a cage in which the animals were. The quantity of fluid was scattered in 10 to 20 minutes. The experiments were made in the open air.

On account of the special importance of these experiments we will describe them individually.*

EXPERIMENT I.

a. Sept. 9. Material—cultivation of human pneumonia of Sept. 7. First generation. Two mice inhaled for 5 minutes.

b. Sept. 16. The animals lively; inhalation repeated. Material from the same case, for about ½ hour. One mouse could not be found on the following day. The other was found dead on Sept. 19.

Autopsy (Frobenius).—Both pleural cavities full of greyish, slimy exudation. Lungs quite compressed, almost unrecognizable, dark red and grey-coloured throughout. In the pleural exudation on both sides large numbers of micrococci with capsules. Jelly inoculated with them gave typical nail-growths, which were bred through several generations, then used for injection and produced positive results in the manner we have indicated.

EXPERIMENT II.

Oct. 2. Material—cultivation from the mouse which died after inhalation on Sept. 19.

4 mice.

One of the mice killed on Oct. 5, quite healthy. Another was collapsed on Oct. 6, and sank at 11 o'clock.

Autopsy.—On both sides several drops of sticky, greyish-yellow fluid in the pleural cavity; several foci of red infiltration in both lungs occupying in all about a third of the lung parenchyma. Spleen swollen to more than three times its normal size, dark red, abundance of micrococci with capsules in the blood, pleural exudation, lungs and spleen. Inoculation of jelly with the splenic juice and pleural fluid gave typical nail-growths.

The other two mice remained well.

EXPERIMENT III.

Oct. 8. Material—croupous pneumonia of Sept. 7, 4th generation.

6 mice. On Oct. 11 two of the animals were suffering from dyspnœa and weakness; both were found dead on the following day.

Autopsy.—Mouse A. Thick slimy fluid in both pleural cavities, more in the right than the left; also grey turbid fluid in the pericardial sac; peritoneal cavity normal; spleen much swollen, dark red. Diffused redness in both lungs, the right lower lobe is, moreover, entirely converted into a dense grey mass; typical lobar, grey hepatization. Trachea empty.

Mouse B. Similar to the above, only that the lower half of the left lower lobe is in a state of dense grey hepatization, which is sharply defined from the upper half of the same lobe.

In both animals abundance of micrococci with capsules in the pleural exuda-

* In these experiments several guinea-pigs also inhaled the spray, but without definite positive results. One of them died, after inhalation, with double pleural effusion, in which no micro-organisms could be found, and the fluid also remained sterile in jelly.

tion, spleen and blood. Typical nail-growth from inoculation of jelly with the pleural fluid.

The grey hepatized parts of the lung are full of micrococci with capsules, just as in early cases in man, but no fibrin.

These experiments are very convincing; that not all the animals, but only some individuals, became ill in every case probably depends on the fact that many of the animals had their backs turned towards the spray, so that only a very little or none of the fluid reached their lungs.

IV.—DIFFERENCES OF THE PNEUMOCOCCI IN DIFFERENT SPECIES OF ANIMALS.

We found already in human pneumonia substantial differences in the size of the individual micrococci as well as in that of the capsules. It has been already mentioned that the shape also varies. In mice the micrococci are generally considerably larger than the average human pneumococcus. Many of the micrococci in mice are far larger than those of man, attaining to twice or even three times their dimensions. The micrococci of guinea-pigs are usually smaller than those of mice, but are surrounded by an unusually broad capsule, which may sometimes reach the diameter of a red blood corpuscle; the size of the capsules varies between wide limits. In dogs the micrococci are, as a rule, hardly any larger than those of man; the capsules are relatively narrow, very little broader than the micrococcus itself. It must be noted, in relation to this question of relative size, that it depends to a great extent on the method of staining, and more especially on the means adopted for their decolorisation. When the specimen is fully decolorised by alcohol, especially with the assistance of weak acetic acid, the micrococci are appreciably smaller; they may by these means lose more than a third of their diameter. It is necessary then for the sake of comparison to deal with preparations treated in an absolutely identical manner.

The differences in dimensions can also be made out in unstained preparations, but with much less distinctness. It is to be noted besides that the micrococci of guinea-pigs very frequently have an irregular instead of a perfectly round or elliptical contour; the micrococci of dogs and mice, on the contrary, always have a sharp margin.

It is further to be remarked that rod-shaped bodies of different lengths, usually of the size of a diplococcus, but many of larger size, are found much more frequently in all three species of animals than in man. They are always surrounded by a characteristic capsule. Similar structures are in fact, as we have mentioned, only very occasionally found in man, whereas they are of frequent occurrence in animals, sometimes in the proportion of one to every ten spherical or elliptical micrococci. An indication is sometimes given that they are composed of individual micrococci, but the margins of the rods are usually bounded by sharp lines.

As we have not yet sufficiently carried out the direct observation of the process of vegetation of the micrococci, we must confine ourselves to the citation of this fact. We had to give up the suspicion which we cherished at first, that we had to do with impure cultivations, as we have seen the same appearance under the most varying combinations.

It must still further be added that a perfectly clear border is usually found around the micrococci of guinea-pigs, outside which is the stained capsule. A similar picture can also be made out in mice and dogs, but only when the excess of colour has been removed by alcohol. Before this is done the capsule in mice and dogs is frequently so intensely stained, that it is scarcely possible to differentiate it from the micrococcus which lies inside it. The alcohol removes the greater part of the stain from the capsules, only the periphery remains as an intensely stained more or less broad border. It has often happened to us that in spreading out the slimy exudation of mice on the cover-glass, which we do with a platinum needle, in many places the micrococci have been torn out of the capsules, so that the micrococcus lies near the empty capsule, which shows a clear space in the middle.

The outer border of the capsule is usually very sharp in mice and dogs, but quite delicate, on the contrary, in guinea-pigs. The capsules have also a tolerably sharp contour in man.

CONCLUSION.

Experiments have already been communicated formerly in which pneumonias were produced by injection of cultivations of micro-organisms, chiefly by Klebs, and in later times by Salvioli and Zäslein.* We will not enter on a closer analysis of the experiments of Klebs. We will only remark that the material with which this investigator worked was not always quite free from objection. Klebs used bronchial contents; Salvioli and Zäslein, on the other hand, used blood, or the fluid from blisters raised on the skin of persons suffering from pneumonia; they cultivated in different fluids, not on solid nutrient media. All these observers stated that they had obtained positive results in rabbits. We must draw the conclusion therefrom that if in their experiments pneumonia was caused by micro-organisms, it could not have been the same organism with which we have worked. Ours produced no effect upon rabbits.

The view that pneumonia is an infective disease has been advanced for a considerable time by investigators. Jürgensen more especially has advocated this hypothesis. During the last few years it has apparently been more and more accepted by our clinical physicians. The former demonstration of the regular occurrence of micro-organisms in this disease strengthened this view. In the present work we have, we believe, made a further advance.

The communications with regard to epidemic pneumonia have become more numerous in the last few years. In large towns, indeed, one has rarely the opportunity of making observations of this kind. Nevertheless in the country and small towns this fact has apparently been more frequently noticed than is generally believed. It is very desirable that observations of this kind should no longer remain unpublished. Those localities in which one or only a few medical men have the whole population under their care are particularly favourable for such observations.

* *Italia Medica*, No. 39. *Centralbl. f. d. med. Wissensch.*, v., Oct. 13.

FURTHER OBSERVATIONS ON THE MICRO-COCCUS OF PNEUMONIA.*

THROUGH the kindness of Dr. Riess we had the opportunity of examining, with Dr. Gram, of Copenhagen, the blood of living persons for the presence of micro-organisms in a series of cases of pneumonia. We must, in the first place, mention that we have hitherto never been able to demonstrate the presence of micro-organisms by direct microscopical examination of the blood of pneumonic patients, whereas we have nearly always found large numbers of micrococci in the blood of our experimental animals. We tried, therefore, to discover the few organisms which were perhaps present by the method of cultivation.

We used for this purpose the blood abstracted by cupping; the skin of the patient was previously purified by washing with corrosive sublimate and alcohol, the spring lancet and cupping-glass sterilized by heat, so that the blood was easily obtained quite pure. Large and small quantities of this blood were introduced by means of a sterilized platinum needle, or pipette, into meat jelly, containing peptone. In all, six undoubted cases of acute pneumonia at various stages of the disease, but mostly in the first days, were examined in this way. In five of these cases the results were negative, but the sixth case gave a positive result; out of a large number of inoculation punctures, in one a development of micro-organisms occurred, which in subsequent generations presented the typical appearance of the nail-shaped cultivation. Further experiments on animals were made with these cultivations, and results attained thereby which were identical with those which were described in our earlier work.

Rabbits proved to be completely refractory, while mice died in a perfectly typical manner of pneumonia and pleurisy. In these

* *Fortschritte der Medicin*, vol. ii., No. 10, 1884.

animals micrococci, with capsules, were demonstrated. The pathological products were used for further inoculation, and we obtained from them the characteristic cultivations, so that we think that we have established the fact that in certain cases of pneumonia, or probably at a definite stage of the disease, the pneumococcus is present in a living state in the blood. It is not to be wondered at that the micrococcus was absent in the blood in a large number of cases.

It was pointed out in my first communication that it is not possible to demonstrate, in every case of pneumonia, the existence of the pneumococcus described by us, and after abundant experience I can only repeat this statement. One always finds, indeed, by microscopical examination, abundance of micrococci (irrespective of the very old cases); in cultivation experiments, however, the results are often quite negative, or one obtains growths of other kinds of micrococci, which behave quite differently from the pneumococci, as regards both their mode of growth on meat jelly and their action on animals.* This may depend on very different circumstances. Either there are several forms of pneumonia, only one of which is dependent on the micro-organism, which we have described, or the micro-organisms may have been present, but at the time of examination have disappeared or be no longer alive. Further investigations must decide these points. I consider it extremely probable that there are different exciters of pneumonia, just as we now know a number of different micro-organisms which cause suppuration. I am inclined to the view of those clinical workers who do not regard the unity of pneumonia as satisfactorily proved; however, this is only an opinion, definite facts on this point have not yet been made out.†

* See Talamon, *Prog. Med.*, 1883, p. 1030; Afanassiew, *Comptes Rendus de la soc. de biologie*; A. Fränkel, *Verh. d. congr. f. innere Med.*, 1884, p. 77.

† A paper of some importance with regard to pneumonia is published by Dr. R. Emmerich in the *Fortschritte der Medecin*, vol. ii., No. 5, 1884, p. 153, "On Pneumococci in the Soil under the Floor of a Hospital Ward as a Cause of an Epidemic of Pneumonia."—E. T.

THE

ETIOLOGY OF CHOLERA.

By Dr. ROBERT KOCH.

TRANSLATED BY

GEORGE LOCKWOOD LAYCOCK, M.B.,

Physician to the Paddington Green Children's Hospital.

I.—PAPER READ BY DR. ROBERT KOCH AT THE CONFERENCE HELD AT BERLIN FOR THE DISCUSSION OF THE CHOLERA QUESTION IN JULY, 1884.

We need for sanitary regulations a scientific basis as firmly established as possible; for these regulations have to do not only with very costly management, but also with the well-being of many men. This is more particularly the case as regards the prevention of epidemics, with which, as can be said without any exaggeration, the most important sanitary endeavours are occupied. On this account it might be presumed that the contest against epidemics is waged on scientific principles, which are quite established and carefully carried out; but unfortunately this is not universally the case; and especially with regard to cholera such a firm basis is wanting. Quite a number of views have been propounded as to the nature, the spread, and the mode of infection of cholera, and different theories have been founded thereupon. But opinions are so divergent from each other—some indeed are directly antagonistic,—that we are unable without further inquiry to accept them as a basis or starting point for our measures for combating this epidemic.

Thus, on the one hand, it is stated that cholera is a specific disease having its source in India; on the other, this is disputed, and it is said that cholera can arise spontaneously even in other countries, and does not depend on a specific cause. The one side only acknowledges that cholera can be transmitted by the sick and their emanations; the other that it can be carried by merchandise, by healthy men, and by currents of air. Just as contradictory opinions exist as to the importance of drinking water as a vehicle for the infective material, as to the influence of the nature of the soil, as to the question whether or not the dejections of the patient contain the infective material and as to the duration of the stage of incubation. All these points are,

however, of the greatest importance in the prevention of cholera, and this disease cannot be successfully combated, until some agreement is arrived at with regard to these preliminary questions in its etiology.

The etiology of cholera has indeed been able to profit little from the progress which we have made in the etiology of other infectious diseases. This advance has been principally developed during the last ten years, and during this time no occasion has demanded an inquiry to be made into cholera, at least not in Europe or in the neighbouring countries; and in India, where cholera could have given continuous material for investigation, nobody has been found to occupy himself with the task by the help of the recent methods of research.

On this ground it was therefore not unfortunate that last year cholera broke out in Egypt and gave an opportunity for studying the nature and mode of infection of the disease before it spread into Europe. This opportunity was made use of by different governments, and expeditions were sent there for the investigation of cholera.

The honourable charge of conducting one of these expeditions fell to my lot.

When I undertook this commission I was well aware of the difficulty of the task which lay before me. Absolutely nothing was known with regard to the virus of cholera. One was at a loss where to seek for it, whether only in the intestinal canal, or in the blood, or in some other place. Further, one did not know whether bacteria would have to be dealt with in this case, whether some kind of fungus or the like, or whether some animal parasite such as an amœba. To be sure the investigation did not in this direction meet with such difficulties as in another, where I expected it the least. I had pictured to myself the pathological conditions entirely after the description in the text-books, namely, that the intestine in cholera would show peculiarly little change, and that it would be filled with a material like rice-water. I had almost forgotten the result of the autopsies which I had formerly seen, so that I was unable to correct from them my erroneous views. I was therefore at first somewhat surprized and uncertain, when I came to see something quite different in the intestine. In the first post-mortem examinations it almost at once became apparent, that in an overwhelming

majority of cases remarkably deep and striking changes existed in the intestine; other cases again showed changes less serious; and lastly, I came across cases which corresponded in some measure to the type which is described in the text-books. But it required time and a number of post-mortem examinations as well, before I succeeded in forming a correct general idea, and in estimating rightly all the different changes which came before me.

I shall here remark that in spite of the most careful examination of every other organ, and of the blood, nothing was found, which warranted the inference to be drawn that there existed any virus in them. The interest was therefore directed finally to the changes existing in the intestine, and these might be roughly grouped in the following manner. There were cases in which the lower segment of the small intestine was stained of a dark brownish red colour, most intense immediately above the ileo-cæcal valve and diminishing upwards, the mucous membrane being studded with superficial hæmorrhages. In many cases the mucous membrane was even superficially necrosed, and covered with a diphtheritic deposit. In such cases the contents of the intestine were not a rice-water like and colourless, but a bloody ichorous, offensive, fluid. Other cases again showed a gradual transition to less deep changes, the reddening being less intense or only in patches; and finally there were cases in which only the margins of the follicles or Peyer's patches were reddened. This last-mentioned group presented a very characteristic appearance, which scarcely ever occurs in other intestinal diseases, and which is quite peculiar to cholera. In a very few cases, however, was there extremely little change in the mucous membrane, which appeared somewhat swollen and less transparent in the superficial layers, the solitary glands and Peyer's patches being more strongly marked. The whole mucous membrane was coloured a light rosy red, but it never went so far as capillary hæmorrhage. In these cases the intestinal contents appeared colourless, though they never could be compared with rice-water, but rather with gruel. Only in a very few cases did I see the contents of the intestine composed of clear water and mucus, with comparatively little flocculent matter in suspension.

If the intestine and its contents were microscopically examined, it then appeared that in some cases, particularly in those in

which the Peyer's patches were reddened at the edges, this redness corresponded to a migration of bacteria. It gave an appearance like what you have seen in one of the preparations before you, which was taken from such a case. (See fig. 4.) The bacteria had partly crowded into the tubular glands, and had partly thrust themselves between the epithelium and the basement membrane, and thus had, as it were, detached the epithelium; in other places they appeared to have penetrated deeper into the tissues. Then there were cases in which, behind these bacteria (which because of their size and shape had a definite appearance, so that they could be distinguished from other bacteria, and have special

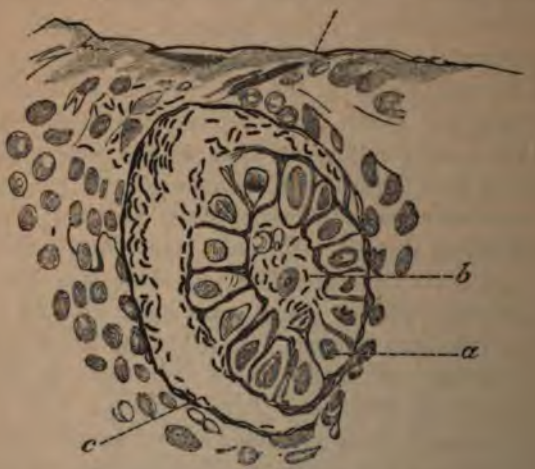

Fig. 4.

Section of the mucous membrane of a cholera intestine. A tubular gland (*a*) is divided transversely. In its interior (*b*) and between the epithelium and the basement membrane (*c*) are numerous comma bacilli × 600.

attention devoted to them), various other bacteria penetrated into the tubular glands and the surrounding tissues, for example, large thick bacilli and very fine bacilli. Similar conditions are found in necrotic and diphtheritic changes of the intestinal mucous membrane and in typhoid ulcers, where the tissues, destroyed by the pathogenic organisms, are afterwards infiltrated with other non-pathogenic bacteria. These first-mentioned bacteria could not therefore be considered *à priori* as altogether unimportant in the cholera process, while all the others gave the impression that they were secondary; for the first described bacteria always pre-

ceded these latter, they penetrated deeper into the tissues, and appeared as if they had opened a way for the other bacilli.

Now as to the contents of the intestine. At first the cases examined were scarcely suitable and afforded no clear picture, for the contents of the intestine had already a decomposed bloody character. In these cases were innumerable quantities of the most different kinds of bacilli, so that the attention could not be fixed on the special cholera bacillus. It was only after I had performed autopsies on a few acute and uncomplicated cases, in which hæmorrhage had not yet occurred, and in which the intestinal contents had not yet become decomposed, that I recognized that the purer and fresher the cases were, the more a definite kind of bacteria prevailed in the intestinal contents; and it very soon became evident that these were the same bacteria which I had previously seen in the mucous membrane. (See fig. 5.) This discovery, it stands to reason, drew more and more the attention to this species of bacteria. I have examined them from every point of view to determine their special peculiarities, and can communicate the following about them.

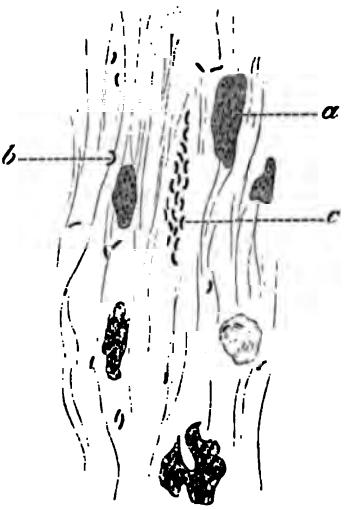

Fig. 5.

Cover-glass preparation from the contents of a cholera intestine. (*a*) remains of the epithelial cells, (*b*) semicircular comma bacilli, (*c*) the characteristic grouping of the comma bacilli × 600.

These bacteria, which, on account of their peculiar form, I have named comma bacilli, are smaller than the tubercle bacillus. As a precise idea of the size, length, and breadth of bacteria can scarcely be got from a statement of the dimensions in numbers, I prefer to compare the dimensions of bacteria with some well-known object, so that a fairly accurate idea can be obtained. As the tubercle bacilli are known to all, I shall compare the cholera bacteria with them. The cholera bacilli are about half, or at the most two-thirds as long as the tubercle bacilli, but they are plumper, thicker, and furnished with a slight curve.

This curve is usually not more marked than that of a comma, but under certain circumstances it can become more pronounced, and even may form a semicircle. Other cases are seen in which the curve is double; one comma may join another, but in an opposite direction, so that the letter S is formed. (See fig. 6.) But I believe that in both cases two individuals remain in connection with each other after division, and thus cause the appearance as if there was a more marked curvature. In the cultivations there is found a very noteworthy form of development of the comma bacilli, which is very characteristic. In one of the preparations, which have been laid before you, there are several well-marked examples of this form, and I took the opportunity, in the demonstration of these preparations, to draw particular attention to them. The comma bacilli frequently form threads of greater or less length. These threads are not straight, as are those of other bacilli, for example the anthrax bacilli, nor simple undulating threads, as might be thought from the microscopical characters, but are long, delicate spirals, which in their length and other appearances have the greatest resemblance to the spirochætæ of relapsing fever. I should not be able to distinguish them from each other if they were placed side by side. On account of the peculiar form of development, I am inclined to the view that the comma bacillus is not really a true bacillus, but an intermediate form between the bacilli and the spirilla. Possibly even it may be a true spirillum, of which we have a fragment before us. In other spirilla, for example the *spirillum undulans*, it is seen that short specimens do not form a complete spiral, but consist of a short rod, which is more or less curved. Later I shall return to this point, which is not altogether unimportant.

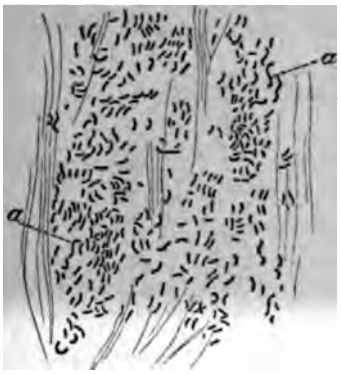

Fig. 6.

Cover-glass preparation of cholera dejecta on damp linen (2 days old). Great multiplication of the comma bacilli with S shaped forms (*a*) × 600.

From the demonstration of the preparation in which the comma bacilli were cultivated in meat infusion, you have already

learned that they can be grown in that material. They increase in this fluid extraordinarily quickly and luxuriantly, and this circumstance can be utilized to study their other properties by suspending a droplet of a meat infusion, in which these organisms are growing, from the under surface of a cover-glass and examining it directly with a high power. It is then seen that the comma bacilli are extraordinarily active in their movements. When they accumulate in numbers at the margin of the droplet, and are swimming about actively, it seems quite as if there were a swarm of midges, between which dive here and there long spiral-shaped threads, which have themselves pretty active motion. The whole forms a peculiar, and in the highest degree characteristic picture. (See fig. 7.)

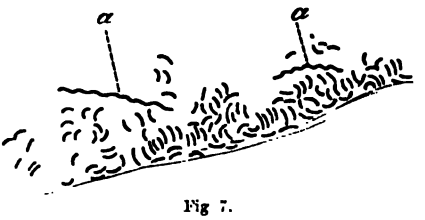

Fig 7.

Cover-glass preparation of the edge of a drop of meat infusion containing a pure cultivation of comma bacilli. Spirillar threads (*a*) × 600.

The comma bacilli also grow in other fluids, and they especially develope in milk very rapidly and luxuriantly. They do not cause the milk to curdle, nor do they separate the casein, as many other bacilli do, which have equally the property of developing in milk. The milk appears quite unchanged, but if a small drop be taken from the surface and examined microscopically, it swarms with comma bacilli. Further, they grow in blood serum, in which they develope very rapidly and increase luxuriantly. A very good soil for the comma bacilli is the nutrient jelly, of which you have already seen a specimen. This nutrient jelly is also of use, as I have explained in the demonstration of the method of cultivation, for rendering the discovery of the comma bacilli easier and extremely certain.* In nutrient jelly the colonies of the comma bacilli take quite a characteristic and definite form, which, as far as I have investigated, and as my experience goes, is like that formed by no other kind of bacillus.

The colony appears, when it is quite recent, as a very pale and tiny drop, which is not completely circular, as is usually the case with colonies of bacteria in gelatine. They have a contour

* A demonstration preceded the reading of this paper.—G. L. L.

more or less irregularly defined, with projections in parts, giving a rough or dentated appearance. They also assume very early a somewhat granular character, and are not of homogeneous consistence like most other colonies of bacteria. (See figs. 8 and 9.)

As the colony become somewhat larger, the granulations become more apparent, till finally it appears as a little mass of highly refracting granules. I would compare the appearance of such a colony best with that of a heap of bits of glass. On further increase of the colony, the gelatine in its immediate neighbourhood becomes fluid, and at the same time the colony of bacteria sinks somewhat deeper into the substance of the gelatine.

Fig. 8.
Colonies of comma bacilli as seen in plate cultivations in nutrient jelly × 80.

Fig. 9.
Natural size of the colonies of cholera bacilli as seen in plate cultivations.

Thus there is formed a small funnel-shaped recess in the gelatine, in the middle of which the colony can be recognized as a small white point. This appearance is quite peculiar, at least it is seen in very few other forms of bacteria, and according to my knowledge, is never so distinctly marked as in the comma bacilli. This sinking in of the colony can be most clearly observed if a pure cultivation is prepared exactly in the way which has been described in the demonstration of the cultivation process. A suitable colony should be looked for under a low power in the layer of gelatine. This should be touched with a

platinum wire which has been previously heated; the bacilli should be transferred on the wire to a test tube filled with gelatine, which is then plugged with sterilized cotton wool. A pure cultivation made in this manner grows in the same way as in the gelatine on the glass plate. I possess a large collection of pure cultivations of bacteria prepared in this way, but I have never seen in them such changes as those which the comma bacillus causes after its transfer to the gelatine. As soon as the cultivation begins to develope a small funnel is noticed, which indicates the top of the needle track. (See fig. 10.) Gradually the gelatine in the neighbourhood of this track becomes fluid, and there is clearly seen the small colony, which is ever enlarging. A deeply sunken spot however always remains, which appears in the partially fluid gelatine as if a bubble of air floated over the colony of the bacilli. It almost gives the impression as if the vegetation of the bacilli had not only caused the liquefaction of the gelatine, but also a rapid evaporation of the fluid formed. We know already a number of different bacteria, which cause the gelatine in test tube cultivations to become fluid just in the same manner around the track of inoculation. But such a depression and bubble-shaped hollowing out of the surface is never found. I have still to mention that the liquefying of the gelatine from a single isolated colony never extends far, as can be best demonstrated in a layer of gelatine spread on a glass plate. The dimensions of this liquefied area around a colony may be estimated at about one mm. Other kinds of bacteria are able, as you have seen in one of the gelatine plates laid before you, to liquefy the gelatine over a greater area, so that a colony may extend to 1 cm. or more in diameter. In the cultivation of the comma bacilli in test tubes the liquefying of the gelatine spreads gradually and very slowly from the track of inoculation, so that in about a week the entire contents of the tube become fluid. To all these peculiarities, trivial as they appear by themselves, particular weight should be attached, because they serve to distinguish the comma bacilli from other varieties of bacteria.

Fig. 10.
Test tube cultivation of cholera bacilli showing the funnel-shaped depression at the surface.

Further, the comma bacilli can be cultivated when placed in agar-agar, to which meat infusion and peptone has been added. This agar-agar jelly is not liquefied by the comma bacilli. They can also be grown on boiled potatoes, and, in regard to certain questions, this is a very important point. They grow on potatoes just like the bacilli of glanders. These last, as you have perhaps seen in the cultivations demonstrated at the Hygienic Exhibition, form a thin pap-like brownish layer on the potatoes. The cultivation of the comma bacilli, if grown on potatoes, is similar to this, but it is not coloured such a deep brown, but rather a light-greyish brown.

The comma bacilli thrive best at a temperature of from 30° to 40° C., but they are not very sensitive to lower temperatures. Experiments have been made, which have shown that they do quite well at a temperature of 17° C., though they grow correspondingly slower. Under 17° C. the growth is very slight, and under 16° C. it seems to stop altogether. In this respect the comma bacilli correspond in a noteworthy manner to the anthrax bacilli, which also require about the same limits as regards low temperature. I once made an experiment as to the influence of lower temperatures on the comma bacilli, to discover if it were possible not only to delay their development by a very low temperature, but even to destroy them. For this purpose a cultivation was placed for an hour in a temperature of —10° C., which completely froze it. Then a cultivation was made therefrom in gelatine, but this showed in its development and growth not the slightest difference. It bore freezing perfectly well. Not so is it with the deprivation of air and oxygen. It stops growing at once if it is deprived of air, and therefore if one accepts the division of ærobic and anærobic organisms it would belong to the anærobic. One can easily convince oneself in this way. After the inoculated jelly has been poured out on a glass plate, and when it has just begun to solidify, a piece of glass (*Marienglass*), or mica split as thin as possible, is laid on the middle of the gelatine so as to cover at least one-third of the surface. The leaf of mica, on account of its elasticity, adapts itself accurately to the surface of the gelatine, and shuts off the air from the covered portion. It is then seen, as soon as the development of the colonies commences, that it occurs only in the uncovered portion of the gelatine, and reaches very little under

the cover, to the extent only of about 2 mm., as far in fact as the air can penetrate by diffusion; but under the mica plate nothing grows. There certainly exist extremely small colonies invisible to the naked eye, which apparently owe their existence to the oxygen contained in the jelly, but these do not increase in size much further after this is exhausted. An experiment was also made in another way. A small glass containing nutrient jelly, which was inoculated with comma bacilli, was placed under the receiver of an air-pump, another glass prepared in a similar way was placed outside the air-pump, as a control experiment. It then appeared that those under the air-pump did not grow, while those outside the pump did well. But if those which had been under the air-pump were afterwards set out in the air, they then began to grow. They therefore had not died, they only wanted the necessary oxygen to be able to grow. The result is similar when cultivations are placed in an atmosphere of carbonic acid; while those cultivations placed for control outside the carbonic acid atmosphere grow in the usual manner, those subjected to a stream of carbonic acid remain without development. However, in this case also they do not die, for after they have been left in the carbonic acid for a considerable time they begin at once to grow as soon as they are taken out of it.

Taken as a whole the comma bacilli increase, as I have several times indicated, extraordinarily rapidly. Their vegetation reaches its highest point very quickly; it remains stationary only a short time, and then quickly diminishes. The dying comma bacilli lose their form; they appear at times shrivelled, at times they have a more swollen aspect. Also in this state they take up colouring matter faintly or not at all. The peculiar conditions of the growth of the comma bacilli can be best observed, if substances which are rich in comma bacilli, but which contain other bacteria, such, for example, as intestinal contents or cholera dejections, are placed on moist earth, or spread on linen and kept in a moist condition. The comma-bacilli increase in a remarkable manner in a short time, for instance within twenty-four hours. The other bacteria occurring alongside them are in the first instance overgrown by them. A natural pure cultivation is thus formed, and there are obtained, by microscopic examination of portions taken from the surface of the moist earth or

linen, preparations which show almost only comma bacilli. You have seen such a preparation obtained from the linen of a cholera patient which was soiled with dejecta and kept moist (fig. 6). The luxurious growth of the comma bacilli does not, however, last very long. After two to three days they begin to die, and the other bacteria then increase in number. These circumstances are similar to what goes on in the intestine itself. A rapid growth takes place, but when the period of vegetation, which only lasts a short time, is over, and especially when there is transudation of blood into the intestine, the comma bacilli disappear again and in their stead other kinds, especially putrefactive bacteria, develope. On this account I would almost assume, *à priori*, that if the comma bacilli are introduced into a putrid fluid containing much of the products of decomposition of other bacteria, and in particular of putrefactive bacteria, they would not develope properly, but would soon die. Upon this point, however, exhaustive experiments have not as yet been made, and it is only a conjecture I should make on the ground of experience gained in the cultivation of other bacteria. This point is of importance so far, because it is not altogether a matter of indifference whether the comma bacilli, when they get into a cesspool, will there find a good or a very bad soil. In the first case they would increase, and must be destroyed by disinfection; in the latter they would die, and the cesspool would need no further disinfection. According to all the experience I have at my command, I am inclined to accept the latter view.

The comma bacilli thrive best in fluids which do not contain too little nourishing material. Several experiments have been made as to this point. A meat infusion, with an alkaline reaction, was diluted and inoculated with comma bacilli. In one of these experiments the meat infusion, after being diluted five times, proved itself to be no longer a suitable medium. In another the comma bacilli grew even after a tenfold dilution. It stands to reason that these experiments must be performed again and carried out on a more extensive scale, in order to find a certain limit; but in any case this result was recognized, that the dilution could not go very far, and that the comma bacilli required a certain concentration of the nourishing material for growth.

In the cultivation experiments it was further found that the nourishing material, at least the nutrient jelly and the meat infusion, should not be in the least acid. As soon as the nutrient jelly showed the slightest trace of an acid reaction, the growth of the comma bacilli became very much diminished. Did the reaction become clearly acid, then the development completely stopped. However, it is worthy of note that not every acid appeared to be antagonistic to the comma bacilli, for the cut surface of a cooked potato gives a marked acid reaction due to the presence of, if I do not mistake, pyromalic acid (*apfelsäure*), in spite of which the comma bacilli grow quite luxuriantly. So that it cannot be said without further inquiry that all acids hinder the growth, but in any case there is a number of acids which have this property, and in the meat infusion it is probably due to the lactic acid, or an acid salt of phosphorus.

Since the influence of development-inhibiting substances on the growth of the comma bacilli is of no small interest, a number of other substances were investigated. At this stage I should like to draw attention to the fact, that the inhibition of the development does not imply disinfection, and these experiments only deal with the determination of the amount of a substance which suffices to hinder the growth of the bacteria. In no way are they killed, as they would be in disinfection. We had something analogous to these experiments in the influence of carbonic acid on the comma bacilli, in which, only so long as the carbonic acid was allowed to act, was the growth inhibited. So also for a number of substances which I shall enumerate to you.

Iodine has been shown by Davaine to be a very intense poison to bacteria, and in certain circumstances this is quite correct. Davaine made the experiment in this manner. He diluted a fluid, which contained anthrax bacilli, (for example the blood from this disease), to an extreme degree; so that he practically had only pure water, in which were suspended a very few anthrax bacilli. To this fluid he added iodine, and it was shown that the bacilli were killed by this in extraordinarily small quantities. But the conditions in practice are quite otherwise. We have never to stop the growth of infective materials in pure water, but in the alkaline contents of the intestine, or in the blood, or in lymph, and in these the iodine would not remain free, but would immediately form a combination with the alkalies. The testing of the

influence of iodine on the comma bacilli was carried out by adding iodine water to a meat infusion, which was carefully prepared, so as to form a good nourishing material. Iodine was dissolved in water in the proportion of about 1 to 4,000. Of this iodine water 1 cc. was mixed with 10 cc. of the meat infusion, but this addition did not hinder the development of the bacilli in the slightest degree. The proportion of iodine, which will prevent the bacilli from developing, would have to be far greater than that used in the experiment. Therefore it did not appear to me to be necessary to perform further experiments, since in practice a greater amount of iodine than this could not be given.

Alcohol first prevents the development of the comma bacilli when one part is added to ten of the cultivating fluid, that is, to the extent of 10 per cent. This is a degree of concentration which in any case cannot be made practical use of.

Common salt was tried to the extent of 2 per cent. without any hindering of the growth of the comma bacilli being caused.

Sulphate of iron prevented development first on 2 per cent. being added to the cultivating fluid. As this material has frequently been used for disinfection during cholera times, I would impress on your memory that 2 per cent. strength is the first limit of hindrance to development, and that by this concentration sulphate of iron does not kill the comma bacilli. The power of sulphate of iron in preventing development depends upon the fact, that on its addition to the cultivating fluid the peptones and the albuminates, which serve for the nourishment of the bacteria, are precipitated, for a copious precipitate occurs on the addition of a 2 per cent. solution of sulphate of iron. Possibly also the acid reaction comes into play in hindering the growth. A specific action on bacteria does not therefore seem to be possessed by this material, and it is not a real destroying or disinfecting material. I consider it even possible that with a material of this kind quite the contrary result may be obtained to what is intended. Given the case in which the contents of a cesspool, into which it is supposed that the comma bacilli have entered, have to be disinfected. According to my idea the putrefactive process going on in the material is quite sufficient of itself to kill the comma bacilli. But if iron sulphate is added to such an extent as to cause an acid reaction, and thus

to interrupt the putrefactive process, nothing occurs except the cessation of the growth of the bacteria, as well as the comma bacilli. The bacteria will not be killed, and as regards the comma bacilli, the prejudicial influence of the septic bacteria being taken away, they will be preserved instead of being destroyed.

This instance is a very good one as showing that the disinfectant must be accurately estimated and tested on this point, and that one has to carefully decide what acts only as a preventive to decomposition, and what is really destructive to the bacteria. Possibly the first may even serve as a preservative to the infective material.

I shall go over only shortly the proportion of other substances necessary to inhibit the development. Alum, 1 to 100. Camphor, 1 to 300; I had particularly expected from camphor a stronger action, but several careful experiments have shown that this substance possesses only a slight influence on the comma bacilli. Carbolic acid, 1 to 400; this number agrees roughly with what we previously knew of carbolic acid and other bacteria. Peppermint oil, 1 to 2,000. Sulphate of copper, 1 to 2,500; this material has a fairly strong effect, but if one calculates how much sulphate of copper must be given to hinder the growth of the comma bacilli in the intestinal canal, one would arrive at a quantity which it would be impossible to administer to anybody. Quinine, 1 to 5,000; and corrosive sublimate, which here again is found to be far superior to all other substances, 1 to 100,000.

In these experiments as to the influence of development-inhibiting materials, the surprising fact was established that the comma bacilli extraordinarily easily die when they are dried. The experiment was done by allowing a small droplet of a substance containing bacilli to dry on a cover-glass, (indeed for a series of experiments a large stock of such cover-glasses should be prepared). On such a cover-glass a small drop of the fluid to be tested is placed, and the development is then studied in a hollow slide. By proceeding in this way it was found that in not a single preparation was there any growth, but what was more remarkable, there was none in the control specimen, in which the fluid employed was pure meat infusion. At first I did not at all know on what the non-appearance of the growth depended, and thought that the cause must lie in the meat infusion, for in similar

experiments with other bacteria I had never met with anything of the kind. For example, the anthrax bacilli can be kept for a considerable time dry on cover-glasses, and in this state they remain capable of growth from a half to a whole week. As the examination of the meat infusion showed that the fault was not due to it, we had to test whether the comma bacilli had not been destroyed through the process of drying on the cover-glass. To get information on this point the following experiment was performed. A number of cover-glasses were provided with a droplet of some bacillus-holding material. The droplet dried up after a few minutes. Then one cover-glass after a quarter of an hour, another after half an hour, another after an hour, and so forth, had placed upon it a drop of meat infusion. It then became apparent in the several series of experiments which were made, that the comma bacilli on the glasses dried for a quarter of an hour, for half, and for a whole hour were capable of development, but that many of those dried for two hours were dead. In none of these experiments did the bacilli retain their vitality for more than three hours. It was only when compact masses of bacillus cultivation (for example, the pap-like substance of a cultivation grown upon potatoes) were dried, that the bacilli remained alive for a longer time, evidently because in these cases complete dryness occurred very much later. But even under these circumstances the bacilli have never been found to retain their vitality in a dry state longer than twenty-four hours.

This result was in so far important, for with its help it was easy to test whether the bacteria had a resting-stage (*dauerzustand*). We know that other pathogenic bacteria which form spores, for instance anthrax bacilli, can be preserved in a dry state in their resting or spore condition for years on a cover-glass without dying. We know also of other infective materials, with the nature of which we are not yet precisely acquainted, for example variolous or vaccine matter, that they can remain active in a dry state for a long time, even for several years. These cases depend on the existence of a real resting-stage. If then, the comma bacilli, which are destroyed in such an unusually quick manner by drying, exist in a resting-stage under any conditions, this fact must very soon be made evident by drying them.

In any case this is one of the most important questions in the etiology of an infective disease, but particularly so for cholera.

On this account, the investigation of this point has been carried out in the most careful way possible, and in all directions, and I believe there is scarcely anything more to be done in reference to it. First, the cholera dejecta and the intestinal contents of cholera cadavera were placed on moistened linen, in order that the comma bacillus might be able to develope under the most favourable conditions. After different periods pieces of the linen were dried, as for instance after twenty-four hours, after some days, after several weeks, in order to see if in this time anything like a resting-stage had been formed. For the infection by cholera linen affords the only undisputed example of the existence of an active infective material which is attached to a definite object. If a resting-stage is to be found anywhere, it must exist in the linen soiled by cholera patients.

In all these experiments, however, a resting-stage has never been demonstrated. When the dried materials were examined, it was found that the comma bacilli were dead. Further, the dejecta were put into the ground, either mixed with the soil, or spread over the surface of the earth, which was either kept dry or moist; also they were mixed with marsh water and left to decompose without anything further being added. In gelatine cultivations the comma bacilli have been cultivated as long as six weeks; the same in blood serum, in milk, and on potatoes, on which the anthrax bacilli, as is known, form spores extremely quickly and abundantly. But a resting-stage of the comma bacilli has never been met with. Since we know that most of the bacilli possess a resting-stage, this result must appear very surprising. But I shall here call to recollection what I have already mentioned earlier, that it is in the highest degree probable that we have here to deal with a micro-organism which is not a true bacillus, but which is more closely related to the group of spiral bacteria, the spirilla. But we do not, however, know that any of the spirilla have a resting-stage. Spirilla are bacteria which are always associated with fluids, and do not, like anthrax bacilli, grow under conditions in which they have to exist at times in a dry state. It seems to me, therefore, at least as far as my experience goes, that one cannot expect to find a resting-stage for the comma bacilli. Besides, as I shall have to explain later, the want of the resting-stage is completely in accordance with the experience of the etiology of cholera.

If all the peculiarities of the comma bacilli, as yet described, are taken into consideration, one must come to the conviction that they belong to a definite, clearly characterized kind of bacteria, and that they may be easily recognized and distinguished from other bacteria by the aid of their characteristic properties.

After this conviction was come to, it was necessary to determine in what relation the comma bacilli stand to the true cholera process; and in the first place it was necessary to inquire whether they occur in all cases of cholera, and whether they are wanting in all non-cholera cases, that is to say, whether they belong exclusively to cholera. From this point of view as large a number of cases as possible have been thoroughly examined. In Egypt 10 post-mortem examinations could be utilized, though these were only microscopically tested; for the characteristics of the comma bacilli, as shown by their growth in nutrient jelly, were not at that time sufficiently well known to me, to enable me to use the gelatine process for the demonstration of the bacilli. But I have convinced myself by careful microscopical examination, that the comma bacilli were present in all these cases. Then in India 42 autopsies were examined microscopically as well as by cultivation in nutrient jelly, and in no case were the comma bacilli absent. In a number of cases having an acute course, almost a pure cultivation of the comma bacilli was found in the intestine. Further, in India the dejections of 32 cholera patients were examined in like manner, and in each case the comma bacilli were demonstrated therein. Also vomited fluids from cholera patients were very often examined, but in these the comma bacilli were only found twice, and in these cases the character of the vomit showed, that it was not the true stomachic contents, but the contents of the intestine, which had been driven up by the pressure of the abdominal walls and so evacuated; the fluid having an alkaline reaction and quite the appearance of intestinal contents. I have also found the comma bacilli in preparations made from eight other autopsies, part of which I had had sent to me previously from India, and part I had received from Dr. Kartuhi and Dr. Schiess Bey from Alexandria; and lastly I made a short time back two autopsies in Toulon, conjointly with Dr. Strauss and Dr. Roux, and in these cases, as well as in the dejections of two other cases, the comma bacilli were discovered. In both the autopsies in Toul

extremely characteristic and acute cases were dealt with. The one, a sailor was to be dismissed from the hospital on that day as convalescent from malaria. He did not, however, go out, for about 11 o'clock in the forenoon he was attacked by cholera; at 3 o'clock in the afternoon he was dead, and the post-mortem examination was made at half-past 3. I shall take this opportunity of remarking, that in almost all the cases examined by me the autopsies were made very shortly after death. In several cases the autopsy was made directly after death, and in most cases only two or three hours after, so that the post-mortem decomposition could not yet have altered the condition of the intestine and its contents. In the above-mentioned case, as well as in a number of former autopsies, it was very evident that in acute cases almost a pure cultivation of comma bacilli was present in the intestine. I was able to demonstrate these facts to Drs. Strauss and Roux, who had not yet succeeded in discovering the comma bacilli either microscopically or by means of cultivation on solid materials. Dr. Strauss informed me that they always thought that there was some knack in the process of colouring and cultivating the comma bacilli. They have, however, convinced themselves that there is nothing simpler, if only a pure and uncomplicated case is chosen for examination.

Also in the second fatal case which was investigated at Toulon the comma bacilli was found in the intestine almost in a pure cultivation. At this opportunity I begged Dr. Strauss to demonstrate the microbe, which according to his statement existed in the cholera blood. But in neither case could these bodies be found.

If we reckon all these cases together they come to nearly 100, which were examined for the presence of the comma bacilli, and in all the cases were they found. But the investigation has not only proved that they were present, but, as I have already indicated, they always stand in a direct relation to the cholera process itself. For where the true cholera process causes the most marked changes in the intestine, namely, in the lower half of the small intestine, they were found most numerously, above they diminished more and more. In the most typical cases they appeared almost as a pure cultivation. But the older the cases were, and the more the secondary changes

had taken place in the intestine, so much the less numerous were the comma bacilli.

From the choleraic matter investigated by me up to the present time, I believe I can now affirm, that the comma bacilli are never absent in cholera. They are something specific to cholera.

For purposes of control a large number of other cadavera, dejections from the sick and the healthy, and other substances containing bacteria, have been examined in the same manner, to find out if these bacilli, though they are never wanting in cholera, might perhaps be present elsewhere; a point which is of the greatest importance in deciding the causal connection between the comma bacilli and cholera. Among the objects which were examined was the body of a man, who had had cholera six weeks previously, and had died of anæmia. In his intestine there was absolutely no trace of the comma bacilli to be found. Further, the dejecta of a man were examined, who had had an attack of cholera seven or eight days previously, and in whom the evacuations had already begun to be more solid. In this case also the comma bacilli were wanting. In order to convince myself that the comma bacilli only occur in cholera, I have thoroughly examined more than 30 cadavera. For this purpose I chose principally the bodies of those who had died of some intestinal affection, such as dysentery, or intestinal catarrh, so frequently fatal in the tropics, also cases of ulceration of the intestine, a case of typhoid, and several cases of typhus. In this last-mentioned disease the changes in the intestine were at first sight very similar to those, which occur in cases of cholera running a severe course with hæmorrhage in the intestine. The small intestine in its lower segment was infiltrated with hæmorrhages, but it was worthy of note, that Peyer's patches were much more changed in typhus than in cholera, where they show only the slightest changes. In all those cases, which were principally intestinal diseases, there were never any of the comma bacilli found; and experience taught that these kind of intestinal affections especially predispose to attacks of cholera. It was therefore to be expected, that the comma bacilli, if they occur anywhere, would be found especially in such cases. Besides, a large number of evacuations from dysenteric patients were examined, without finding the comma bacilli in any one of them. These investigations I have continued later in Berlin in conjunction with Dr. Stahl, my

untiring fellow-worker, from whom much was expected in bacterial investigations, but whose activity death has unfortunately brought to an early termination. We tested for the presence of the comma bacilli a large number of evacuations, chiefly from the diarrhœa of children, but also from that of adults; further, we tested the saliva, as well as the material rich in bacteria adhering to the teeth and the tongue, but always with negative results. After that different kinds of animals were examined. Because in arsenic poisoning there appears a group of symptoms very like those of cholera, animals were poisoned with arsenic and afterwards examined. A number of bacteria were found in the intestine, but no comma bacilli; nor were they found in the sewage from the drains of the town of Calcutta, in the extremely impure water of the river Hughli, in a number of tanks which were situated in the villages and between the huts of the natives, and which contained very dirty water. Wherever I could obtain a fluid containing bacteria, I have examined it for the presence of the comma bacilli, but I have never found them. Only once in the water, which at flood time overflows the lands near the salt-water lakes lying to the east of Calcutta, have I met with a kind of bacteria, which at first sight had a certain resemblance to the cholera bacilli, but on more exact examination it appeared larger and thicker, and its cultivations did not liquefy gelatine. Besides this observation I had already at my disposal a pretty considerable experience of bacteria, but I cannot call to my recollection, that I have ever seen previously any bacteria which resemble the comma bacilli. I have spoken with many who have made a large number of cultivations, and have had much experience, but they have all declared to me, that they have not yet seen such a form of bacteria. I believe, therefore, I can say with certainty, that the comma bacilli are the constant companions of the choleraic process, and that they are present nowhere else.

It will be now time to answer the question, What can we suppose to be the connection between the comma bacilli and the choleraic process? In answering this question, three different propositions can be advanced. First, it may be said that the cholera process is favourable to the growth of the comma bacilli, because it provides a soil for them, and as a consequence a surprising increase in this kind of bacteria takes place. If this

assertion is made, then one must go upon the supposition, that everybody has the comma bacilli in himself, before he has the cholera, for they have been found in the most different places, in India, in Egypt, and in France, and in men of the most different race and nationality. According to this supposition this kind of bacteria must be one of the most widely spread and the most common. But the opposite is the case, for they occur, as we have seen, neither in those who suffer from other diseases, nor in healthy people, nor apart from man in places most favourable for the development of bacteria. They always appear only when cholera has broken out. This proposition cannot be considered as admissible, and therefore we must let it drop.

Secondly, one may attempt to explain the regular coincidence of the comma bacilli and the cholera process thus:—that during the disease conditions arise, in consequence of which, among the many bacteria which are present in the intestine, one or other undergoes alteration, and assumes the form and peculiarities which we have learnt to recognize as belonging to the comma bacillus. With regard to this explanation I must confess that it is without any foundation in fact, and is a pure hypothesis. We do not yet know such a change of one kind of bacteria into another. The only example of change in the peculiarities of the bacteria affects their physiological and pathogenic action, but not their form. The anthrax bacilli, for instance, lose their pathogenic action, if they are treated in a certain way, but they remain quite unchanged in their form. In this example, then, we have to do with a loss of their pathogenic properties. But this is exactly the contrary to what would take place, if the harmless intestinal bacteria changed to the dangerous cholera bacilli, and of this kind of alteration, viz., from harmless to hurtful bacteria, there does not exist one well-authenticated example. Some years ago, when bacteric investigation was yet in its infancy, one might have suggested such an hypothesis with some degree of justification. But the more the knowledge of the bacteria has advanced, the more has it become apparent, that as regards their form the bacteria are extraordinarily constant. In reference to the comma bacilli specially I shall here notice, that if they are grown outside the human body they retain completely all their previously described characters. For

instance, they were cultivated in jelly to the 20th cultivation, and if they had not been as constant in their properties as other bacteria, they would have returned in the course of this experiment to the usual form of the ordinary intestinal bacteria. But this was in no way the case.

There now remains only the third proposition, namely, that the cholera process and the comma bacilli stand in direct dependence on each other, and with regard to this, I know no other explanation than that the comma bacilli cause the cholera process, that they precede the disease, and cause it. The reverse of this—that the cholera process produces the comma bacilli—might be advanced, as I have explained, but this, as was shown, is not possible. To me it is proved that the comma bacilli are the cause of cholera.

But it can be fairly demanded that, if this be so, further proof should be brought forward, and above all things that the cholera process should be produced experimentally by the comma bacilli. To satisfy this demand attempts have been made in every possible way. The only possibility of obtaining such direct proof of the cholera-engendering action of the comma bacilli is afforded by experiments on animals, which, if one could give credence without reserve to the statements of authors, should be possible without any difficulty. It has been stated that cholera attacks cows, dogs, cats, poultry, elephants, and many other animals, but if these statements are examined somewhat more minutely, it will be found that they are altogether untrustworthy. Up to the present time we possess no really trustworthy example at all of the lower animals being spontaneously affected by cholera during cholera epidemics. Further all the experiments, which have been made up till now with choleraic material on animals, either have turned out directly negative, or, when they were stated to be positive, were only so nominally, for the evidence was not complete, or was upset by other experimenters. In spite of this we have experimented on animals in the most thorough manner. In particular, because much importance is attached to the results on white mice obtained by Thiersch, I took with me 50 white mice from Berlin, and performed on them every kind of infection experiment. They were fed more especially with the evacuations of cholera patients, and the intestinal contents of cholera cadavera. We carried

out the method of research of Thiersch as accurately as possible, and used as food not only the material when fresh, but also after it had become decomposed. Although these experiments were performed over and over again with material from different cholera cases, our mice remained quite healthy. Then monkeys were experimented on, as well as cats, dogs, poultry, and various other animals which were available, but we have never been able to obtain anything like the cholera process. In the same way we experimented with cultivations of the comma bacilli, and used them as food in every stage of their development. We also found that when we fed animals on large quantities of the comma bacilli, then killed them, and searched the contents of their stomach and intestine for comma bacilli, that the comma bacilli were destroyed in the stomach, and did not usually reach the intestine. Other bacteria behave differently under these circumstances, for there was accidentally discovered at Calcutta a micrococcus, giving a beautiful red colour, which on account of its striking colour was easy to recognize, and therefore was particularly suitable for such an experiment. This micrococcus, at my suggestion, was used as food for a mouse by Dr. Barclay of Calcutta, and the intestinal contents of the animal were placed on potatoes. There again red colonies of micrococci were found, showing that it had passed through the stomach of the mouse uninjured. The comma bacilli, on the contrary, are destroyed in the stomach of animals. As it had to be decided whether the failure of the feeding experiment depended on this peculiarity of the comma bacilli, the experiment was altered, and the substances introduced directly into the intestine of the animals. The abdomen was opened, and the fluid was injected directly into the small intestine by a subcutaneous syringe (Pravaz's). The animals bore this interference very well, but they did not become ill from it. Then we tried in monkeys to throw the cholera dejections as high up as possible in the intestine through a long catheter. This was easily done, but the animals remained well. Also I have to mention that purgatives were first given to animals to excite a certain amount of irritation in the intestine, and then the infective substance was introduced, without a different result being obtained. The only experiment in which the comma bacilli showed a pathogenic effect, and which therefore made us hope at first that some

result might be got from it, was the injection of pure cultivations directly into the circulation of rabbits, and into the abdominal cavity of mice. The rabbits appeared to be very ill, though they recovered again after a few days. The mice, on the other hand, died after from twenty-four to forty-eight hours, and comma bacilli were found in their blood.

One must, of course, use a fairly large quantity in animals, and it is not as in other infective experiments, which require only the use of the smallest amount to obtain a result. As regards the possibility of being able to infect animals with cholera, I have inquired all over India, to obtain evidence of a similar disease being observed in animals, but I have been assured that even in Bengal nothing of the kind has ever occurred. In this province there is an extremely dense population, and many kinds of domestic animals, and it must be allowed that in that country, where cholera is universally and continually present, animals, just as often as men, get the infective material of cholera into their digestive tracts in quite as active a form; but it has never been observed that animals fall ill with attacks like cholera. I believe therefore, that not only all those animals which are at our disposal for experimental purposes, but those which commonly come in contact with mankind, have all of them an immunity from cholera; and that a true cholera process cannot be artificially produced in them. Hence we must give up this method of proof.

Nevertheless one cannot therefore say that no proof at all of the pathogenic action of the comma bacilli can be brought forward by this means. I have already explained to you that as far as I am concerned, I cannot, even excluding these experiments on animals, form any other opinion, except that there exists a causal connection between the comma bacilli and the cholera process. If later attempts to produce in animals something similar to cholera should be successful, they would not be more convincing to me than the facts which stand now at our disposal. Moreover, we know other diseases which do not spread to animals, for instance, leprosy; and yet we must assume, from what we know of the leprosy bacillus, that it is the cause of leprosy. In this disease also we cannot avail ourselves of experiments on animals, for up till now no species of animal has been found susceptible to leprosy. Apparently it is just the same with typhoid fever,

I do not know that anybody has been successful in infecting animals with it. Hence we must be satisfied if we can establish the constant presence of a certain kind of bacteria in the disease under consideration, and the absence of the same bacteria in other diseases. The bacteria in question must always go hand-in-hand with the infective material of the disease, and what I especially lay stress upon, the occurrence of the pathogenic bacteria must correspond to the pathological changes in the body, and to the course of the disease. On the other hand, we are aware of diseases of the lower animals, which cannot be transferred to man, for example, rinderpest and pleuro-pneumonia. We here meet with one of the most widespread phenomena in nature. Almost all the parasites attack only one or a few species of animals which serve as its host. I would remind you of tape-worms; many species of animals have their own especial tape-worm, which can develop only in that species and in no other.

We must, therefore, do without this part of the proof in a large number of infective diseases, including the exanthemata; and we can do this the more easily, because we are already acquainted with a whole series of other diseases which are caused by pathogenic organisms, in which, moreover, the conditions are similar, and with regard to which we know with complete certainty that the disease is produced by the organisms which are found in it, while on the other hand we have never yet seen that a disease produces a specific organism. I think that after we have learnt to recognize a number of diseases produced by such parasites a deduction can be fairly made from analogy.

Besides, we have at our disposal some observations quite as good as experiments on men. We can, in fact, regard as experiments what occurs under natural circumstances. The most important of these observations is the infection of persons having to do with cholera linen. I have had several opportunities of examining cholera linen, and have always found, (as you could convince yourself in one of the microscopical preparations,) the comma bacilli in enormous numbers, and almost as a pure cultivation in the mucus-like substance, which is on the surface of the linen soiled with dejecta.

If then an infection occurs through the cholera linen, as the comma bacilli is the only organism in question, the infection

can be caused by it only. Supposing the transfer has taken place thus: the washerwoman brings her hands, dirtied with the comma bacilli, in contact with her food, or directly with her mouth; or the water used for washing containing the comma bacilli is splashed about, and a few drops fall on the lips. In any case the conditions are exactly like an experiment, in which a man has been fed with a minute quantity of a pure cultivation of comma bacilli. It is in fact an experiment which a man performs unwittingly upon himself, and it affords quite the same proof as if it were designedly done. This observation has been made so frequently, and by such different medical men, that there can be no doubt about it. Besides, I can bring forward a similar observation made by myself. I have succeeded in finding the comma bacilli, with all their characteristic properties, in a tank supplying the drinking and service water to the entire surrounding population, and in the immediate neighbourhood of which a number of fatal cholera cases had occurred. It was afterwards ascertained, that the linen of the first of these fatal cases had been washed in the tank, and this is the only instance in which I have as yet been able to find the comma bacilli outside the human body. On the banks of this tank were situated 30 to 40 huts, in which dwelt from 200 to 300 persons. Of these, 17 died of cholera, though how many sick there were could not be exactly determined. A tank of this kind supplies drinking and service water to the neighbouring inhabitants, and receives all the refuse from the households as well. The Hindus bathe daily in such a tank, they wash their apparel in it, and the personal excrement is placed by preference on its bank, and should a hut by any chance be provided with a latrine, it has its outflow to the tank. Just such a tank was the one in question. The little epidemic had already reached its height, when the comma bacilli were found, at first in a fairly abundant amount, and at several parts of the bank; but shortly afterwards, when only single cases were occurring, only at one place, and in lesser amount. On the first discovery they were so abundant that their number could not have depended on the dejections and the washing water from the cholera linen thrown at one time or another into the tank. Some growth must have taken place. On the other hand, the small amount found at the second examination did not correspond to the numerous cases occurring just

previously. If these cases had furnished the bacilli in the tank water, then the bacilli would have been far more numerous on the second occasion than on the first. In this case, therefore, it cannot be said, that the presence of the comma bacilli in the tank was only a consequence of the cholera epidemic. On the contrary, the circumstances were such that the epidemic followed the bacilli. To these kinds of observations, and especially to infection through cholera linen, we must attach the greatest importance, for perhaps we may never be able to carry out direct infection experiments with any result.

For my view, that the comma bacillus is the cause of cholera, I find further important evidence in the fact, that the whole etiology of cholera, as far as it is known to us, is completely in accord with the characteristics of the comma bacilli.

We have seen that the comma bacilli grow extremely rapidly, that their vegetation quickly reaches a climax, then ceases, and that finally they are supplanted by other bacilli. This corresponds exactly to what goes on in the intestine in cholera.

It may be assumed that, as is the case with other bacteria, very few of the comma bacilli, (under certain circumstances even one,) might suffice to cause an infection. Correspondingly, we can well picture to ourselves single comma bacilli being admitted opportunely to the intestinal canal, and increasing there very rapidly. As soon as they have increased to a certain degree, an irritation of the intestinal mucous membrane and diarrhœa occurs; then as the increase goes on and reaches its height, the peculiar group of symptoms, which we designate as the true cholera attack, appears.

We have formerly seen that it is highly probable, that the comma bacilli cannot pass the stomach under ordinary circumstances, at least in animals. This also agrees with all our experience of cholera. For it appears that predisposition plays an extremely important part in cholera infection. It may be presumed that of a number of individuals, who are exposed to cholera infection, only a fraction fall ill, and these are almost always those who had previously suffered from some disturbance or other of the digestion, for instance catarrh of the stomach or intestine, or who had overloaded their stomach with indigestible food. Especially in the last case masses of food, more or less

undigested and incompletely acted upon by the stomach, could pass into the intestine, and possibly could carry with them the comma bacilli not yet completely destroyed. Certainly the frequent observation is well known, that most of the cholera cases happen on Monday or Tuesday, these being the days which succeed excesses in eating and drinking.

Now it is certainly a peculiar phenomenon that the comma bacilli should be confined to the intestine. They do not spread to the blood, nor even to the mesenteric glands. How does it happen, then, that this vegetation of bacteria in the intestine can cause death? To explain this it must be recollected that the bacteria in their growth do not only use up matter, but also produce substances of a very different kind. We already know a number of these products of bacteric fermentation, which are of a very peculiar nature. Many are volatile, and give off an intense odour, others form colouring matter, others again form poisonous substances. The decomposition of albuminous fluids, such as blood, produces poisons which, since the decomposition is only a consequence of the growth of bacteria, must be the products of change of these bacteria. Many appearances are in favour of these poisons being produced only by a given kind of bacteria, for we see that putrid fluids, which at one time can be injected into an animal without having any effect, at another time will prove to be highly poisonous. Thus I should explain the action of the comma bacilli in the intestine as being due to the poisonous products of decomposition. In support of this view I possess certain facts. In one of the cultivation experiments the gelatine had a fairly large number of blood corpuscles in it, as well as comma bacilli. After the gelatine had been poured out on a glass plate a number of colonies of comma bacilli grew. The plate had the appearance as if a red-coloured dust were suspended in it, as the effect of the individual blood corpuscles could be clearly made out by transmitted light. In this red granular layer the colonies of the comma bacilli appeared even to the naked eye as small colourless bodies. When it was examined microscopically, it showed the surprising phenomenon that the colonies of the comma bacilli had destroyed all the blood corpuscles in a fairly wide circle around them, which even extended far beyond the limit within which the gelatine had been liquefied. Thus it appears that the comma bacilli can exercise

a destructive influence on the formed elements of the blood, and most probably on other cells as well.

Moreover, another observation has been made by an Indian physician, Dr. Richards, of Goolundo, which likewise is in favour of the existence of a poisonous material in the contents of the cholera intestine. Dr. Richards first fed dogs on a large quantity of cholera dejecta without producing any effect on the animals. He then performed the same experiment on pigs, which, according to his account, died of convulsions in a short time—fifteen minutes to two and a half hours after the meal. Here, then, was evidently an intoxication, and not, as Dr. Richards supposed, an artificial inoculation of cholera. The fact that this was the case, was markedly shown by an experiment, in which the contents of the intestine of a pig, killed through feeding it on cholera dejecta, and having had the cholera according to Dr. Richards, was given as food to a second pig. The second animal remained healthy, and therefore there could not have taken place a reproduction of the supposed infective material in the intestine of the first pig. If a true cholera had really been produced in pigs, then a second pig could have been infected with the intestinal contents of the first, and from this a third, and so on. If the experiment did not exactly prove what Dr. Richards intended, it is at least so far interesting as showing, that under certain circumstances substances can exist in the cholera dejecta which are poisonous to pigs. Dogs appeared not to be affected by it, nor are mice and many other animals, as our experiments show. The power of resistance of other animals to this poison, and the susceptibility of the pig to it, should not cause surprise, if it is remembered that a poison, which is sometimes found in the salt meat and herring brine, also only appears to be fatal to pigs.

If it be accepted that the comma bacilli form a specific poison, the phenomena and course of cholera may be explained thus. The action of the poison shows itself, partly directly in the destruction of the epithelium, and in the more severe cases, of the superficial layers of the mucous membrane, partly indirectly when it is absorbed, and acts on the entire organism, but especially on the circulating system, which is brought to a state of paralysis. The group of symptoms in a regular attack of cholera, usually thought to be the consequence of the loss of

water and thickening of the blood, should be in reality considered, according to my idea, poisoning. For it not unfrequently happens, that even though a comparatively small quantity of fluid has been lost during life, through vomiting and diarrhœa, the intestine also will be found to contain only a little fluid after death.

If death occurs in the stage of cholera poisoning, the post-mortem appearances correspond to those cases, in which the intestinal mucous membrane is very little changed, and the intestinal contents consist of a pure cultivation of the comma bacilli.

On the other hand, if this stage is prolonged, or if it is survived, then the consequences of the necrosis of the epithelium and mucous membrane make themselves evident. Capillary hæmorrhage in the mucous membrane occurs, and the constituents of the blood mix more or less abundantly with the intestinal contents. Then the albuminous fluid in the intestine begins to decompose, and under the influence of the bacteria of decomposition other poisonous products are formed, which are likewise absorbed. These, however, act differently to the cholera poison, and give rise to symptoms corresponding to those commonly designated as cholera typhoid.

In accordance with the theory, that the comma bacilli can grow and develop their action in the intestine only, the seat of the infective material must be sought for in the dejecta of the patient, and exceptionally in the vomit. I believe on this point I am in entire agreement with the more recent views. Certainly this theory is still contradicted by some investigators, but we possess such incontestable examples of it, especially infection by cholera linen, that, without regarding the cholera bacilli, there can be no doubt at all that the cholera dejecta really contain the infective material of cholera.

For the further spread of the infective material, the main condition is, that the dejecta should remain in a moist state, for as soon as they dry up they lose their activity.

One of the most frequent paths by which the infective material spreads, is water, of which we have had an example in the tank epidemic. How easily can cholera dejections, or the water used for washing cholera linen, contaminate wells, public water-courses, or other places from which drinking or service

water is taken! From them the comma bacilli find many opportunities to return to their human habitation, either in the drinking water, or in the water used for diluting milk, for cooking food, for scouring dishes, for cleaning vegetables or fruits, for washing, bathing, and so forth.

Besides, the infective material can reach the digestive organs by another way. For the comma bacilli can undoubtedly remain capable of life for a longer space of time in provisions, which have a moist surface; and it can be easily imagined, that they are not unfrequently propagated through the contact of dirty hands and such like. I also think it not at all improbable, that the infective material is spread through food by means of insects, such as the common fly. In most cases certainly the infective material is deposited in the ground with the dejections, and finds a path some way or other into a reservoir or cistern.

I also go on the supposition that only moist substances, (and they may be very different, I do not at all confine myself to drinking water,) which in any way become contaminated by dejections, can carry the infective material to the body. On the other hand I do not believe that the infective material of cholera can retain its vitality in a dry state, or, what is the same thing, that it can be transported by the air. For the spread of an infective material by the air can, as a general rule, take place only when it is in a dry dust-like condition. Also it is in accordance with experience, that the infective material cannot be spread in a dry state, for we know that up till now cholera has never reached us from India by merchandise; nor have letters and parcels sent by post ever brought cholera, even when they were not perforated and fumigated, as has happened frequently lately. Cholera has always, if we investigate more closely the origin of isolated epidemics, come to us in no other way than by mankind himself, and if in isolated epidemics we do not succeed in finding the individual who brought the cholera, we must not suppose that an exception has taken place. For we must remember that not only the individual, who died of cholera, or who has had an undoubted cholera attack, is capable of spreading infection, but that there is every possible gradation between the most severe form of the disease, and quite an unimportant diarrhœa, which apparently is just as able to infect as the most severe case of cholera. However, absolute certainty will be obtained as to this

important point only, when the least severe cases come to be diagnosed as cholera cases by means of the comma bacilli.

There still remains a very important question to answer, namely, whether the infective material can reproduce itself, and multiply outside the human body. I think that this is the case. As comma bacilli grow on a layer of gelatine, as they can grow on a piece of linen, in a meat infusion, or on a potato, so they ought to be able to grow outside the body, especially as we have seen that a comparatively low temperature makes their development possible. I certainly should not like to say, that the increase of the comma bacilli outside the human body goes on directly in spring or river water, for these fluids do not possess that concentration of nourishment which is requisite for the growth of the bacilli. But I can easily imagine that, though the water in a reservoir as a whole is too poor in nourishment for the growth of the bacilli, certain places might have a sufficient concentration of nourishing material, for instance where a gutter, or the waste-pipe of a water-closet discharges into standing water, or when vegetable matter or animal evacuations, or the like, are deposited and are exposed to decomposition by bacteria. At such points an active life can be developed. Formerly I made many such investigations, and it has often happened to me that water contained almost no bacteria at all, while vegetable refuse, especially roots or fruit, which floated therein, swarmed with bacteria, particularly of the bacillus and spirillar varieties. Even in the immediate neighbourhood of such objects, the water was rendered muddy by the crowds of bacteria, which evidently obtained the necessary nutriment from the material which as the result of diffusion surrounded it for a short distance.

I think that we can then very easily demonstrate the connection between the ground water and the spread of cholera. Everywhere, where water stagnates on the surface, or in the ground, in marshes, in docks which have no outlet, in places where the ground is formed like a trough, in sluggish rivers, and the like, the conditions, which have been described, can be found. There a concentrated nutrient solution can form in the neighbourhood of animal and vegetable decaying matters most easily, and give the micro-organisms opportunity for growth. On the other hand, whenever the water has a swift current, or is in a constant state of change both on the surface and in the ground, these

conditions occur less easily, or sometimes not at all. For the continuous current prevents a localised concentration of nourishment in the fluid, sufficient for the pathogenic bacteria. The connection between the sinking of the ground water, and the increase of many infective diseases, we might explain thus, that with the sinking of the ground water the current which exists in it becomes very much lessened. Besides, the mass of water lying superficially at disposal will be considerably diminished, and therefore such a concentration as I have described as necessary for the growth of the bacteria, will be produced much sooner.

If we grant that cholera depends on a well-defined specific organism, then we could not admit a spontaneous (*autochtone*) origin of cholera in any place. Such a specific organism, even if it is only a comma bacillus, follows just as much the laws of growth as a highly developed plant. It must always develope from its like, and cannot be produced from other things or out of nothing. But since the comma bacilli do not belong to the universally distributed micro-organisms, we are compelled to trace the disease dependent on them to well defined localities, from which these micro-organisms have been brought to us. We could not, therefore, imagine that the cholera can arise of itself, even though exceptionally, in the delta of the river Nile, because this is in some respects similar to the delta of the Ganges, as was last year asserted in all earnestness. Just as little can we think, that cholera could arise here among us in Europe without previous importation of the comma bacilli. An attempt was once made to prove, that a cholera epidemic occurring in Europe, which apparently had broken out in an isolated manner in Poland, had arisen spontaneously, but later it became evident, that this mode of origin could not be admitted. For the cholera in this case had existed in various parts of Russia in small unobserved epidemics, and had been carried to Poland by troops. A short time back a somewhat similar example came to my notice. About ten years ago the cholera broke out suddenly in the town of Hama in Syria, and nobody knew how it had come there. It was repeatedly declared that it had arisen spontaneously. I was lately asked about it by a French physician in France, and since there was nothing definite to be found on record as to the origin of this epidemic, I could only answer, that the

mode of introduction in this case had not yet been made clear; but I gave as my conviction, that the origin of the cholera in Syria must be traced back to India, while I pointed out at the same time, that the epidemics in Syria and Egypt, apparently arising spontaneously, had occurred in the trade route between India and Europe, or in its immediate neighbourhood, and that it had never risen in places which had no connection with India. Soon after this I was accidentally put into the position to get a satisfactory explanation of the origin of this epidemic. In Lyons Professor Lortet, who had been himself in Hama during this epidemic, and had instituted inquiries as to the origin of the cholera, informed me that the cholera had been brought to Hama from Djedah by Turkish soldiers.

Undoubtedly, as far as we have gone we are unacquainted with a cholera epidemic, which has arisen spontaneously outside India, and on this point experience agrees with the proposition, that cholera is dependent on a specific organism whose habitat is in India.

Now the relative conditions of cholera in India are of quite a peculiar nature. I do not believe that the whole of India is the fatherland of the comma bacillus. Formerly, indeed, it was asserted that cholera was at home in Ceylon, in Madras, in Bombay, and had subsequently spread over almost the whole of India, but this has been rightly denied. Only in regard to the province of Bengal there exists no difference of opinion. All authors are agreed, that the delta of the Ganges is the true home of cholera, and I have come to the conviction that this is the case, and that there are no other places of origin of cholera in India. For the only district in India, where cholera prevails continually year after year in a uniform manner, is the delta of the Ganges. In all other places it shows marked variations, or it may even disappear altogether for a shorter or a longer time. In certain places, for example in Bombay, it never entirely disappears, but it is highly probable, that on account of the unusually active trade with the rest of India it is constantly being imported there afresh.

On this map of the province of Bengal you see the delta of the Ganges, which is bordered on the west by the Hughli river, an arm of the Ganges, and on the east by the Brahmaputra.

Over the whole of this district, and on the banks of the Ganges as far up as Benares, cholera continually prevails. On closer examination of the map it must strike one, that the upper part of the delta is thickly covered with townships, while the base of the triangle appears quite uninhabited. This uninhabited stretch of land, called the Sunderbuns, embraces an area of 7,500 English square miles, and is separated from the densely inhabited northern part by quite a sharp line. Here the great rivers the Ganges and the Brahmaputra break up into a network of water-courses, in which the sea water, mixing itself with the river water, flows hither and thither with the tide, and at flood time places large tracts of the Sunderbuns under water.

A luxuriant vegetation and an abundant animal life have developed in this uninhabited region, which is inaccessible to man not only on account of the floods and the numerous tigers, but is avoided principally on account of the pernicious fever, which attacks everybody who remains there even for quite a short time. One can easily imagine how dense the vegetable and animal matter is which is given up to decomposition in the marshy districts of the Sunderbuns, and that here an opportunity is afforded for the development of micro-organisms, such as exists in scarcely any other place on the globe. Peculiarly favourable for this are the regions between the inhabited and the uninhabited parts of the delta, where the excrements of an unusually thickly populated country are washed away by the current, and flowing here and there, are mixed with the brackish water of the Sunderbuns, already teeming with decaying matter. Under these peculiar conditions quite a distinct fauna and flora of micro-organisms must be developed there, to which in all probability the cholera bacillus belongs. For everything points to the cholera having its origin in this district. All the greater epidemics have begun with an increase of cholera in the southern portion of Bengal. Jessore, from which the first intimation of the epidemic of 1817 came, lies on the borders of the Sunderbuns; and Calcutta, which is now the fixed home of the cholera, is connected with the neighbouring Sunderbuns by a marshy and sparsely inhabited tract of land.

Now the comma bacillus finds in this district, contiguous to its presumptive home, the most favourable conditions imaginable to implant itself and to be spread from one individual to another;

Lower Bengal is a perfectly flat country, which rises only slightly above the sea level, and during the rainy season is under water to almost its entire extent. Every one, who builds there, protects himself from these yearly floods by placing his house on elevated ground. This form of building is seen in all the villages of the delta, even in Calcutta itself, though chiefly in its immediate neighbourhood and in the suburbs, which bear more or less a village-like appearance. Each house or group of houses stands on a flat elevation, which is formed by earth being taken from some spot lying near the site, and piled on the building ground. The excavation thus formed becomes filled with water and forms the so-called tank.

Each house or group of houses must have therefore a more or less large tank, and their number is correspondingly very great. In the town of Calcutta there were a short time ago about 800 tanks, although many had already been shut up for sanitary reasons. In the suburbs of Calcutta there still exist more than 1,000. What part the tanks play in the household economy of the native population, and how favourable they are for spreading cholera, I have already previously indicated.

It must be evident that an improvement in the state of the water in those districts will exercise a decided influence on the cholera situation. In truth all testimony shows this in Calcutta. This town, situated on the Hughli river, has about 400,000 inhabitants, and its suburbs have at least as many more. Until the year 1870, Calcutta, that is, the main town, had yearly from 3,500 to 5,000 deaths from cholera, and the suburbs had a corresponding proportion. Already in 1865 they had begun to drain the town, first in that part which is inhabited by Europeans, and which is most extensively built upon; later also the east of the town was gradually provided with a system of drains, but until the year 1874 there were not many houses in the native part of the town really connected. Only in some quarters was the network of channels completely built, as may be seen in the plan before you. Since then the completion of the drainage has been continually worked at, and is now pretty far advanced.

I must mention at this point one peculiarity of Calcutta. In the central parts of the town, among massive houses and palace-like villas, there are groups of huts like villages, thickly crowded

together, which are exclusively inhabited by the natives. These villages lying in the town are called Bustees. Closets, latrines and the like, the huts of a Bustee do not possess. All the dirt collected between the dwellings can, on account of the closeness of the buildings, be only incompletely removed, and therefore is carried directly or washed by the rain to the tanks, the natural cesspools for all the fluid filth. Connection between such huts and this system of drainage is out of the question.

At the same time with the drainage, the construction of water-works for Calcutta was taken in hand. Water was taken from the Hughli several miles above Calcutta, was well filtered, and then conducted to the town. The water-works were opened in the year 1870.

From 1865 to 1870 the effect of the gradually extending drainage on the cholera mortality was not noticed. But immediately after the opening of the water-works the cholera diminished, and since that time has remained on an average at about a third of the previous amount. The drainage also has considerably advanced towards completion since 1870, but, as it has not yet further increased the remission of cholera which had suddenly begun with the entrance of good drinking water, the favourable effect in this case can only be ascribed to the water-works. If in spite of this, cholera still continues relatively frequent in Calcutta, it is due to the fact, that a great part of the population take what water they need, not from the water conduits, but in the old-fashioned way from the Hughli, or from the numerous tanks.

In the suburbs, which stand in direct connection, and the closest intercourse with the town, but which do not participate in the water supply, the cholera mortality remains as before.

Still clearer does the influence of the water arrangements show itself in Fort William, which stands in the middle of the town on the Hughli. The fort itself is not drained, and because of the distance from the nearest town drains, cannot be included in the system of drainage. The relations of the ground water must be exactly the same as they were at the time of the construction of the fort. Formerly the garrison of the fort was every year severely visited by cholera. But since the beginning of 1860 the attention of the officials was drawn to the drinking water, which was guarded as far as possible from impurities, and

since then the cholera has markedly declined. The fort received an absolutely reliable water at the same time as the town, and from that time forth cholera has disappeared from the fort. This case may be regarded as a regular experiment, in which all the conditions have remained unaltered except that of the drinking water. If the cholera does not visit the fort any more, it can only be ascribed to the change in the drinking water.

There are similar if not so striking examples of the influence of drinking water on cholera in other Indian towns. It is so in Madras, where cholera has declined to a marked extent since a water supply was introduced; similarly in Bombay. But particularly interesting in reference to this, is what has occurred in Pondicherry. Formerly cholera occurred very frequently in this town. For a number of years artesian wells of a depth of 300 to 400 metres have been in use, and from that time cholera has disappeared from Pondicherry. Last spring nevertheless it was unexpectedly announced that the immunity of Pondicherry, already assumed to be certain, had proved by no means to be relied upon. In consequence I communicated with Dr. Furnell, of Madras, who had occupied himself mainly with the behaviour of cholera in Pondicherry, and obtained from him the intelligence, that it was a fact that a number of cases of cholera had occurred there, but exclusively in those portions of the town, which had not yet been provided with artesian wells.

Though I have cited to you some examples of the advantages of a good supply of drinking water, the assurance, that I am not a supporter of the exclusive drinking-water theory, is scarcely necessary after my former deductions. I want specially to avoid any prominent point of view, for I consider that the ways in which cholera can spread itself are extremely different, and that almost every place has its own peculiarities, which have to be thoroughly investigated; and the regulations, which are to serve for the prevention of infection in the place in question, must be drawn up accordingly.

Moreover, in India the spread of cholera depends on human intercourse, and this is principally due to the very unusual extent to which pilgrimages are developed there. We can scarcely imagine to what a degree pilgrimages are carried in India. To give you an example, I shall only mention the two principal places of resort for pilgrims, Hurdwar and Puri. These

are places in which every year hundreds of thousands, often over a million of men, congregate together from all parts of India. The pilgrims remain there for several weeks, they are packed together in the narrowest space, and live in the most miserable condition. In these places, also, tanks are everywhere found, in which thousands of men bathe, and out of which they drink. These are circumstances which do not make it appear at all wonderful that this disease, when it develops among the crowds of pilgrims, quickly spreads out over the whole of India.

Cholera originally travelled beyond the boundaries of India, through North India to the interior of Asia, from there to Persia, and thence to the south of Europe. But this has become altered since commerce does not go any more by the caravan route through Persia, but by sea through the Red Sea and the Suez Canal. I scarcely think that an invasion of cholera by the land route through Asia is now to be feared. It is not absolutely impossible that it may take this route, but it is not probable. But, on the other hand, the other way, the sea route from India through the Red Sea, principally from the chief harbour of export Bombay, will become in my opinion from year to year more dangerous. From Bombay, which is seldom free from cholera, Egypt can be reached in 11 days, Italy in 16 days, and the south of France in 18, or at the most 20 days. These spaces of time, in comparison to what was the case formerly, have become so extraordinarily short, that the dangers of the direct importation of cholera from India to Europe will become continually greater. As in reference to this, the manner in which cholera behaves on board ship is of special interest, I should like to have permission to make a remark about it.

It has always surprised me, that true cholera epidemics only occur in ships, which have on board a large number of men, while on ships manned to a less extent, and on all merchant ships, cholera epidemics lasting for more than a week never arise, even if cholera cases occur on the first day of the voyage. Because this point is of the greatest importance not only for the etiology of cholera, but also for maritime trade, I have made inquiries about it as far as possible, and have found that this observation is completely confirmed.

If the question which arises from ship cholera is discussed, we

must cast our eye on ships which serve as transports for masses of men, such as troop ships, pilgrim, coolie, or emigrant ships. On those which start from cholera-infected ports cholera does not occur so seldom as is frequently supposed. The attempt has sometimes been made to show that marine traffic is, as regards the spread of cholera, quite without danger, for it is calculated that to such and such a number of ships free from cholera, there is only one on which cholera breaks out. To this calculation must be opposed the fact, that even if out of a thousand ships only one has cholera on board, it stands to reason that this one cholera ship can do just as much mischief as if all the thousand had been infected. If the calculation of the proportion between ships free from and those infected by cholera be limited to the crowded transports, the result, as I have said, is much less favourable than people imagine.

In the Reports of the Sanitary Commissioner with the Government of India for the year 1881, there is found a highly interesting table of cholera on the coolie ships which sailed from Calcutta. These ships are not very large, though they carry from 300 to 600 Indian labourers, so-called coolies, mostly to the British colonies in America. Of such ships 222 made the voyage during 10 years; of these 33 had cholera, and in 16 of them the epidemic lasted more than 20 days. One can thus easily imagine how great the danger of a cholera invasion must be to the more nearly situated parts of Europe, if a similar coolie transport should go to Egypt, for instance, or to any of the Mediterranean ports.

There is one question in the etiology of cholera, which is rather of a theoretical interest, and on which I have not yet had the opportunity of expressing my opinion, and which therefore I shall only shortly touch upon. This question is the explanation of the noteworthy fact, that cholera outside India disappears after a relatively short space of time.

The extinction of the infection appears to me to depend on different factors.

First, I consider it settled that, as in many other infective diseases, an individual obtains a certain immunity by a previous attack of cholera. This immunity does not appear to last very long, for there are instances enough of people, who had been attacked during one epidemic, falling ill of cholera a second

time during another; but one has seldom heard of anybody being attacked twice in the same cholera epidemic. Repeated attacks of cholera, however, ought often to occur, for the individual who has recovered from an attack of cholera usually returns again after some days to the same surroundings, and will be continually exposed to the same hurtful influences, and to the same source of infection. Further, some observations, which have been made in India, indicate that a certain immunity is obtained after an attack of cholera. Now, as a single individual can obtain an immunity, so can a whole district become more or less safe for a certain space of time, as many instances teach us. It is often seen that if the cholera attacks a place, and infects it through and through, and afterwards appears again in the next year, this place is almost entirely spared, or is only very slightly attacked.

As a second reason for the extinction of a cholera epidemic, the absence of a resting-stage of the cholera bacilli is of importance, for with its help the infective material could outlast the duration of the immunity of the inhabitants, which would be an unfavourable time for its further progress.

Finally, there is still to be considered the circumstance, that temperatures which are under 17° act so prejudicially on the growth of the bacilli outside the body, that an increase no longer takes place. If all these factors work together, if, therefore, winter comes and there is only left a population more or less immune to cholera, then, since there exists no resting-stage in the infective material, the epidemic must become extinct.

Before I finish I should like to say a few words on the practical use we can put the discovery of the comma bacilli to. It is commonly said, What is the use of such a discovery? We certainly know that cholera arises from bacilli, but in spite of this we cannot cure the disease any better than formerly. I remember that similar opinions were frequently expressed with regard to the discovery of the tubercle-bacillus. He, who looks at these things exclusively from the point of view of the prescription-writing physician, is certainly right, for he has still no palpable application before his eyes, but yet these critics ought to remember that a rational treatment for the majority of diseases, and especially for infective diseases, cannot be carried out, until their cause and nature has been recognized. I have

hopes that even without this, the discovery of the cholera bacilli will be of very great use. First, I think it may be of use from a diagnostic point of view. It is extremely important that the first case, which occurs in any country or place, should be correctly diagnosed. In my opinion one can now determine with certainty whether cholera is present or not by the demonstration of the cholera bacilli. This appears to me to be a very important advantage.

Further, I believe that after we have recognised the true cause of the disease and its peculiarities, the etiology of cholera can be constructed on definite and established lines, and something final can be formulated out of its many contradictions. Now we shall obtain an established basis for an harmonious course of action, the scope of which will be definitely known. I hope to get especial advantage from the observation that the comma bacilli are destroyed by drying. Certainly the fact that the choleraic infective material is destroyed by drying should have been made use of in practice earlier than now, but it wanted experimental support, and there was never any certainty about it. Now we can put down the peculiarities of the infective material as quite decided facts, and can reckon on them in the future. But the greatest advantage we obtain from it is, that a limit is once and for all put to the frightful waste of disinfectants, and that millions of money will not be again poured into the gutters and water-closets, as in the last epidemic, without being of the smallest benefit.

Moreover, I lean to the hope, that therapeutically recognition of the comma bacilli will be of use. In the future a diagnosis can be made in the milder cases, and at the commencement of the disease. Therapeutic attempts will become correspondingly more certain, if it is known that the patient really suffers from cholera. An early diagnosis must be indeed of the greatest value, for the prospects of a therapeutical result are brightest at the commencement of the disease.

II.—THE SECOND CONFERENCE ON CHOLERA, HELD IN BERLIN ON MAY 4, 5, 6, 7, AND 8, 1885.*

[The whole discussion is well worthy of study by those who are interested in this subject. We can only give here the part of Koch's first address which has reference to the further experiments on animals which complete the proof that the cholera bacilli are causally connected with cholera. After criticising the work of those who have opposed this view, Koch proceeds as follows] :—

Only in the direction of experiments on animals has an advance been made; since Nicati and Rietsch† have succeeded in infecting dogs and guinea-pigs by injection of choleraic intestinal contents and cultivations of the comma bacilli into the duodenum. These experiments have been repeated and confirmed here in the Sanitary Institute as well as by Babes,‡ Flügge,§ and Watson Cheyne.‖ Rietsch and Nicati at first believed that the infection could only take place if the bile were excluded, and therefore they ligatured the ductus choledochus; but they found later that the experiment would succeed without such ligature. Our experiments also were carried out partly with and partly without ligature of the ductus choledochus, and gave the following results. Of 10 guinea-pigs operated on with ligature of the gall duct and injection of cultivations of the comma bacilli into the duodenum, 6 died of cholera in the first two days, the rest died later, in consequence of the ligature. You see here preserved in alcohol the abdominal organs of such a guinea-pig which survived the infection, but rapidly wasted, and died on the ninth day after the operation. The gall bladder is enormously distended, as well as the ductus choledochus above the point of ligature. No trace of peritonitis is to be observed in this case. In another guinea-pig

* *Berliner Klinischer Wochenschrift*, No. 37A, 1885.

† *Comptes Rendus*, vol. 99, &c.

‡ Virchow's *Archiv*, 1885.

§ *Deutsche Med. Wochenschrift*, January, 1885.

‖ Report on the Cholera Bacillus, *Brit. Med. Journal*, April 25th, May 2nd, 9th, 16th, and 23rd, 1885.

which died twelve days after the operation, the gall bladder was ruptured, and the peritoneal cavity filled with bile. The two remaining guinea-pigs perished from twisting and obstruction of the intestine, in consequence of peritonitic adhesions in the immediate neighbourhood of the point of ligature. In these experiments it soon struck me, that the better the operation was performed, and the less severe the manipulation, so much the less was the prospect of the death of the animal from cholera.

Of 18 animals, which had only an injection into the duodenum, without ligature of the bile duct, 13 died of cholera. At the same time also control experiments were made, by the injection of other kinds of bacteria, such as the micrococcus prodigiosus, different kinds of bacilli, &c., into the duodenum of guinea-pigs. Of these animals, in which the ligature of the gall duct was omitted, not one died. This showed that the operation is not of itself dangerous to the animal. Klein declared that the guinea-pigs died in these experiments, not of cholera, but of septicæmia. But according to my experience, the danger of septicæmia in this extremely simple operation may be excluded with certainty. One must set to work very awkwardly to lose any of the animals from that disease.

In the experiments done without ligature of the gall duct, the results were also the less positive the less severe the operation, and the less the intestine was bruised and stretched by the search for, and dragging out of the duodenum. On this account, also, the experiment only succeeded occasionally when one contented oneself with opening the peritoneal cavity only to a slight extent, and with injecting the material, not into the deep lying duodenum, but into the loops of the small intestine which first came into view. Of 6 guinea-pigs which were operated upon in this manner only one died of cholera; the rest lived. Then the same experiment was made on 4 rabbits without one of them dying, or even becoming ill.

Now though, by means of the injection of the comma bacilli into the duodenum in animals, a process analogous to cholera in man may be set up in the digestive tract, one must nevertheless allow that this mode of infection corresponds very little to the natural process. It is by no means a small matter to open the peritoneal cavity, and search for, and draw out the duodenum, in order to introduce the infective material into this part of the

intestine. I have on this account endeavoured to infect the animals in a natural manner, and have been finally successful. It appears to me not unimportant to describe the way by which I was at last led to a positive result. The earlier experiments had already shown that the comma bacilli are killed in the stomach, for if the animals are fed on choleraic fluids and cultivations and killed after some time, no comma bacilli can be found in the stomach and intestine, they have been destroyed in the stomach. But the non-appearance of the infection could not be due to this alone, for in the injection into the duodenum the gastric digestion is avoided, and nevertheless the infection did not always succeed. Now in order to get an insight into the conditions which come into play, I have made a number of preliminary experiments, and as guinea-pigs appear to be particularly susceptible to the cholera infection, I have confined myself to these animals. First of all I endeavoured to find out somewhat more minutely the natural digestive arrangements in guinea-pigs. If a freshly killed guinea-pig is examined, the stomach is always found completely crammed with a firm mass of food; so that if anything, for example a fluid, is introduced into the stomach, it cannot pass through it directly. I had at first thought that if a large quantity of fluid containing bacilli was injected into the stomach at one time it might be possible to force some through it. But I convinced myself very soon that this was quite impossible, and that the stomach would burst before the solid firm mass which distended it could be forced aside. The small intestine in guinea-pigs in contrast to the stomach is almost empty. The gastric contents are strongly acid, the mucus-like contents of the small intestine alkaline, while the contents of the cæcum, which is very large in these animals, have again a markedly acid reaction; so that it is only in the small intestine that an opportunity is afforded for the comma bacilli to grow and multiply. In order to find out something about the length of time which the ingesta take to pass the stomach and intestine, guinea-pigs were fed alternately with different kinds of food, as, for example, with carrots and hay. In animals which were killed in one to two hours after the change of food, one saw that the foods had not become mixed in the stomach, as occurs in digestion in man, but had been slowly pushed through it in layers, in the order in which they had been taken, so that on a sharply defined dark green layer of hay

there followed a red layer of carrots, or *vice versa*. The foods then pass surprisingly quickly through the small intestine to the cæcum. This was demonstrated very clearly by feeding with coloured materials. If for instance a mixture of chinese ink was injected into the stomach of the animals, one could follow and notice still more accurately the stratified progress of the food; and the colouring material, as soon as it had passed the pylorus, was found in a very short time in the cæcum. The same experiment was made with small blue glass beads, when it appeared that the beads traversed the stomach comparatively quickly, but only with the layer of food with which they had entered; and they then very rapidly, probably in a few minutes, passed down the small intestine to lodge for a longer time in the cæcum. In one experiment, for example, 250 beads were administered, and the animal killed after three hours; only about half of the beads were present in the stomach, the small intestine did not contain a single one, while there was a large number in the cæcum. An experiment of feeding with the spores of the anthrax is also worthy of mention. One might assume that beads are bodies by the behaviour of which in the stomach and intestine that of bacteria could not be judged. Four guinea-pigs were therefore fed with a large quantity of anthrax spores; one of these animals was killed after two and a half hours, and the contents of the stomach, small intestine, and cæcum were examined for the development of the easily recognizable colonies of the anthrax bacilli by means of cultivations in nutrient jelly on plates. In this case many anthrax spores were still found in the stomach, likewise in the small intestine, and some spores had already reached the cæcum; the mass of food also had passed through the stomach and small intestine in the short space of two hours. A second animal was killed after three hours. The number of anthrax spores in the stomach was already markedly smaller, in the small intestine they were still abundant, in the cæcum likewise abundant. The third guinea-pig was killed after three and a half hours, and had in the stomach only a few anthrax spores, in the small intestine also very much fewer than the foregoing animal, but in the cæcum there was a considerable number. In a guinea-pig killed after five hours, only one or two anthrax spores were found in the stomach, a very few in the small intestine, on the other hand they were still very numerous in the cæcum.

It became apparent, in these experiments, that the pathogenic bacteria, which were used as food for the guinea-pig, passed through the stomach and small intestine surprisingly quickly, nevertheless they always remained sufficiently long in the stomach to be destroyed by the gastric juice, unless they existed in a resistant resting stage, like the anthrax spores.

The next point was to enable sporeless bacteria, like the comma bacilli, to pass through the stomach uninjured. To render this practicable the fluid containing the bacilli was made up in the form of pills and covered over with keratin, and as this experiment did not lead to a positive result other enveloping substances, which were insoluble in the stomach, were made use of, such as collodion, caoutchouc, paraffin, and so forth; but all in vain.

Thereupon I tried to neutralise the acid reaction of the stomach, if only for a short time. At first those doses of the alkaline fluid were fixed upon which could be borne without any detriment to the animal. A 5 per cent. solution of carbonate of soda proved most suitable for our purpose, and 5 ccm. of this solution could be imbibed by the animals without causing any disturbance. If a specimen of the contents of the stomach was taken by a fine catheter, it was found in a series of experiments that the reaction was still alkaline after three hours. When we had made out this, we proceeded to feed animals, whose gastric contents had been thus made alkaline, with cholera cultivations, or to inject the same through a catheter directly into the stomach. In the first experiment 7 guinea-pigs were used. These received 5 ccm. of the soda solution, and some time after, so as not to bring the cholera bacilli into direct contact with the soda solution, 10 ccm. of meat infusion in which cholera bacteria were growing. The animals remained quite lively after this. As later also no effect was apparent, they were killed after twenty hours, and the gastric contents, the intestinal contents, and the contents of the cæcum were examined with gelatine plates. In 6 of the 7 animals the cholera bacilli were demonstrated in the small intestine. The experiment was thus so far successful, that the comma bacilli passed through the stomach uninjured, but without having produced disease in the animals. This experiment was performed over again, but in this way: 2 guinea-pigs receiving a 2 per

and 6 guinea-pigs a 5 per cent. solution of soda, and then the injection of comma bacilli. These animals also remained quite healthy, and from this result it was at least evident that it is quite a harmless procedure to introduce into the stomach of guinea-pigs a syringeful of a 5 per cent. solution of soda. The animals were not even ill from it. Finally, a third series of experiments was made on 4 guinea-pigs, which first received a 5 per cent. soda solution, and then the cholera bacilli. These animals likewise remained healthy. On the next day, however, one of them seemed to be ill, was out of sorts, and did not eat. On the day after it was seriously ill, and showed the very peculiar symptoms which were already known to me in animals infected by injection into the duodenum. It had a paralytic weakness of the hinder extremities, supported itself no longer on its hind feet, and in consequence lay quite flat with its legs stretched out. The respirations were weak and prolonged, the head and extremities felt cold, the heart's pulsation was scarcely perceptible, and the animal died after it had been in this condition for a few hours. It was examined immediately after death, and the most pronounced choleraic signs were found in the intestinal canal. The small intestine was deeply injected, and filled with a flocculent colourless fluid. Also the stomach and intestine did not as usual contain firm masses, but a large amount of fluid instead. Diarrhœa had not occurred, and, in accordance with this, firm scybalæ were still present in the rectum. The examination by the microscope and with gelatine plates showed that the small intestine contained a pure cultivation of numerous comma bacilli. Now it was very remarkable that of 19 animals the infection was successful in 1 only, and this by chance in an animal which had aborted immediately before the infection. In the autopsy the abdominal walls were found to be very flaccid, and the uterus still greatly enlarged. This led me to the idea that either the abortion of itself, or perhaps its unknown cause, might have acted on the other abdominal organs, and especially on the small intestine, in such a way as to produce a temporary relaxation of the intestine with cessation of the peristaltic action, and that in consequence of this the comma bacilli which were present in the intestine were enabled to remain there longer, and thus obtain a footing there. In order to produce experimentally a similar condition,

alcohol, chloral, morphia, atropia, and opium were employed in the form of subcutaneous or intra-abdominal injection, and it was found that opium answered best for this purpose. Opium in guinea-pigs must be employed in a special way. Incredible doses can be given internally to the animals without any noteworthy effect. Up to a certain point this circumstance may find its explanation in the distended condition of the stomach described previously. The dose of the tincture of opium which is given to the animal cannot come into action at once, since at first it remains among the masses of food which the animal has in its stomach, and is only gradually absorbed. In consequence of this, an accurate dosing is not possible, and I have therefore preferred to introduce the material into the abdominal cavity of the animal by means of injection, which can be done in guinea-pigs very easily and without danger. I use opium in the form of tincture of opium, and in doses of 1 ccm. to each 200 grammes weight of the animal. In a short time after this dose a deep narcosis sets in, lasting from a half to one hour, after which the animal becomes as lively as before. 35 guinea-pigs were experimented on by the administration of the soda solution and cholera bouillon, with subsequent injection of tincture of opium. Of these, 30 died of cholera. The clinical symptoms and the post-mortem appearances were the same as in the guinea-pigs in which the injections had been made into the duodenum, and also in the one mentioned previously, which had died after the administration of the soda solution and the cholera bouillon alone. If the dose of the soda solution or of the cholera fluid is reduced, the result is not so certain. For example, 14 guinea-pigs were treated thus: they received 5 ccm. of the soda solution, and then a fluid to which only one-third of a drop of the cholera bouillon was added. Of these animals only 7 died of cholera, the remaining 7 remained healthy. Again, in another experiment, where the dose was still more reduced, there died only 7 out of 24 guinea-pigs. On the whole, up till now 85 guinea-pigs have been infected in this way with cholera, and have died always with the same characteristic symptoms and post-mortem appearances. I shall only just mention, in addition, that the infective material was successfully transmitted from one animal to another. In the place of cholera bouillon the intestinal contents of a guinea-pig, which had died of cholera,

were administered to other animals, which in consequence died of cholera also.

If other bacteria are administered to animals in the same way as the cholera bacilli, with the assistance of the soda solution and tincture of opium, very remarkable results are likewise obtained.

The Finkler comma bacilli can also destroy guinea-pigs by this mode of infection, though they are not so virulent as the cholera bacilli; for of 15 animals infected with them only 5 died. The post-mortem condition of these animals was different to that of animals which had died of cholera. The intestine was likewise largely filled with watery fluid, but it appeared pale grey. The vessels were not nearly so markedly injected as in the cholera animals, and the intestinal contents had a penetrating putrid smell, which corresponded exactly to the smell developed by the Finkler bacteria in nutrient jelly.

Further, the bacilli cultivated by Deneke from old cheese, which also possess a curved form, were experimented with, as also those found by Miller in a hollow tooth, which to all appearances are identical with Finkler's bacilli. Of 15 animals infected with Deneke's bacilli, 3 died; of 21 infected with Miller's bacilli, only 4.

The guinea-pigs which survived these experiments received subsequently cholera bacilli, and the whole of them died of the cholera infection.

Pathogenic bacteria also, which, under ordinary conditions, do not act from the intestine, may produce effects when used in this manner, as, for example, sporeless anthrax bacilli and Brieger's bacteria. Other organisms, such as the bacteria of fowl cholera, of osteo-myelitis, of rabbit septicæmia, and of erysipelas, fail to act even under these conditions. The bacilli of typhoid fever, which have not as yet been successfully inoculated on animals, gave a doubtful result, and on this account the experiments with them should be repeated.

I may here only mention, that attempts have been made in other ways to set up in the intestine a condition favourable to the development of the cholera bacillus. For example, we gave the animals croton oil and castor oil, or they were fed with lees (*Hefe*), in order to set up an intestinal catarrh. Further, we have injected into the abdominal cavity turpentine, tincture of

iodine, glycerine, alcohol, &c., and thereby have had successful results. The use of alcohol was the most successful in making the animals susceptible to the cholera infection, though taken as a whole the action of alcohol appeared to fall short of that of the tincture of opium.

From these experiments we can now decide that the cholera bacteria have extremely energetic pathogenic properties, and are able to show them, if they reach the small intestine uninjured, and find it in a condition which allows them to obtain a firm footing and to develop. In guinea-pigs these conditions can only be realized artificially; but in man the relations in reference to the gastric digestion are quite different to those in guinea-pigs. The human stomach is not constantly filled with strongly acid masses of food, like the stomach of our experimental animals. Probably its contents very often have a neutral or even alkaline reaction; for instance, always after the completion of the true gastric digestion and the emptying of the chyme into the small intestine.

I am indebted to Professor Ewald, who has lately been investigating this question, for some interesting communications with reference to it. He found, that if water is introduced through an œsophageal tube into the empty stomach, it remains for a considerable time neutral, or even takes on an alkaline reaction. At the same time it was found that the quantity of water in the stomach gradually diminished—that is to say, that the stomach constantly passes on a certain quantity of its contents into the small intestine. Possibly this slow decrease was also due to absorption from the stomach. But in about an hour or an hour and a half a rapid diminution of the fluid in the stomach suddenly occurred, even before its reaction had become acid. Evidently the pylorus had then opened, and permitted the exit of the gastric contents into the small intestine in large quantities. If we were now to assume that cholera bacilli accidentally existed in this water, then they could undoubtedly have reached the duodenum of the individual in question in a living condition, and there might possibly have caused a cholera infection. An artificial preparation, as in the experimental animals, is not then necessary in order to infect man.

But it may be further concluded that, as a rule, the behaviour of man to the cholera infection will not be always the same

according to the condition of their gastric digestion. The different individual predisposition of man depends perhaps to a great extent upon the state of the gastric digestion at the time when the infective material reaches the stomach; and further, on what happens to be the condition of the intestine, whether perhaps it approximates more or less to the condition of the intestine of the guinea-pig after the injection of the tincture of opium. We obtain in this way a certain amount of insight into the nature of the process of infection, and I do not doubt that by means of further experiment in this direction a still greater advance will be made, and we shall perhaps be able to clear up much which is now dark to us. Since the infection of animals through the stomach has succeeded, one will be able to test experimentally the effect of drugs on the cholera process.

As I have previously mentioned to you that the cholera bacilli do not pass into the blood, we can only explain their action by supposing that they generate poisonous substances belonging to the group of ptomaines, which are absorbed, and then act on the entire organism. In order to give this idea a foundation of fact, I have endeavoured to demonstrate directly these poisonous products of the cholera bacilli, which we must suppose to exist, but these investigations have not yet yielded much result. Only so much has already been made out that it is possible to prepare cultivations of the comma bacilli which are intensely poisonous, and which, if they are injected into the animal either subcutaneously or into the peritoneal cavity, set up in a few minutes the same group of symptoms which occur in animals suffering from cholera a day or two after infection. These symptoms are the paralytic weakness of the hinder extremities, coldness of the head and legs, and prolonged respiration, a condition which usually leads after some hours to death. I should like to add to these communications a few remarks upon other experiments, which do not stand in direct connection with the experiments on infection. First, on the capability of resistance, and the durability of the cholera bacteria. On this point many investigations have been lately made by Nicati and Rietsch and by Babes. We have also again studied this question ourselves, and tried in the first place to find out how long the cholera bacteria remain alive in spring or river water, in sewer water, in excrement, and in water from a cesspool. Our experiments have shown that

cholera bacilli mixed with spring water could be found up to thirty days afterwards; in the Berlin sewer water they lived only six to seven days; mixed with excrement, only twenty-seven hours; and in cesspool water they were no longer alive after twenty-four hours.

Experiments were then made to try and preserve cholera bacteria for a longer time on clothing materials, such as linen cloth, &c., kept in a moist state. So far as we have as yet gone with these investigations, the result is that the capability of life of the cholera bacteria is not of very long duration. They were already dead after three to four days.

In our first conference I mentioned to you, as you will remember, the surprisingly rapid death of the cholera bacilli when in a dry state, and pointed out to you the practical importance of this peculiar circumstance. My statements at first met everywhere with doubt, but all reliable observers have convinced themselves of their accuracy, and I would at this opportunity positively reiterate them, with the request at the same time that the most extended use of this experience should be made in combating infection.

With regard to disinfection, I can report to you the result of experiments with carbolic acid. The cholera bacteria were killed in a few minutes in a solution of ½ per cent. of carbolic acid. Also sulphate of iron, sulphate of copper, and other metallic salts are active, but not nearly so sure, since a more or less large quantity of them are precipitated by some of the constituents of the nutrient solution. This would naturally make itself felt in the disinfection of cholera dejecta, and therefore I give carbolic acid decidedly the preference.

Lastly, I should like to draw your attention to an observation on the action of the cholera virus on man.

The infection of those persons who have to do with cholera linen gives us, as already mentioned in our former conference, numerous experiences in this direction; and the last epidemic in France and Italy has afforded us many further examples. That an unintentional infection could occur through the manipulation of the cholera bacilli, was not therefore improbable. In consideration of this, every precautionary regulation possible was employed to ward off this danger during the cholera courses, which were held here in the Sanitary Institute. But spite of all our care one case of infection nevertheless occur

which happily ended without a bad result. Before I give more exact details of the case, I shall remind you of some investigators who have already made infection experiments on themselves. Thus Bochefontaine, in Paris, has swallowed choleraic dejecta in pills without becoming sick of cholera in consequence. Klein, according to an announcement in the *Indian Medical Gazette*, when he had just arrived in Bombay, drank a fluid which was said to contain cholera bacilli. Apart from the fact that in these cases it was not proved that the true cholera bacillus was present in the fluids swallowed, it stands to reason that these experiments, having a negative result, prove nothing at all; since a healthy stomach most probably destroys the bacilli during the period of digestion, and therefore it is not to be expected that if cholera bacilli are introduced into the human stomach an infection must result in every case. But if these experiments had turned out positively, they would have afforded just as little evidence, because they took place in localities where an infection could occur in other ways.

An experiment of this kind, carried out in a cholera locality, is only of real importance if the infection attacks not one single individual but a number of individuals at the same time. Because the greater the number of the infected persons the less the probability that all of those who became ill were infected in consequence of an accident unconnected with the experiment. On this account I lay great stress on the instance of cholera infection reported by Macnamara, which I would bring to your recollection on this occasion.

I have corresponded about the case with Macnamara himself. The accounts of it in his work on cholera are somewhat meagre; for instance, he does not state where the case occurred, nor does he give any names; however, he may have quite satisfactory reasons for not doing so. I have been always assured in Calcutta, where there was much talk about this case, that it was quite a reliable observation, and that the facts occurred exactly as Macnamara reported. He has written to me himself to the effect, that he is ready at any moment to make privately further and more exact communications, which must set at rest any doubt. On this account I am convinced that everything happened as described by Macnamara, and that these observations can be scientifically made use of without any hesitation. In the

Dictionary of Medicine Macnamara makes the following communication about it.

Through an accident—what kind of accident it was is not stated—cholera dejecta became mixed with water. This water remained exposed to the heat of the sun the whole day, then 19 persons drank of it and 5 of them fell ill of cholera within thirty-six hours.

I am assured on special inquiry that almost no cholera prevailed at that time, and particularly at the place where the accident happened. Further, all the persons, who were familiar with the Indian conditions, to whom this case was known, and whom I asked about it, were in no doubt that these men had really become ill in consequence of the use of this water contaminated with cholera dejecta.

In our case of cholera infection observed during the cholera course, we had not to do with a wide-spread disease, but only with the infection of one individual. Nevertheless the observation is of great importance, because it occurred at a place and at a time when cholera infection, from any other source than that of the manipulation of the cholera bacilli, was absolutely excluded; and because until now it is the only case in which within the borders of Germany the true cholera bacilli have been demonstrated in the dejecta of one suffering from cholerine.

The medical man in question, whose name and residence you will allow me to omit to mention, had been in Berlin for eight days, when he became affected with a slight disturbance of digestion associated with diarrhœa. The evacuations had a thin soupy appearance, and occurred several times daily, so that his condition caused him no anxiety. But on the last day of his stay more frequent thin watery evacuations made their appearance. Nevertheless he believed himself able to travel from here, did so and arrived safely home, and then sickened with a true attack of cholerine. He had for two days very frequent watery and colourless evacuations, great weakness, unquenchable thirst, and the urinary secretions were reduced to a minimum. True cramp of the calves of the legs did not show itself, but strong contraction of the soles of the feet, and spasmodic flexion of the toes occurred. As he felt himself too weak to examine his own evacuations, he placed a small quantity in a well-cleaned bottle and sent it here. The vessel was sent off in the evening, arrived here on the following morning, and was at once examined. The parcel

thus been only a night on the journey, and that during a cold season of the year, so that its contents could not have altered materially in transit. The examination of the dejecta, which was made on cover glasses, and at the same time by cultivations on hollow slides and on plates, showed in each case the presence of very numerous true cholera bacilli. One of the pure cultivations exhibited to-day came from the dejecta of this patient. I shall only add that the patient got better. The diarrhœa stopped, but there remained for a long time a surprising weakness.

I must not neglect to hold up this case as a warning to those who experiment with the cholera bacilli, and who do not work with the greatest care.

Since the question of the existence of a permanent form (*dauerform*) of the cholera bacilli is still mentioned in our programme, I shall express my opinion about it in a few words. On account of the importance of the question I have been constantly endeavouring as far as possible to discover something, which could be looked on as a resting-stage of the cholera bacteria, analogous to the spore formation of other bacilli. But I have arrived at only a negative result, as was the case in all the earlier investigations in this direction. All statements made by other observers up till now with regard to a permanent form and spore formation depend evidently on errors. Thus for example Ceci believes that he has seen spores in the cholera bacilli. He looked on those bacilli occurring almost always in old cultivations, which after staining by aniline show an uncoloured spot in their middle, as spore-bearing. I also came across these peculiarly coloured bacilli in my first cultivations, but I very soon convinced myself that the part remaining uncoloured did not do so through the formation of a spore, but arose when the bacillus became thicker and coarser than usual. Probably a swelling occurs in consequence of the absorption of moisture, and a division of the plasma takes place between the thicker and more deeply coloured substance found at the end of the bacillus and the less concentrated substance lying in the middle. A similar thing may be observed in the bacteria of rabbit septicæmia, which constantly take up the colouring matter, so that the middle is only slightly or not at all coloured. As this appearance in the cholera bacillus occurs only in older cultivations, it must be regarded as a kind of involution or change in

the dying or dead bacilli. Confirming this idea is the fact that cultivations, which contain such bacilli, are not in the least more capable of resisting hurtful influences, such as dryness, heat, and chemicals, than the ordinary comma bacilli. Ceci himself has found out that his apparent spore-bearing bacteria were destroyed in a very short time if they were dried. Hence they could not have existed in a durable form.

At this opportunity I may mention as curiosities that Klein has observed a longitudinal division of the cholera bacilli; and that according to Ferran the cholera bacilli belong to the cycle of development of a mould (peronospora). Both these supposed discoveries depend upon an erroneous interpretation of the forms of involution of the cholera bacillus.

Though a true resting-stage of the cholera bacilli has not yet been found, and does not even appear to exist, we are already acquainted with other facts which may explain the passing slumber of a cholera epidemic, which may last months, sometimes even a whole winter. Cholera bacilli, in contrast to their small capability of resisting dryness, can under certain circumstances remain capable of life in a moist condition for a long time. It has been already stated by Nicati and Rietsch, that the cholera bacilli remained alive in the harbour water of Marseilles for 81 days. We found on testing old cultivations, which were grown in agar, that even after 144 days, cholera bacilli, still capable of development, were present. In an examination after 175 days the cultivations were found however to be dead. Hence one can easily imagine that in superficial layers of earth, in marshes and so forth, the cholera bacilli may find conditions in which they can exist preserved from death for five months, or even longer, just as well, or even better, than on our moist agar jelly.

ON THE

BACILLUS OF GLANDERS.

By Dr. LÖFFLER and Prof. SCHÜTZ,

(*Deutsch. Med. Wochenschr.*, Dec., 1882.)

TRANSLATED BY

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PRELIMINARY REPORT BY DR. STRUCK ON THE RESEARCHES CONDUCTED IN THE IMPERIAL SANITARY INSTITUTE, WHICH LED TO THE DISCOVERY OF THE BACILLUS OF GLANDERS.

THE researches referred to were carried out by Dr. Löffler and Professor Schütz, and were an outcome of the experiments which the Imperial Board of Health were conducting for the determination of effectual methods of disinfection. They promise to become of great practical importance not only for sanitary science but also on account of the definite knowledge obtained as to the essential nature of glanders. They indicate also the proper direction in which further researches should be carried on, as well as the preventive measures which may be adopted against this disease. I desire to publish in the following paper only those portions of the researches which may prove useful in assisting those who wish to pursue further the investigation of this subject.

In the first instance search was made for definite forms of bacteria among the specific products of glanders contained in the so-called glanders nodules. Sections were also made of the lung, spleen, liver, and the septum nasi of a horse which had suffered from glanders, and after being treated by different staining reagents they were examined under the microscope. Fine rods about the size of a tubercle bacillus were occasionally found in those preparations which had been stained in a concentrated watery solution of methylene blue and afterwards treated with a very weak solution of acetic acid; the water being extracted by alcohol and the preparations cleared in cedar oil. (See Plate VI., figs. 17, 18, 19.) No other forms of bacteria were present in these specific products, and in order to ascertain

with certainty whether these rods were the cause of glanders the culture method was employed. If we suppose that a special bacterium is the cause of glanders, it is naturally to be expected that it would thrive best in the serum of the blood of those animals which it is well known possess a great susceptibility to the contagium of glanders, such for example as the horse and sheep.

In accordance with Koch's method of cultivation for the bacillus of tubercle, a number of small particles, carefully selected from glanders nodules taken from the lungs and spleen of a horse which had suffered from glanders, were placed, on the 14th of September, in a series of sterilized test tubes containing the blood serum of a horse or sheep. During the first two days no changes were observed on the inoculated surface of the serum. On the third day however numerous transparent droplets which had formed in places on the surface of the serum were observed in the majority of the tubes. Cover-glass preparations of these droplets, stained in the usual manner, demonstrated bacilli of the above-mentioned size. Similar droplets were found in almost all the test tubes inoculated with glanders material, and they always contained the one kind of bacterium. Such being the case, one was naturally induced to test these bacilli as to their causal relation with glanders by inoculation of healthy animals susceptible to the disease. When a successful result was obtained the cultures were further continued through four generations for one month, in order that the objection might not be raised that particles of the original material were present in the vaccinating fluid. On the 14th of October, a small quantity of this fourth cultivation, consisting only of the above described bacilli, was inoculated on the mucous membrane of the nose, and on the two shoulders of an old and apparently healthy horse. In forty-eight hours the animal began to show signs of high fever. At the inoculated spots deep ulcers were developed, from which knotted lymphatic cords extended to the nearest lymphatic glands, so that in about eight days after the inoculation the horse exhibited the pronounced clinical appearances of glanders. In about four weeks the ulcers began to cicatrise, the glandular swellings diminished, and the animal appeared so much better that it became doubtful whether the symptoms which occurred after the inoculation were really due to glan-

It was resolved therefore to kill the animal on the 25th of November in order to ascertain whether changes, due to glanders, existed in the internal organs.

The post-mortem gave a very surprising result. There were found on the septum of the nose, and also in the posterior nares, numerous white striated nodules. In the lung there existed old fibrous and calcareous nodules, but fresh grey nodules surrounded by a red zone were also observed. At the root of the lung there was found a glanders growth of about the size of an apple, and we naturally concluded that the animal had previously suffered from glanders. It could not, therefore, with certainty be stated that the fresh eruption was caused by the artificial inoculation, and consequently the experiment could not be considered absolutely conclusive. The fresh glanders material taken from this animal was employed for obtaining new cultivations, and these developed in three days transparent little drops similar to the previous cultivations, and contained only the above described bacilli. The same bacilli were found after treating with methylene blue the fresh glanders products taken from the dead horse. During November the fresh organs from another horse suffering from glanders were examined, and the same transparent little drops containing bacilli were successfully cultivated from the glanders nodules present in the liver. Again on the 1st of December, in a fourth case, cultivations were successfully made from fresh glanders nodules, and the result was in all cases the same.

Rabbits, mice, and guinea-pigs were also successfully inoculated by pure cultivations of these bacilli. The action of the virus on rabbits was various. At the post-mortem examination of some of them only local ulcerations and swellings of the neighbouring glands were found, while others exhibited a perfect picture of glanders, such as ulcers on the septum nasi and glanders nodules in the lungs. The inoculation of white mice which are otherwise exceedingly susceptible to infective diseases of all kinds, gave negative results with these cultivations. On the other hand the inoculation of field mice gave satisfactory results. At the post-mortem examination of those animals which had died within eight days after the inoculation, the spleen and liver were found infiltrated with small yellowish-grey nodules, and in these fine bacilli were present.

The result of the inoculation of guinea-pigs was remarkable. The course of the after symptoms varied in duration according as small or large quantities of the culture were used for injection. On the third or fourth day after the inoculation there was always developed at the seat of inoculation an ulcer with a markedly indurated base. The neighbouring lymphatic glands then began to enlarge, and became as large as a hazel-nut or even a chestnut. In some animals the process remained stationary at this point for weeks, the virus probably being retained in the glands. In those animals where especially large quantities of bacilli had been introduced subcutaneously, some developed nodular swellings of the testes, ovaries, or vulva; others nodular swellings of the feet or at different parts of the skin, or even ulcerative processes in the nasal passages which led to perforation of the bone; finally, in some of the animals an acute general affection appeared which speedily ended in death. The spleen and lungs were found to be infiltrated with innumerable submiliary grey nodules, which showed great resemblance to the miliary tubercle. They were distinguished from the latter by the fact that the tubercle bacillus could not be demonstrated in them by the characteristic staining method. By other colouring materials, however, fine bacilli were found such as exist in the glanders products of horses. All these changes naturally pointed to glanders, because the same symptoms were observed in horses suffering from that disease. Among other symptoms may be mentioned the metastasis to the testicles, and the osteo-myelitis, which has chiefly its seat in the ribs. The cultivations from all these organs, testicle, spleen, lung, &c., produced always the same pure cultivations, identical with those which had been obtained from the different organs of four horses suffering from glanders.

Although it was highly probable that the above described bacilli were the cause of glanders the decisive test by the inoculation of horses with the pure cultivation had yet to be performed. Accordingly two healthy horses were procured, one about 20 and the other about 2 years of age, and they were both inoculated on the 28th of November with pure cultivations of bacilli.

The material employed for the elder animal was the eighth cultivation in succession of the pure cultivation obtained on the 14th of September, and had been grown for ten weeks outside the

animal body. For the 2-year old animal a cultivation was used which had been continued for five generations outside the animal body. This had been previously obtained from the testicle of a guinea-pig which died on the 8th of November, and which had been inoculated with the fourth generation of the culture of the 14th of September.

In order to produce an infection as rapidly as possible both animals were inoculated by injections on both sides of the neck, the breast, and the flanks, and in the case of the younger animal also on the bridge of the nose. The nasal mucous membrane was not touched, in order to see whether secondary eruptions would develop on the intact mucous membranes.

In a few days diffuse boggy swellings appeared in both animals at the seat of inoculation. The animals ate badly, and the legs became stiff and the hair rough. In about 8 days beaded string-like cords which extended to the neighbouring glands could be felt in the skin. The swellings had by this time opened and secreted a turbid yellowish green fluid. On the twelfth day there was observed on the skin of the forehead of the younger animal, besides the above-mentioned symptoms, an ulcer about the size of a shilling with everted edges, and which had extended as deep as the frontal bone. In both animals there was a discharge from the nostrils, which dried upon the margins and formed thin yellowish crusts. On the mucous membrane of the nose there were little ulcers with raised margins which distinctly pointed to the glanders nature of the disease. They grew weaker from day to day, and on the 12th of December the elder one died.

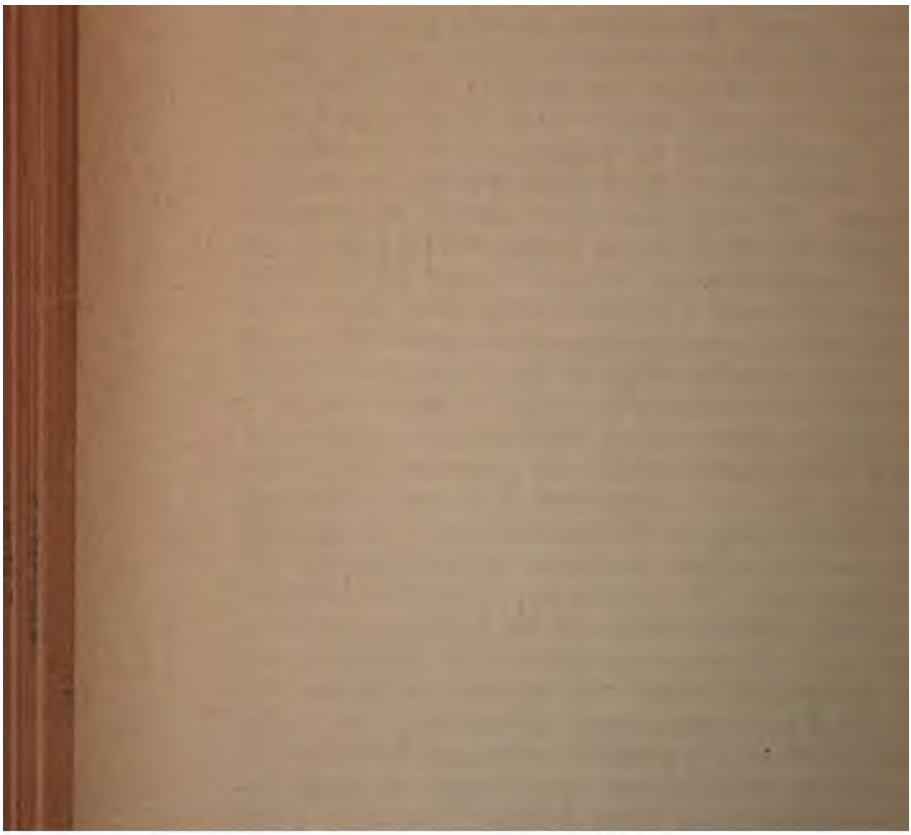
The post-mortem gave the following results. At all the inoculated spots ulcers of about the size of a shilling had formed. On each ulcer there lay a thick crust which consisted of dried secretion products and hair, and a white turbid fluid was exuding from the margins of the crust. The soft parts in the vicinity and under the ulcers were broken down and almost fluctuating. The subcutaneous tissue surrounding the ulcers was infiltrated with a purulent fluid and was easily separable from the underlying parts. The ulcers on the neck were connected with one another by means of lymphatic cords of about the thickness of the finger, extending as far as the neighbouring glands. These were almost the size of a fowl's egg, and the

reddened tissues contained little yellow or yellowish white deposits. From the other inoculated spots lymphatic cords could be traced extending to the neighbouring lymphatic glands, and there could be occasionally demonstrated on them soft yellowish white almost fluctuating nodules about the size of a pea or bean. The axillary and inguinal glands were swollen and infiltrated by the aforesaid yellowish deposits. Ulcers with sinuous margins were seated on the mucous membrane of the septum nasi and turbinated bones. In the margins and the bases of these ulcers there were found small yellowish or grey nodules. The submaxillary glands contained nodules about the size of a bean or hazel-nut, and were infiltrated with yellow deposits. An ulcer about the size of a sixpence with a raised margin was discovered on the anterior surface of the epiglottis. Numerous nodules about the size of a millet seed or pea were found in the lungs, the smaller ones containing a grey turbid centre surrounded by a reddish area. Nodules of various sizes consisting of a yellowish white pulpy mass were found imbedded in various muscles of the body. Since the result of the experiment was a decisive one in the elder animal, and as the younger one was showing distinct signs of failing health it was killed on the 18th of December.

A post-mortem was directly made, and the following changes were observed :—At the inoculated spots there were large ulcers secreting a thin yellowish-white fluid. The ulcer on the bridge of the nose was about the size of half a crown and extended to the periosteum of the upper jaw and nasal bone. The ulcer on the forehead was somewhat smaller and its base was covered with blood clots. Several little ulcers were also discovered on the skin of the right hind thigh, and one was found on the skin of the penis. From the ulcers formed in the upper part of the neck thick knotty lymphatic cords could be felt extending down to the swollen shoulder and axillary glands. The nodules, which lay partly in and partly near the lymph vessels, contained a pus-like fluid, and the glands themselves were about the size of a hen's egg, soft and infiltrated with little yellowish grey deposits. From the ulcers which had formed on the breast the ulceration extended deeply into the subcutaneous tissues and muscles, and several lymphatic cords about the thickness of a quill extended as far as the axillary glands

These glands were about the size of a walnut, and contained yellowish white deposits as large as a millet seed. The ulcers situated at the inoculated spots in the vicinity of the flanks were flat, and the contiguous tissue was infiltrated with a turbid fluid. The lower extremity of the right hind thigh was swollen, especially near the ulcers which had their seat on the external surface of the tarsus. The glands in the right groin, the size of a hen's egg, were soft and fluctuating, and of a reddish colour. The glands in the left groin were similarly affected, but of a smaller size. The subcutaneous tissue of the penis had a gelatinous appearance, and the lymphatic glands were enlarged. The ulcer situated on the penis extended deeply into the subjacent tissue. The right sterno-cleido-mastoid, the pectoralis major of each side, the abdominal muscles, the left gracilis, and the right semimembranosus contained several large cavities which were filled with a yellowish white turbid fluid. The cavity situated in the semimembranosus was about the size of the fist, and contained in addition to the aforementioned fluid a necrosed piece of muscle about the length of a finger phalanx.

The mucous membrane of both sides of the nose was studded with grey and yellowish nodules and numerous ulcers. The ulcers arising from the breaking down of the nodules were full of fresh nodules lying on the base and under the excavated margins, and the mucous membrane surrounding them was of a reddish colour. The most severely attacked parts were the margins of the turbinated bones and the upper portions of the posterior nares. No ulcers were found in the pharynx, larynx, trachea, or bronchial tubes. The submaxillary glands were about as large as a walnut, tough and movable. On section they were found to be of a reddish colour, and infiltrated with several yellowish white deposits. In the lungs six nodules the size of a millet seed were found, showing a grey centre and a reddened periphery, and the parenchyma of the lung surrounding them was tough and moist. The anterior mediastinal and bronchial glands were enlarged, soft, and engorged. The spleen was somewhat enlarged, and its parenchyma was soft and dark brown. The liver, kidney, heart, and all the muscles of the body appeared congested.



RECENT

RESEARCHES ON MICRO-ORGANISMS

IN RELATION TO

SUPPURATION AND SEPTIC DISEASES.

TRANSLATED AND ABSTRACTED BY

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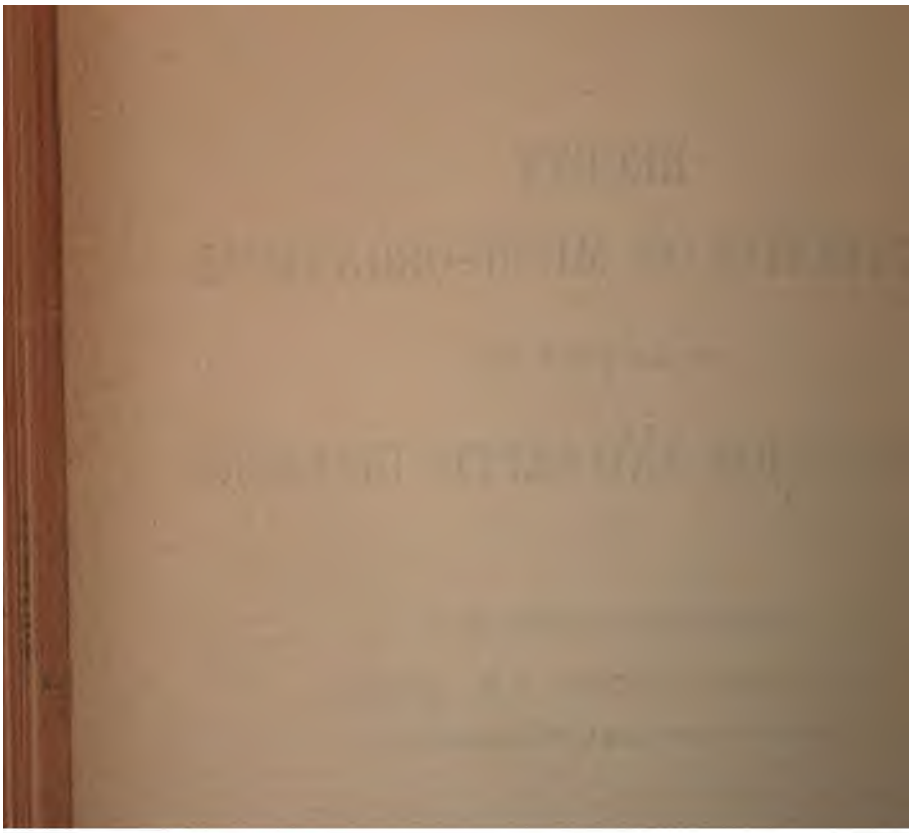

I.—MICRO-ORGANISMS IN HUMAN TRAUMATIC INFECTIVE DISEASES.

By Dr. FRIEDR. JUL. ROSENBACH, *Professor Extraordinary and Assistant-Surgeon at the Surgical Policlinique at Göttingen.*

INTRODUCTION.

"R. Koch, in his investigations on the etiology of traumatic infective diseases, has discovered a number of diseases in animals which have in some respects an unmistakable likeness to the surgical infective diseases of man. The diseases which Koch induced experimentally in animals differed in a very important manner from those described by former experimenters, more especially in that they ran a typical course, and showed their specific nature by the regular reproduction of the definite symptoms. Koch has further demonstrated, by the aid of entirely new methods, that each of these diseases is caused by a definite microbe which always with certainty reproduces the disease whether it has been inoculated directly from animal to animal, or has first been cultivated on a dead nutrient soil for a number of generations. Thus we have become acquainted with a septicæmia of mice, a progressive necrosis of the tissue in mice, a progressive abscess formation in rabbits, a pyæmia and a septicæmia in rabbits, and have seen how each of these diseases is caused by a distinct microbe. At a later period there has been discovered in addition a bacteric septicæmia of rabbits, mice, birds, &c., a malignant œdema, &c. I will, however, only remind the reader of these facts, I do not propose to discuss the far reaching discoveries of Koch and his scholars in this place; they have attracted the attention due to them, and I must take it for granted that they are well known to each of my readers.*

* See *Investigations into the Etiology of Traumatic Infective Diseases.* By Dr. Robert Koch. Translated by W. Watson Cheyne, M.B., F.R.C.S. London: The New Sydenham Society. 1880.

I would also fain believe that, on first reading Koch's investigations, the reader said to himself as I did, that it would now only be a question of time before the surgical infective diseases of man were investigated, and their etiology known, in like manner as had been done by Koch in his investigations on animals.

"In fact the etiology of a number of surgical infective diseases in man has already been made out. In erysipelas, gonorrhœa, glanders, &c., the microbes which cause them have been discovered. Koch, by the discovery of the tubercle bacillus, has not only made a discovery which affects to a great extent medical knowledge, but has also made us acquainted with the pathogenic microbe of many surgical chronic infective diseases which daily come before us in practice. But with regard to that class of diseases of wounds which have a less specific character, and the infective material of which is present everywhere—I refer to suppuration, phlegmon, sepsis, pyæmia, &c.—the investigations have not reached a similar point, although here also, more especially as the result of very excellent researches carried on in England, important advances have been made."

I.—METHOD OF INVESTIGATION.

After recalling the three points which according to Koch should be made out, if possible, in every case of infective disease before the conclusion is drawn that a particular microbe is the cause of the disease (viz., that the bacteria in question are present in the affected parts in sufficient numbers to account for the disease, that they can be cultivated outside the body, and that after being thus separated from the morbid material they reproduce the disease when reinoculated into animals of the same species), Rosenbach goes on to say, in regard to septic diseases in the human being, "The two first conditions can be carried out in the case of man as well as in the case of animals. The reinoculation experiment has indeed been also carried out in exceptional cases, as in erysipelas and gonorrhœa, but as a rule this link in the chain of evidence must be omitted. In spite of this, however, in those cases where the disease can be reproduced in the lower animals, as in tuberculosis and erysipelas, the experiment is quite convincing; and this is still the case even where the affection in the animals is more or less abortiv

long as it is thoroughly characteristic. Where, however, the reinoculation of the cultivated microbe cannot be carried out, the absolute proof is wanting. And unfortunately, as we shall see, we meet with this difficulty in the case of sepsis and pyæmia in man, particularly in those forms in which a severe constitutional affection follows the introduction of a minimal quantity of the virus. Nevertheless, when we see how the extremely infective bacillar septicæmia of mice only attacks house mice while field mice are unaffected by it, how can we expect that similar diseases of man must necessarily be inoculable on the lower animals, although of course the possibility of this occurrence cannot be set aside? Even the simple observation of a particular micro-organism in an infective disease is of the same value as any other pathological observation. A constant coincidence is further a weighty reason for supposing a more or less direct etiological connection with the disease. Of course under such circumstances a definite proof of such connection can only be established by prolonged clinical and pathological experience. By such observations alone we may by degrees obtain a substitute for the absence of the single conclusive experiment. It is naturally necessary in such a case so to make the observations with regard to the presence of the microbe in the affected parts that gross errors cannot arise, more especially that micro-organisms which have accidentally entered after death be not mistaken for the pathogenic ones. Where, however, it is possible to obtain, with exclusion of all impurities from without, the material for investigation for microbes (whether for microscopic observation or cultivation experiments) from masses in the interior of the living body, or from the diseased but as yet unopened tissue, the significance of the result is such that it is no longer the number of similar results in a given disease which is of importance, but rather each individual and well observed instance. Opportunities for such observations are at the present time offered in surgical practice, as we now not uncommonly cut down upon deep unopened infective masses with antiseptic precautions which prevent the entrance of microbes from without. The results detailed in the following pages have been obtained from such cases."

II.—SUPPURATION AND ABSCESS FORMATION.

"The fact that suppuration hardly ever follows injuries except when there is solution of continuity of the skin or mucous membranes has at different times led observers to refer the cause of suppuration to injurious matters coming from without, for example, to the oxygen of the air. The view, also, that suppuration was due to infection with microbes has been more especially brought to the front by Hüter's work on inflammation. But it is only lately that the views with regard to suppuration and abscess formation have taken definite shape. On the one hand, the antiseptic method of treatment, the more it has become perfected in the hands of surgeons, shows us that even the most lacerated wounds, wounds with comminution of bone, wounds into serous cavities, &c., where the antiseptic treatment is successful, heal without suppuration or abscess, although a certain amount of inflammation with swelling occurs. The necessary conclusion from this is that suppuration and phlegmon are the consequences of infection. On the other hand, while these views ripened as the result of clinical experience, the same problem was being studied from the experimental side. In an experimental work on acute suppurative osteomyelitis I have, as the result of investigations on the medulla of bone, been led to propound the view that every spontaneous suppurative phlegmon occurring in connection with wounds, indeed every inflammation in wounds passing beyond the degree necessary for repair, is caused by ferments (micro-organisms) which have penetrated into the wounds."

Then follows a short historical sketch in which reference is made to the works of Kocher, Uskoff, Councilman,* Pasteur,† Doléris,‡ Ogston,§ and myself ‖ Of these researches the most important is Ogston's, who was the first to demonstrate the constant presence of micrococci in acute abscesses. He further pointed out

* Virchow's *Archiv*, vol. xcii. p. 217.

† *Bulletin de l'Acad. de Med.* 2 Sér., Tome vii., 1878, p. 447.

‡ *La fièvre puerpérale et les organismes inférieurs.* Paris, 1880. Ballière et fils.

§ "Report on Micro-organisms in Surgical Disease," *Brit. Med. Journal*, March 12, 1881, p. 369; also "Micrococcus Poisoning," *Journal of Anatomy and Physiology, Normal and Pathological*, vol. xvi. p. 526, and vol. xvii. p. 24.

‖ "Relation of Organisms to Antiseptic Dressings," *Trans. Path. Soc.*, vol. xxx. See also "Report on Micrococci in relation to Wounds, Abscesses, and Septic Diseases," *Brit. Med. Journal*, September and October, 1884.

that in some cases these micrococci were in chains (streptococci), in others in groups (staphylococci), and in his later papers he looked on these as distinct organisms. Rosenbach also in his earlier researches noticed these two forms, but did not come to a definite conclusion with regard to them as the result of microscopical observation. With regard to this he says :—

"Fortunately Koch has provided us with another mode of recognizing and differentiating these different organisms as simple as it is ingenious, viz., cultivation on solid materials. When I began to cultivate the microbes of abscesses on such media I learned for the first time that they were of different kinds. From the macroscopic appearances of the cultivations alone the same forms could always be readily recognized, and in every case the conclusion thus come to was corroborated by the result obtained by the microscope. At first I made the cultivations on peptonized meat jelly (which I will in future shortly designate by the letters P. F. G. [*Pepton-Fleischextract-Gelatine*]). I now only employ this material for certain purposes and not as a general rule, because most of the micrococci found in pus quickly render the gelatine fluid so that it is often quite liquid before the colonies have assumed the characteristic form or colour. On the other hand none of the micro-organisms to which I shall here allude have the property of rendering agar jelly fluid, and hence I found it as a general rule more suitable. On it is best seen the different modes of growth of the bacteric colonies, the shades of colour, transparency, &c. I have chiefly cultivated on this material, but I have also employed solidified blood serum and more seldom potatoes. This material (in future indicated by the letters F. P. A. [*Fleischpepton-Agarnährboden*]) was prepared by the method employed in the Imperial Sanitary Institute.

"To 1,000 grammes of infusion of meat (1,000 grammes of minced beef macerated in 1,000 grammes of distilled water for twelve hours in the cold, the fluid poured off, boiled and filtered) were added 10 grammes of pepton, 6 grammes of chloride of sodium, and about 20 grammes of agar agar. This mixture was boiled till the agar agar broke up, it was then rendered slightly alkaline by means of phosphate of soda and filtered through cotton wool in a water bath.

"It is not possible to obtain this material always of the same consistence, because the agar agar is only partially dissolved even after long boiling. For the macroscopic appearance of the cultures

the degree of consistence of this material is not an indifferent matter, and hence when one wishes to compare cultivations of different organisms with one another it is necessary to grow them side by side in tubes containing portions of the same specimen of the agar jelly.

"As the result of cultivations from the pus of thirty unopened acute abscesses I found five different kinds of microbes. (I do not here include abscesses containing foul pus, in which there were, in addition to the organisms of pus, bacilli, spirilla and various kinds of micrococci.) Of these five kinds I must for the present omit one as uncertain. The very first experiment which I made was with pus which came from a praepatellar abscess, which showed a tendency to further spread, but nevertheless healed without any difficulty, and from it I obtained, both on solidified blood serum and also on F. P. G. in three different tubes, an oval coccus (bacterium ?) twice as long as broad, which quickly rendered the jelly fluid. A little of the cultivation was injected into the eye of a rabbit, and the result was subacute suppuration without any marked constitutional affection. I mistrust this observation only because it was the first, and because I have never again found this microbe in unopened abscesses. However, Ogston also speaks of similar oval cocci in pus, and this organism reminds me more especially of Pasteur's *microbe pyogénique*. The organism which I have most frequently found is a micrococcus which occurs in pretty large groups, or, according to Ogston, in masses of the appearance of fish roe or of bunches of grapes. I will here adopt the name of *staphylococcus* proposed by Ogston, not, however, as the name of only one kind but for a group which as yet seems to consist of two different kinds. These two varieties are so similar in their mode of growth, their microscopic form, their mode of grouping, and their effects on animals, that it would be difficult to distinguish them were it not that they are at once recognizable by producing different colours when cultivated. The most common kind produces golden yellow, opaque colonies; the other white but also opaque masses. This difference in colour is constant through any number of generations and on soils of the most different kind—agar jelly, potatoes, blood serum, egg albumen, meat with or without air, &c. In ... these two kinds not uncommonly occur together, and pro... more often than I believed at the commencement of these i...

gations, for the colonies on agar agar have a tendency when they coalesce to grow so intimately together that the observer may think that he has a pure cultivation of the yellow coccus, while as a matter of fact two kinds are present. I would for the present propose to designate these two kinds by their colour under the names '*Staphylococcus flavus vel aureus*' and '*Staphylococcus albus.*'"*

a. Staphylococcus pyogenes aureus.

"I have found this organism more frequently than any other. If sown in the form of lines on F. P. A., whether from pus or from a pure cultivation, there is seen after twenty-four hours, or even earlier (the temperature being 30° to 37° C.), a faintly opaque line which soon becomes more apparent, and then looks as if it had been drawn with a whitish yellow, or, when older, with an orange yellow oil colour. The growth increases in breadth, forming round masses (up to 3 to 4 mm. in diameter) and becomes of a more and

* In reference to other organisms in pus see a paper by Dr. Passet of Munich, "Ueber Mikro-organismen des eitrigen Zellgewebs-Entzundung des Menschen," *Fortschritte der Medicin*, vol. iii. 1885, Nos. 2 and 3.

In his cultivations from acute abscesses Passet has found, besides staphylococcus pyogenes aureus and albus, six other organisms, of which the most frequent form differed from the two former in producing a sulphur or citron colour, and was, therefore, called *staphylococcus pyogenes citreus*. In addition he found a streptococcus which differed in some respects from Rosenbach's strept. pyogenes. He also obtained a coccus which grew on the surface of the jelly like a drop of white wax, and this he called *staphylococcus cereus albus*, and one of similar character, which, however, produced a yellow colour and was hence named *staphylococcus cereus flavus*. He further found a coccus which in many respects resembled the pneumonia coccus, described by Friedlænder and Frobenius, but which nevertheless could be differentiated from it. Lastly he obtained a bacillus from an abscess near the anus, to which he gave the name *bacillus pyogenes fœtidus*. The relative frequency with which he found these organisms was as follows :—

Staphylococcus pyogenes albus alone	4 times
Streptococcus alone	8 „
Staphyl. cereus albus alone	2 „
Staphyl. cereus flavus alone	1 „
Staphyl. pyog. aureus + albus	11 „
Staphyl. pyog. albus + citreus	2 „
Staphyl. pyog. albus + streptococcus	1 „
Staphyl. pyog. albus + citreus + streptococcus	1 „
Bacillus pyogenes fœtidus	1 „
The pneumonia-like coccus alone	1 „
And with another organism	1 „

As regards the origin of these cocci outside the body Passet found staphyl. pyog. aureus in house water used for rinsing, and staphyl. pyog. albus in raw beef which had lain exposed for some days in a kitchen and was beginning to smell.—Ed.

more deep orange colour; after a time it ceases to extend. It does not spread deeply. It grows more slowly in the cold. When inoculated by plunging an infected needle into the jelly it also grows well and forms an opaque, yellow, in parts irregular, column. In F. P. G. the line of inoculation becomes very quickly fluid, and later the whole mass of jelly liquefies, even before the micrococcus has spread through it. The growth then falls to the bottom and becomes gradually of a dark orange colour. On solidified blood serum it grows rapidly, at first of a faint yellow colour, but later of a darker hue. It also grows well on potatoes. Exposed to the air the cultivation gradually dries up, loses its colour, and becomes more difficult to inoculate, without however, even after a year (in one case), completely dying out. Without air it retains its vitality for a long time. Microscopically it is a very small coccus, quite spherical in shape. In young cultivations the cocci lie very regularly imbedded in a ground substance and present a very pretty picture (fig. 30, Plate VIII.). This is their only mode of arrangement. The very young cocci seem to me to be smaller than the older ones, and in old cultivations different sized forms are present (fig. 31, Plate VIII.). Injections of cultivations of this organism are very deleterious to animals (rabbits, dogs). After the injection of about ·5 grammes of a mixture of the cultivation with water into the knee or pleural cavity, rabbits are commonly found dead next morning. If they are still alive a very severe inflammation developes. When these organisms are injected into the knees of dogs, the animals generally survive, but an abscess quickly forms. On dead putrescible soils this microbe does not cause putrefaction, whether air be present or not. Nor does it produce any gas, or only very slight traces of it. I have cultivated them in large quantities on egg albumen and boiled beef at a temperature of 30° to 35° C. in vacuo, in flasks. The waterhammer test can still be got, even after the lapse of years. Nevertheless, the meat as well as the albumen break up. I have examined such a flask, (in which the egg albumen had become entirely broken up,) for pepton by boiling the fluid, filtering it, boiling with oxide of lead and removing the lead by means of sulphuretted hydrogen. The remaining fluid contained large quantities of pepton."

b. Staphylococcus pyogenes albus.

"This grows on F. P. A. in luxuriant, opaque white masses which look like drops of white oil colour drawn out. The line of growth increases rapidly in breadth, and in one to two weeks may reach 4 mm. After some time the cultivation dries up, becomes smooth, and can with difficulty be reinoculated. F. P. G. rapidly becomes liquid. In flasks exhausted of air I have preserved a pure cultivation for three and a half years, and lately have obtained good cultivations from it. Not uncommonly the cultivations of this microbe are scanty and imperfect, and scarcely spread beyond the place where it was sown. Fresh cultivations, made early in such cases, grow luxuriantly. Microscopically this organism cannot be distinguished from the yellow one. Its pathogenic action is also similar."

From a case of acute suppuration of the knee joint, apparently spontaneous, pure cultivations of this organism were obtained. "A portion of a culture of the fourth generation on F. P. A. about 4 mm. broad and 3 cm. long was thoroughly mixed with 2 ccm. of sterilized water, and 5 ccm. of this mixture were injected into the right knee joints of each of two rabbits. On the following day the knees were drawn up to the side of the body. The whole knee and the inner side of the lower half of the thigh were considerably swollen and hot. On the next day the swelling had extended beyond the groin to the abdominal wall. One animal died seven days after the injection. The right knee was full of thick pus. In the thigh and abdominal wall there was no suppuration. Lungs, kidneys, and other organs normal; a little fluid in the pleural and abdominal cavities. Cultivations from the pus from the knee gave luxuriant growth of staphylococcus albus. The same organisms also grew from the blood of the heart, though only in small numbers. The second animal died ten days after the injection. It had become very emaciated. On examination, nine hours after death, the knee was found to be distended with pus, which had burst through the capsule both upwards and downwards. Upwards the abscess reached as far as the fold of the groin. The pus had everywhere penetrated between the muscles of the thigh. At the lower part the pus had burrowed between the muscles of the calf as far as the middle of the leg. The pus had the consistence of semi-fluid flour (*Griesbrei*) and

the characteristic smell of osteomyelitic pus. Over the abdomen there was a flat collection of pus above the fold of the groin. The lungs were normal, with the exception of diffusely scattered dark points. Pleura, peritoneum, heart, kidneys, and liver were normal; spleen dark but small. On F. P. A. inoculations were made from

"1. The dark coagulated blood from the right ventricle. Result: pure cultivations of the white staphylococcus, which were further cultivated through three generations.

"2. The blood of the left ventricle. Result: staphylococcus albus with impurities.

"3. The purulent infiltration on the abdominal wall.

"4. Pus from the knee. The result in both 3 and 4 was a luxuriant growth of staphylococcus albus.

"The cultivation result (the pure cultivation of the original coccus) in both these experiments shows that the pathological changes were due entirely to this coccus. The cultivation from the pus in the abdominal wall shows that these organisms can pass along the lymphatics, just as we see in man lymphatic abscesses following peripheral suppurations. Finally, the cultivation from the blood shows that the organism can pass into the circulation."

c. *Micrococcus pyogenes tenuis.*

"I have given this name to a microbe which is apparently only seldom found. I have seen it three times; in each case it was unmixed with other forms. At first however it escaped my observation, because its colonies are so delicate as to be hardly visible (hence the name which I have given to it). This micrococcus is essentially different from both the foregoing. In the cultivations, the cocci lie together in very small numbers, and are not arranged in definite groups. I have, it is true, not seen them in the tissue, but I do not think that they can be placed among the staphylococci. On agar there forms around the line of inoculation quite thin deposits, almost as transparent as glass, which look as if one had surrounded the line of inoculation for about the breadth of a millimeter with a very thin layer of transparent varnish. When inoculated by plunging the needle into the jelly, and more especially when the organism grows between the glass and the jelly, it grows more luxuriantly, and forms a somewhat

thicker, slightly opaque layer. Microscopically the individual cocci are irregular in size, perhaps somewhat larger than the aforementioned staphylococci, and they not uncommonly show two dark ends, with less stained material between (fig. 34, Plate VIII.). I have not yet made any experiments on animals with this coccus."

d. Streptococcus pyogenes.

"By the term streptococcus we mean cocci which arrange themselves in characteristic chains or groups, but this term only indicates a genus, for there are several species which have this arrangement. So far as we at present know we have three different kinds of streptococci in connection with traumatic infective diseases. Apart from the form which Koch has shown to be the cause of a progressive necrosis of the tissue in mice, we know two forms in connection with infective diseases of wounds in man. The one is the microbe of erysipelas discovered by Fehleisen, which I would for the present designate by the term streptococcus erysipelatosus (Fehleisen). The other is that to which I am about to refer, which is sufficiently distinguished by the name streptococcus pyogenes. As I have already said, I have been unable to find any characteristic microscopical distinction between these two kinds of cocci (figs. 32 and 33, Plate VIII.); on the other hand, however, as Fehleisen has already pointed out, the cultivations are different and sufficiently characteristic to enable us to distinguish them by parallel experiments. On F. P. G. streptococcus pyogenes forms at first slightly whitish, somewhat translucent round dots of the size of small grains of sand, which only grow sparingly on this medium, even when the temperature is kept as high as can be done without liquefying the gelatine. On F. P. A. this coccus grows much more rapidly when the temperature is from 35° to 37° C. Here also it has the same tendency as in the gelatine to grow in little points, which, however, may increase to the size of a pin's head. If one sows these organisms in a line they grow in a continuous streak, but here also the tendency to form centres appears. As the culture becomes older it grows most in the middle, and assumes here a slight brownish colour; at the periphery it quickly falls to the surrounding level. But the outermost

border is again somewhat thicker, and has often a wavy, dotted appearance, produced by the formation of little heaps of cocci; not uncommonly, also, one sees new points appearing around the border. On further growth a still flatter terrace forms around the first border, and this eventually becomes surrounded by a third, and so on. On the whole the growth of the cultivation is slow, and in two or three weeks reaches a maximum breadth of 2 to 3 mm. The older the culture the more difficult it becomes to reinoculate it. . . . It grows well on solidified blood serum, and in the same manner as on agar agar. It does not cause liquefaction of any of these soils. In airless vessels it quickly breaks up egg albumen and beef without the production of any foul smell, or of any marked amount of gas; it leads to the formation of large quantities of pepton.

"At the first glance the cultivations of this organism seem to be very similar to those of the micrococcus of erysipelas. The latter has often the tendency, though to a much less marked degree, to the formation of flat rings, the borders of which are, however, decidedly thicker and more irregular, and the colonies are whiter and more opaque than in the case of streptococcus pyogenes. On further growth of the streptococcus erysipelatosus there is a marked formation of processes often to such an extent that the culture acquires a dendritic appearance resembling a fern leaf, while that of the streptococcus pyogenes resembles rather the leaf of an acacia. When inoculated by plunging an infected needle into the substance of the jelly the erysipelas organism forms whitish yellow masses; the growth is somewhat more marked than that of streptococcus pyogenes. . . . Microscopically cultivations of the erysipelas organism show similar coils and network arrangement to the pyogenic one, but somewhat better marked. The chains, as well as the individual cocci, seem also to be somewhat larger. . . .

"Rabbits are not seriously affected by the streptococcus pyogenes. Injections of cultivations on agar agar diffused in water caused the formation of abscesses, inoculations of agar cultivations into a small pocket of the skin caused a local inflammatory nodule. Mice are much more sensitive. I have seen two out of six mice die of progressive suppuration on the third and fourth days after introduction of a minute quantity of the culture into a small skin wound. . . .

"I have thus mentioned and described the characteristics of the cocci which I have found in unopened abscesses. The question naturally arises whether these different microbes have different actions; whether one can find any difference in the clinical symptoms of abscesses and suppurations according to the species of microbe which has caused them. Undoubtedly there is a difference, and I have not unfrequently been able to diagnose the micro-organisms from the clinical symptoms."

III.—CLINICAL DETAILS OF THE CASES OF ACUTE ABSCESS EXAMINED WITH THE MICROBES FOUND IN EACH.

Rosenbach had notes of 26 cases, in which the result was as follows:—

a. Staphylococcus aureus (with or without albus)

was found in the following:—

1. Abscess of the scalp following eczema.
2. Præpatellar abscess.
3. Submental abscess of 8 days' duration.
4. Submaxillary abscess (glandular) of 8 days' duration.
5. Deep acute subfascial abscess at lower end of radius.
6. Abscess of submental glands after eruption in mouth.
7. Abscess of mamma of 14 days' duration.
8. Abscess of crural glands, after a graze over the internal malleolus 4 weeks previously.
9. Præpatellar abscess.

Also in two cases of furuncle of the upper lip, one of them having been opened before the commencement of suppuration, after 2 days duration.

b. Streptococcus alone in

1. Subcutaneous phlegmon over the knee after graze of skin 7 days previously.
2. Abscess of axillary glands after graze of the thumb. Thin creamy pus obtained. Of 8 days' duration.
3. Deep-seated abscess of the upper arm after graze of the back of the hand, of 8 days' duration.

4. Large abscess, with much inflammation, at the angle of the jaw. Swelling had lasted for 3 weeks.

5. Abscess of præjugular glands after scarlatina. Several glands inflamed, but only these suppurated.

6. Deep-seated abscess of the shoulder. Painful for 11 days before the swelling was noticed.

7. Abscess, with much inflammation, at the inner side of the knee, of 3 weeks' duration. Very little pus.

8. Small lymphangitic abscess of the hand.

9. Abscess of glands at angle of jaw. Great induration. Of 8 days' duration.

10. Lymphangitic præpatellar phlegmon after graze over knee.

11. Lymphangitic abscess of the arm with marked inflammatory induration. Healing slow.

c. Staphylococcus and Streptococcus together.

1. Lymphangitic abscess at the upper and inner part of the upper arm after frequent scratches and pustules on the hand. Of 8 days' duration.

2. Acute spreading suppuration after excision of the shoulder. Slow recovery. Fever did not subside till 12 days later.

3. Extensive inflammatory induration of neck yielding turbid serous fluid when opened. Streptococci were most numerous.

d. Micrococcus pyogenes tenuis in

Large abscess at upper part of thigh. Symptoms not severe (no elevation of temperature, reddening of skin, &c.), though the abscess formed rapidly.

IV.—PUS FROM CHRONIC ABSCESSES.

Ogston came to the conclusion that the pus of chronic abscesses did not contain micro-organisms. Rosenbach says: "I have already in my work on Osteomyelitis (1878) expressed the opinion, to which I was led by observations in the clinique here, that all the so-called chronic (fungous) inflammations of bones which were not the residua of acute inflammations, even though of but little intensity, were due to tuberculosis; naturally one

must except from this list those caused by syphilis, glanders, actinomycosis, &c. I have lately made a series of observations by injecting the pus from chronic abscesses in connection with fungous inflammations of bones into the knees, pleura, peritoneum, and subcutaneous tissue of animals. The greater number of these animals became affected locally with typical tuberculosis, and this usually ended in general tuberculosis. These experiments have since been repeated by various observers with like results. Ogston also, had he kept his animals longer under observation, would have come to similar conclusions. As the result of the classical clinical observations of Volkmann and König this extremely important fact has been established, a fact which introduces a new era into modern surgery, viz., that the so-called chronic fungous inflammations are etiologically identical with tuberculosis; and Koch has conclusively proved this view by his observations on the tubercle bacillus. Although the microscopical examination of such pus for tubercle bacilli has perhaps oftener given negative than positive results, nevertheless one must conclude, as Koch does, that at least the germs —spores—are present in the pus. The above-mentioned experiments on animals lend great certainty to such a conclusion. I have incidentally made some attempts to cultivate the bacilli from such pus on solidified blood serum. In two out of five cases I obtained a positive result, viz., undoubted cultivations of tubercle bacilli in several tubes. In one case the cultivations were spoilt because the pus was impure, and in the other two cases the result was negative."

V.—ABSCESSES AND SUPPURATIONS WITHOUT THE PRESENCE OF MICRO-ORGANISMS.

1. On removing an echinococcus cyst from the abdominal cavity some thin but decidedly purulent fluid was found behind it. No micro-organisms could be demonstrated in it by cultivation on F. P. G. and F. P. A.

2. A suppurating echinococcus cyst in the abdominal cavity. No micro-organisms could be found either by microscopical examination or by cultivation.

After pointing out that analogous inflammations have been

observed in connection with cysticercus cellulosæ, observations which led Prof. Leber* to the conclusion that the cysticerci excreted an irritating substance, Rosenbach goes on to say: "Although in the two cases mentioned micro-organisms could not be found, I nevertheless hesitate to ascribe the inflammation to the parasites (echinococci) as such, for on the one hand echinococci can exist in the body for a long time without causing suppuration, indeed they may die and become converted into a gelatinous mass without the occurrence of suppuration, and on the other hand, because on opening the abscess microbes were not found, it does not follow that at some time or other they were not present and did not die out later. The following investigations on sepsis will show that there are bacilli which may cause suppuration and then very quickly die. In some cases of suppurating hydatid cysts bacilli have in fact been observed, while in other cases the presence of a foul smell shows that microbes, probably belonging to the class of bacilli, have been at work. Undoubted cases of pus formation without micro-organisms are only known to me as the result of experiments on animals." He then goes on to refer to cases of suppuration resulting from the injection of turpentine oil and metallic mercury.

VI.—CULTIVATIONS FROM THE PUS FROM EMPYEMATA.

1. Child. 6 years old. Taken ill 14 days previously. Large empyema on the right side; healed in 5 weeks after it was opened. *Gave pure cultivations of staphylococcus pyogenes aureus.*

2. Empyema after pistol shot wound of lung in boy aged 15. Antiseptic dressing applied. Five days later chest opened and a litre of stinking fluid blood evacuated containing a microbe not previously known to Rosenbach. Eight days after the injury the discharge was thin and without smell, and 5 days later it was purulent and *contained staphylococcus pyogenes albus and aureus, and also another yellow coccus.*

3. Child. 3 years old. Ill for 2 weeks. Large quantity of pus. Rapid healing. *Pure cultivation of micrococcus pyogenes tenuis.*

* "Ueber die Wirkung von Fremdkörpern im Innern des Auges," International Medical Congress, London, 1881.

4. Man. Aged 68. Became ill with bronchitis 1¼ months previously. Then followed symptoms of pleuritic effusion, but no fever. *Pure cultivation of micrococcus pyogenes tenuis.* Died of bronchitis and cardiac disease.

5. Boy. Aged 17. After being punctured several times, pus only being got on the last occasion, the chest was opened 17 days after the commencement of the illness. *Pure cultivation of streptococcus pyogenes.* It is worthy of note that in this case each seat of puncture inflamed and became like a furuncle. As antiseptic precautions were employed the infection here must have come from the fluid evacuated. Three weeks later rheumatism of the joints and endocarditis. The wound was more than a year in healing.

VII.—SEVERE SUPPURATIONS AND PHLEGMONS.

1. Perinephritic abscess. Ill for 3 weeks. A large quantity of pus and necrotic tissue evacuated. Healed well. *Pure cultivation of staphylococcus pyogenes aureus.*

2. Large abscess in abdomen, possibly in connection with carcinoma of the intestine. *Both forms of staphylococci.*

3. Spontaneous suppuration of knee joint of 3 weeks' standing. Symptoms severe but healing rapid after antiseptic incision. *Pure cultivation of staphylococcus albus.*

4. Suppuration of the sheaths of the tendons in the forearm following a small suppuration over the last joint of the right thumb. Great redness, pain and fever. Comparatively little pus found. In spite of the incision the process spread as far as the elbow, making further incisions necessary. The tendons did not become necrosed. *Pure cultivation of streptococcus pyogenes.*

5. Erysipelatoid phlegmon of the forearm. No history of prick, &c.; diagnosed as erysipelas; fluctuation noticed 6 days after the commencement of the affection. This collection was opened, but only a few drops of turbid fluid were evacuated. *Pure cultivations of streptococcus pyogenes* were obtained. Portions of the subcutaneous tissue necrosed, but the symptoms subsided on opening the abscess.

6. Phlegmon of the forearm and sheaths of the tendons. For some time a small suppurating wound had existed over the

thumb. Symptoms severe, and erysipelatoid. An incision was made seven days after the commencement of the affection, and a little turbid, serous fluid, with small whitish flakes, was evacuated. *Pure cultivation of streptococcus pyogenes.* Cultivations made from the blood remained sterile.

7. Penetrating wound of the knee in a child. Suppuration had been going on for some time before the child was seen, and the tissues around were much inflamed. *Cultivations gave both streptococcus and staphylococcus.*

8. Suppuration after comminuted fracture of the patella and suture of the bone antiseptically. *Both staphylococcus and streptococcus.*

VIII.—DIFFERENCES IN THE CLINICAL COURSE OF THE INFLAMMATIONS AND SUPPURATIONS CAUSED BY THE DIFFERENT MICROBES.

"The frequency of occurrence of the different organisms in the foregoing cases was as follows:—

Staphylococcus alone	16 times.
Streptococcus alone	15 times.
Staphylococcus and streptococcus together.	5 times.
Micrococcus pyogenes tenuis	3 times.

"In the case of the simple abscesses there is no constant or striking difference. This is not to be wondered at, for abscesses are for the most part only seen when they are already fully formed. Then, however, the invasion—the true disease—is past. If, on the other hand, we look at the phlegmons, we notice differences which correspond closely with Ogston's observations. The three cases of phlegmon, which were caused by streptococcus alone, are marked out by a, so to speak, erysipelatoid character. This was best seen in Case 5. At first erysipelas was diagnosed, but six days later it was necessary to make an incision, and then the inflammation subsided without marked suppuration. Case 4 also showed erysipelatoid swelling with marked constitutional symptoms, but comparatively little formation of pus or necrosis of the tissue. Case 6 had the same characters. Redness and slight swelling, which I at first diagnosed as lymphangitis, existed for seven days, and then an incision was made and only a few drops of turbid fluid evacuated. Also among the empyemata that caused by streptococcus is peculiar; here also

there was the same slowness in the formation of pus. At first only serum with a few whitish flakes was evacuated, the same again after four days, and only on the eleventh day was true pus present. The slow subsidence of the fever, the slow improvement in the lung infiltration, and the length of time required for healing, are very peculiar. I might also refer to the small inflammations which occurred along the needle tracks, showing the infectivity of the material. Naturally these observations must be further extended, more especially as there is a difficulty here in regard to the observations on animals. Should these differences be established by further observations, we must ascribe to streptococcus pyogenes special characteristics which would correspond to Ogston's observations on the different modes of invasion of streptococcus and staphylococcus. The most important of these characteristics is the mode in which sterptococcus can penetrate into the living tissue, grow through it, and live in it for a considerable time before suppuration occurs and the tissue breaks down. This property of penetrating into the living tissue, without destroying it or causing suppuration, is most marked in the case of the erysipelas organism. It can spread for a long time through extensive tracts of the tissue, and live in it without leading to suppuration or to necrosis (unless where it causes the latter mechanically). I believe that this similarity in their action shows a relationship between these two kinds of streptococci. However, there is nothing more than a similarity, either in their infective action or in their morphology. As the result of Fehleisen's beautiful observations, one cannot for a moment suppose that the cocci of pus can cause erysipelas, or, on the contrary, that the erysipelas cocci can cause suppuration, or, at least, not without great difficulty. But I have made observations which lead me to suspect that the streptococcus pyogenes very readily associates itself with erysipelas, and penetrates into the body, and so may cause suppuration beneath skin affected with erysipelas, or indeed, in some cases, give rise to constitutional symptoms—even metastases and pyæmia. Also in those cases where erysipelas spreads over a joint, *e.g.*, the knee joint, and a suppurative gonitis results, I do not believe that this is due to the erysipelas organism, but rather to the streptococcus pyogenes. The facts narrated with regard to the staphylococci are less

characteristic. These organisms may also occasion violent and spreading inflammations, which may possibly show an acute character, but at any rate much more easily and quickly tend to the formation of pus. The staphylococci appear to have considerable power of penetrating into the living tissue, but much less than the streptococci of growing through and living in it without causing its destruction.

"Micrococcus pyogenes tenuis seems to me to have only a local pus-forming character, while it excites but little fever, and that at the commencement, and hardly at all phlegmon. We see it in Case *d* (III.) occurring alone in an enormous abscess in a little child. The course must be described as very harmless. It also occurred alone in two empyemata, and both of these were without fever, at least in their further course. One healed rapidly; in the other, the fatal termination was due only to the complications."

IX.—Here follows a paragraph on acute osteomyelitis, which has of late been ascribed by Loeffler, Becker,* and others,† to a specific micrococcus producing a yellow colour, this organism being supposed to be confined to acute osteomyelitis, and to be different from those found in other suppurations. As the result of his elaborate observations, however, Rosenbach comes to the conclusion that this organism is none other than the staphylococcus pyogenes aureus previously described, and is not confined to acute osteomyelitis. He has examined 15 cases of osteomyelitis, and found staphylococcus pyogenes aureus present alone in 12 cases, in 1 mixed with staphylococcus pyogenes albus, and in 1 with streptococcus pyogenes. In the 15th case, which was also quite typical, he found staphylococcus pyogenes albus alone. Rosenbach has compared the behaviour of the coccus of acute osteomyelitis obtained from the Imperial Sanitary Institute in Berlin (Loeffler) with the staphylococcus pyogenes aureus obtained from the 2 cases of furuncle before

* *Deutsche Med. Wochenschrift*, No. 46, Nov. 14, 1883.

† "Uber einen bei der acuten infectiösen Osteomyelitis des Menschen vorkommenden Mikrokokkus," by Feodor Krause, *Fortschritte der Medicin*, Nos. 7 and 8, 1884. Krause found this orange micrococcus in a number of cases of osteomyelitis, and he also found it in three cases of carbuncle of the neck. He also examined twelve acute abscesses, and found streptococcus pyogenes in all of them. In all the cases but one it alone was present; in the exceptional case it was mixed with staphylococcus pyogenes albus.—ED.

mentioned, and has found that they are identical, both in their modes of growth and in their effects on animals. Loeffler found, in his experiments with the organism obtained from acute osteomyelitis, that when injections of it were made into the veins after previous fracture or bruising of the bone, acute osteomyelitis occurred at the seat of injury, if the animals lived long enough. Rosenbach has obtained precisely the same effect by using staphylococcus pyogenes aureus, cultivated from the furuncles of the lip.

X.—SEPSIS.

"It must have struck every one who has followed the literature of the last few years on this subject that at present morbid processes are included under the name 'sepsis' which, etiologically as well as clinically, differ greatly from each other. Apart from the fact that many employ the term 'septic' for any foul condition of a wound, even though very slight, clinicians still apply the terms 'septicæmia,' 'septhæmia,' 'sepsis,' &c., to certain severe constitutional affections which for the most part occur in connection with putrid wounds. In Gussenbauer's last work on Septhæmia, Pyohæmia, and Pyoseptthæmia, septicæmia is defined as 'that general affection of the body which arises from the absorption of products of putrefaction into the circulation, and is characterized by a definite alteration of the blood, a typical series of inflammatory processes and a continued fever with peculiar nervous phenomena and critical secretions.'

"In order to give a complete picture of these diseases, and of the clinical conception of them, a very much fuller description would be necessary. Here, where we are only concerned with observations on the occurrence of micro-organisms and on their effects in cases of sepsis in man, I must premise a knowledge of the full meaning of the term, and confine myself to mentioning those points which come into consideration in the following pages, and which bear on the present teaching on the etiology of sepsis. Formerly the etiology of sepsis was very simple. Even at a time when the idea of a *contagium animatum* as the cause of infective diseases was fairly widely spread, the symptoms, which were observed after the absorption of putrid materials into the blood, appeared so much a matter of course that one did not

inquire further as to the nature of the disease. The introduction, experimentally, of putrefying materials into the tissues of animals furnished a direct proof of the connection between the two by causing in the animals affections similar to those seen in man, and which were also called septhæmia, sepsis, and putrid intoxication. But very soon there were added to these experiments the very important results obtained by Panum and others, which very greatly complicated the existing views. Fermentation and putrefaction had been ascertained to be processes set up and carried on as the result of the vital action of lowly organisms, and as a consequence similar organisms were held to be at work in sepsis in the living tissues; but the researches to which I have just alluded showed that strictly analogous septic affections could be experimentally induced in animals by the injection of boiled putrid fluids—that is to say, without the action of living organisms. Our knowledge of these active inanimate septic materials (the sepsins, ptomaines, &c.) has made marked progress at the present time. Several different materials of this kind have been obtained, partly from putrefying materials, and partly from the bodies of patients which have died of septic affections, although we are just commencing to gain a more minute knowledge of these bodies, of their chemical constitution, their toxic effects, their connection with definite microbes, &c. On the other hand, Koch and his pupils have experimentally become acquainted with forms of sepsis which are of quite a different nature. They consist in infection by specific microbes, which, when inoculated even in the minutest quantities, rapidly multiply and invade the whole body. These are typical diseases which end fatally, with definite symptoms recurring in every case. When Koch applied the term sepsis to these diseases he did so quite correctly, for the original infective material was a putrid one (putrid blood, &c.), and, in contradistinction to pyæmic affections, it acted on the tissues without causing suppuration. Hence it is at once apparent that if we, with Gussenbauer, refer the etiology of sepsis to the absorption of products of putrefaction, it is possible that there may be different kinds of noxious agents. It is, for example, quite conceivable that among the multitudes of micro-organisms present in a putrid wound, just as in the foul water which Gaffky injected into animals, there may be present one or several which are capable of multiplying in the living human

tissues, and thus causing a general septic affection. It is, however, just as conceivable that in man this does not occur in most cases, but rather that the constitutional symptoms are due to the absorption of inanimate poisons, ferments, ptomaines, &c., or both these forms of sepsis might be combined. And further, just as in the case of the different kinds of microbes, so in the case of the ptomaines, it may be that in one case this kind, in another case that kind, in a third case some combination of them, may be the active noxious agents. In short, the question of the cause of human sepsis is by no means such a simple one as it appeared to our forefathers.

"Although in general one may accept the occurrence of cases of pure sapræmia, *i.e.*, pure ptomaine poisoning, it does not follow that this is a frequent occurrence. Whether there are cases in men which correspond to Koch's mouse septicæmia, or Gaffky's sepsis, is as yet an unanswered question. Pasteur has discovered a *vibrion septique* which, as his very interesting cultivation experiments show, grows only in the absence of air—a bacillus which has been held by Koch and Gaffky to be identical with that of malignant œdema. For the latter reason, and also because in Pasteur's sepsis we have only to do with experiments on animals, these observations cannot be reckoned of great importance. On the other hand, the investigations of Doléris on septic puerperal affections in the human being are of great interest. He investigated two cases of true, intensely acute (*foudroyant*) sepsis, without other notable pathological appearances. His results would lead one to expect a sepsis caused by diablastic bacilli. He says, however, that in this acute sepsis no organisms can be found in the blood till just before death, often only after death. Nor did he observe one form alone, but speaks of 'èléments allongés, minces, cylindriques, rémuants,' which swarmed in the tissue and in the lymph spaces in the uterus and in the peritoneum. Doléris also found similar organisms in putrid infarcts, and believes that one form can change into the other. He also considers the question under what circumstances the blood can become a suitable soil for these organisms. These circumstances are, firstly, absence of oxygen from the blood (*anoxémia*), because this gas is noxious to the *vibrion septique*; and, secondly, the presence of a mass of organisms outside the blood continually supplying it. The first condition can only be

fulfilled by the presence of other organisms (for example, the pyogenic cocci), which use up the oxygen, or during the death struggle, or after death itself. Hence it is evident that Doléris has not found a septic bacillus which plays even in a slight degree a rôle such as that played by the bacilli of mouse septicæmia, &c. If his bacilli cannot penetrate into the body during life, if the pyogenic cocci must prepare the way for them, if the presence of a mass outside the blood constantly supplying fresh bacilli is necessary, then, as far as regards the action of these bacilli, his cases can hardly be reckoned as other than cases of ptomaine poisoning. Naturally it is quite wrong at once to identify with the *vibrion septique* the various bacilli found in septic putrid deposits in man. At the present time I am of opinion that the etiology of the symptoms which clinicians group together as sepsis is not a simple one, nor is it always the same, and it is certainly not to be found in the existence of one definite pathogenic bacillus. The following investigations also will show that the symptoms which are usually as yet designated clinically as sepsis may be produced by the pyogenic cocci, where they penetrate rapidly and in large numbers without the production of metastases, by certain bacilli which spread progressively, as those of malignant œdema or of spreading gangrenous emphysema, also by organisms which do not spread into the living tissue, or at least not much further than the surface, but attack the body by the formation of ptomaines, peptonizing ferments, fibrin ferment, &c. It may perhaps be possible for a future generation to analyse the etiology of such cases when the knowledge of the symptoms peculiar to the organisms in question, to the ptomaines and ferments, are fully known. As yet we can scarcely venture to diagnose in any particular case even the two extremes—pure intoxication or pure infection. On the other hand, we now know a large number of pathogenic effects, caused by micro-organisms, which differ fundamentally from each other, and which we must bear in mind in our further discussions on the etiology of septic diseases. At first, in the case of the specific infective diseases, and of those belonging more especially to the department of medicine, such differences in the pathogenic results were explained by the view, now firmly rooted, that a distinct microbe corresponded to each one of these typical diseases. But in the non-typical wound diseases, to which the greater part of those in

question here belong, one can distinguish from one another certain general pathological effects due to microbes, and these effects may be caused by each of a number of micro-organisms, while conversely one and the same microbe may produce several of them. Thus, for example, I know six different microbes which can cause suppuration in man. On the other hand, there are organisms which have two different properties, *e.g.*, the micrococcus of erysipelas. Besides its property of spreading locally in the living tissue, and thus producing locally intense inflammation without suppuration, it has also the property of causing a severe constitutional affection, fever, &c. A third example will show that these two properties do not necessarily go together; the coccus of finger erysipeloid spreads in exactly the same manner, and causes local inflammation, also without suppuration, but it has no effect on the general condition of the patient.

"For the sake of the clearness and brevity of the following observations it is necessary to have short expressions for these different pathogenic effects, and hence I will tabulate them, and, where possible, designate them shortly, without, however, intending these designations for general use.

"1. A micro-organism can exercise a pathogenic action in that the poison produced by it may be absorbed when the microbe grows in contact with an absorbing surface of the body. It may not possess any other pathogenic property, such as that of penetrating into the tissue, of causing inflammation or suppuration, &c. It may, however, possess such properties. The poisons formed (sepsin, ptomaine, septic alcaloids) may be of very different kinds. I believe that in some cases, though extremely seldom, quite simple chemical substances, such as ammonia or sulphuretted hydrogen, may act as ptomaines. In other cases it is an organic poison, such as Bergmann's sepsin, Sonnenschein and Zuelzer's narcotic alcaloid, or the paralysing diphtheritic poison, &c. In other cases, again, it consists of less known ferments, which act like pepsin or like the fibrin ferment. Possibly still less known organic poisons come into play; at least earlier writers have likened the septic poison to the snake poison. All these dead, pathogenic materials I group together under the name ptomaine.

"2. As regards extension in the body a micro-organism may have the property of penetrating into the tissues of the living

body—invading property. We shall become acquainted with such microbes which penetrate into the surface of the living tissue and then die.

"3. Others can continue to live in the tissues for a shorter or longer time, even for the life of the host, as in syphilis, tuberculosis, &c.—parasitic property.

"4. They may spread slowly or quickly in the living tissues.

"5. They may spread slowly or quickly in the tissue, or even over the whole body (diablastic property), as in bacillary or bacteric septicæmia, anthrax in mice, acute glanders, &c.

"6. Locally the microbe may cause a simple inflammatory affection as the result of its spread, as, for example, in erysipelas; hyperæmia, migration and exudation with formation of vesicles, sometimes even with fibrinous contents. (This is by no means a necessary accompaniment of the growth of bacteria; in the bacillary sepsis, for example, the tissue remains almost without inflammation.)

"7. A frequent property is that of causing suppuration, a property also common to certain acrid poisons (turpentine oil) —pyogenic property.

"8. It may happen that as the result of the specific action of the microbe on the tissue the latter may die—gangrenous action, as, for example, in Koch's progressive gangrene in mice. Naturally I do not include here those cases where, owing to unfavourable conditions, a piece of thin cutis dies as the result of an erysipelas or a phlegmon.

"In connection with these statements and with what follows I should like to recall some well-known facts with regard to other conditions which affect the course of a bacterial disease. In the first place I would mention the attenuation or increase in virulence of bacteria, a fact definitely made out by Pasteur and Koch in the case of anthrax. Further, there are conditions which, apart from the properties of the micro-organism, concern, for example, only their number—cumulative action. In the second place, the individual predisposition of the infected organism plays one of the most important rôles. This has been definitely made out in certain internal diseases, such as measles, scarlatina, &c. And I believe that these conditions are also very important in regard to the non-typical infective diseases to which I am now referring."

A. PUTRID INTOXICATION, SAPRÆMIA.

After referring to Dr. Matthews Duncan's definition of this term,* and to König's views on the subject,† Rosenbach points out that no one has worked out this subject fully, and that from his own results he has come to the conclusion that the idea of a single putrefactive organism is erroneous, but that on the contrary there are several, indeed there may be many. It was only after many observations and experiments that Rosenbach found an organism which he thinks is the common producer of putrefaction. He had made a cultivation of blood on solidified serum in a hospital ward. Three days later an offensive smell in his laboratory called his attention to this tube, in which he found a pure cultivation of a bacillus which he designates as *bacillus saprogenes No.* 1 (fig. 36, Plate VIII.). He thinks that this organism is the most common cause of putrefaction. Grown on egg albumen in air it produces an intensely disgusting putrid smell. It is worthy of note that if the cultivation was carried on till the putrefaction of the albumen was well advanced the microbe could not be recultivated. Without air its action is very slight, showing that it is an aërobic organism. Portions of cultivations on agar agar were injected into one knee of each of two rabbits. One of these animals was affected at the time with a suppurating wound of the lower lip and abscess in the submental glands. It died four days after the injection, but the knee was found quite normal without any trace of putrefaction, suppuration or infiltration of the tissue. In a second experiment an injection was made into one knee in one rabbit, and into one knee and the right pleura in another. There was slight swelling of the knees, which rapidly passed off; no constitutional symptoms. The second animal was killed by a cat ten days after the injection, but nothing abnormal was found in knee or pleura. Hence this bacillus and its products were almost entirely harmless to rabbits. A quantity (9 ccm.) of a cultivation in vacuo was injected subcutaneously into a dog. On the following day there was a little elevation of temperature which then passed off, but almost the same effect was produced by a subsequent injection of distilled water in the same animal.

* M. Duncan, "Puerperal Fever," *Lancet*, 1880, vol. ii. p. 681.
† *Lehrbuch der Allgemeinen Chirurgie*, Berlin, 1883, p. 133.

Bacillus saprogenes No. 2 was a second putrefactive organism which Rosenbach obtained from foul-smelling feet. (See fig. 37, Plate VIII.) The foul smell it produced was not quite so intense as in the case of No. 1, but it was equally disgusting. Without air its putrefactive action on albumen was more marked than in the case of No. 1. Nevertheless it did not at all equal the result obtained when air was freely admitted. Nor was it so harmless to rabbits; it had decided invasive and pyogenic properties.

Rosenbach has also investigated the cause of the putridity in carious teeth, and is inclined to refer it to small micrococci, which will not grow in air, but are anaërobic (fig. 37, Plate VIII.).

He then gives two cases of apparent sapræmia in man, in connection with which he found another bacillus, which he terms *bacillus saprogenes No.* 3.

"1. J. Ebeling, 61 years old, was severely injured by a stone falling on him four days before his admission into the ward, and he was brought in with only a splint on. The injury was seventy-two hours old before antiseptic treatment was begun. There was a compound fracture of the upper part of the left tibia. The finger passed forwards and inwards through a lacerated wound to the head of the tibia, which was completely broken up, and hence through the fragments into the knee joint. There was also a simple fracture of the thigh: thigh and leg were much swollen as far as the groin. The seat of fracture was exposed by a free incision, the various fragments, which were already discoloured, were removed, the little bits of bone washed out, upper end of tibia trimmed with bone forceps, free drainage arranged for behind, and the whole very carefully washed out with carbolic acid and sublimate solution, and then powdered with iodoform. An incision was then made over the fracture of the femur, to make sure that it did not communicate with the knee joint. In the evening there was fever. On the following day, 18th April, the dressing was changed; no putrid smell. Two days later, 20th April, the wound stank; high fever. On the 21st the thigh was amputated at its upper third. 23rd April, general condition much improved: the temperature had fallen. 24th April, temperature elevated again, patient quite delirious; stump putrid, was disinfected. 25th April, high fever, patient delirious; a specimen of blood, taken with various precautions, was sown on agar agar. Only in one of the tubes, and that not at the seat of inoculation, there grew a large bacillus . . . which I look on as an accidental impurity. 26th April, patient constantly delirious, shouts out when touched, high fever, &c. The stump does not smell, but pulpy necrotic portions of tissue are now separating. 30th April, died in a state of high delirium. On post-mortem examination the wound was full of thick yellow pus mixed with oil globules; heart fatty, flabby, brownish red; on cutting through an old adhesion of the lung a considerable quantity of clear reddish fluid was evacuated. In the chief artery of the left lung there was a large embolus, which extended towards the lower lobe. Microscopically the heart showed brown pigment in the nuclei, and

fatty degeneration of the muscular fibres. In the lungs many capillaries were blocked with fat; kidneys large and soft; in the convoluted tubercles fat globules were found."

Immediately after the amputation on April 21st, Rosenbach made cultivations from the seat of injury. A number of organisms grew, but chief among them was a bacillus previously unknown to him, which he terms bacillus sap. No. 3. (See fig. 39, Plate VIII.) It rapidly breaks up egg albumen, and produces a disagreeable foul smell. Where air is absent it acts like bacillus sap. No. 2. Injected into rabbits it causes inflammation and slight suppuration, and may even cause death. Probably the same bacillus was found in a second case, the nature of which was not very clear during life.

"H. Binnewis, 59 years old, a labourer, not very intelligent, so that but little weight can be attached to the history, asserted that for six weeks he had had an ulcer on the leg. Thirty-eight years ago he had broken the leg, and since that time it had often ulcerated. For fourteen days the pain had been considerable. *Present condition*—On the left leg, which is almost entirely swollen, are several old pigmented scars adherent to the thickened and irregular bone, and besides that there are two large ulcers, at the base of which the tibia lies exposed but not necrosed. I concluded that these ulcers were scars adherent to the bone, which had broken down. Close above the ankle, on the outer side, an abscess has burst spontaneously, and discharged a little intensely stinking pus. There is a second abscess over the inner ankle. No albuminuria.

"The abscesses were freely opened, and two quite superficial exfoliations were removed. However, the same foul smell of the pus continued. The fever increased; evidently the ankle suppurated; the patient would not agree to amputation at that time. Amputation was however performed through the upper third of the leg two and a half weeks after his admission, the fever having continuously increased, and delirium having supervened. The cut surface of the tibia was healthy, but pus welled out from the centre of the fibula. This was scraped out and disinfected, nevertheless death occurred on the following day. On examination of the portion amputated, putrid suppuration in the medulla of both fibula and tibia was found. The interior of both bones was filled with a greenish-white, foully-smelling pus. The shell of bone surrounding this pus was not necrotic; but at the lower articular surface of the tibia there was a small necrosis which had caused the suppuration of the joint. On post-mortem examination, there was found a greyish-yellow embolus in the branches of the right pulmonary artery, firmly adherent to the wall; but there was no coagulation, either in the heart or in the veins of the affected limb. There was also blenorrhœa of both bronchi, catarrhal broncho-pneumonia in the left lower lobe, dilatation and hypertrophy of the right ventricle."

When the abscess was opened, the stinking pus was sown on agar agar, and a bacillus grew (fig. 38, Plate VIII.) which Rosenbach considers to be identical with the one found in the

former case (Ebeling). A few colonies of staphylococcus pyogenes aureus also appeared.

"I have thus obtained pure cultivations of three putrefactive organisms (of a fourth also imperfectly) which came from different sources, and had different properties. While one of them was very innocent as regards the living tissues, the other two had invasive and pyogenic, and probably also toxic properties; but they were not at all parasitic, much less diablastic. Before I pass on to discuss the role which the latter bacilli could probably play in the production of septic disease, I must mention another observation which I made in the first case (Ebeling), viz., that no development occurred in agar agar inoculated from the blood, although it was taken at the height of the disease, not long before death. I may add yet another analogous observation:—

"W. Mook, 51 years old, a day labourer, had suffered for four days from irreducible femoral hernia, which had previously been kept up by a truss, with symptoms of pain, vomiting, &c. For the last two days there had been no evacuation. January 22, 1884—Hernia very tender, skin over it red. Herniotomy: dark, distinctly turbid fluid in the sac. The loop of intestine was of doubtful appearance, dark red, slate-coloured on the convexity, with some yellow points: site of strangulation healthy. After dividing the stricture and washing the gut with sublimate it was returned, and a radical operation performed. January 24—Abdomen distended; vomiting. Temperature high, almost 40° C. January 25—Dressing changed; wound looking well. Abdomen very distended, painful; high temperature; vomiting has ceased. January 30—For the last two days patient gradually sinking; temperature always about 40° C. Since yesterday metastatic parotitis. Diagnosis: acute septic peritonitis. No more vomiting. Patient died at mid-day."

Rosenbach intended to make cultivations from the blood during life, but arrived ten minutes too late. He, however, took blood from a vein in the arm, and also some of the purulent infiltration from the parotid, and inoculated therewith agar agar jelly. Nothing grew from the blood; but from the parotid he got large numbers of colonies of staphylococcus pyogenes aureus.

"On post-mortem examination: intestines very much distended and very red where the loops touch one another. No other sign of peritonitis. The piece of intestine which had been strangulated is so adherent to the surrounding tissues that its lumen is narrowed; nevertheless there are now fæces below it. The coil is of a bluish red slaty colour; as it is adherent, it is not dead. The mucous membrane shows deep ulcerations, and the yellow points mentioned before correspond to the deepest parts of these ulcers. There is also marked ulceration in the dilated intestine above." No other morbid appearance.

"If we may conclude from the fact that in these two cases of sepsis microbes did not grow from the blood, that there was not in them a general spread through the body of a definite microbe, as, for example, is the case in mouse septicæmia, &c., then in these instances the main factor must have been ptomaine poisoning from a local source. In the case of Ebeling we find an enormous comminution of the bone and of the soft parts, including also the knee joint, without any antiseptic treatment in the first instance; the soft parts and bone soon became foul, and the stump also became putrid and suppurated. There was also embolism of the pulmonary arteries and œdema of the lungs. This state of matters agrees very well with the assumption that either the invading and poisonous bacillus No. 3, or the staphylococcus pyogenes aureus or both together, attacked the tissue which was bruised and infiltrated with blood, and from thence caused a general poisonous action, partly by ptomaine poisoning, and partly also by the formation of blood ferment, as occurs in cases of burn or cauterization of extensive portions of the living tissues. Also in Mook's case we must suppose similar conditions. Here the appearance of the staphylococcus pyogenes aureus alone in the metastatic deposits is of interest. Ogston supposes that staphylococcus produces acrid chemical substances. We see animals die with symptoms of rapid sepsis, after injection of this organism into its blood or serous cavities. The staphylococcus pyogenes aureus was present in all three cases.

"Nevertheless, the above conclusions are not at present warranted from the fact alone that the blood cultivations remained sterile. We know how difficult it is to cultivate the specific pathogenic organisms, how different staining and culture methods were necessary to demonstrate and cultivate the tubercle bacillus. We know how many microbes whose existence we must assume, *e.g.*, in scarlatina, measles, syphilis,* &c., have as yet escaped our observation. And so also it may be that in the future the microbe of human sepsis may be discovered as the cause of such cases as the two before narrated. But such an assumption is not probable when we see how, in other cases, the general septic disease is evidently due to invasion of the known pyogenic cocci,

* As regards the organism of syphilis, Lustgarten seems to have discovered a bacillus which is very probably the cause of the disease. See the *Lancet*, March, 1885.—Ed.

or of easily demonstrable bacilli. Such cases are the four which follow—two of progressive grangrene, and two of progressive gangrenous emphysema :—

b. Spreading Gangrene.

"K. Sandross, a shoemaker, aged 54, injured the index finger of his right hand four days before admission. On the following day the finger and hand were swollen, there was great pain and shivering. *Present condition*—Back and palm of hand show marked inflammatory swelling; index finger shapeless, blue. An abscess was opened in the palm of the hand and drained, and several incisions were made over the index finger to relieve tension. On the following day more fever; forearm much swollen and livid; punctures and large incisions made; limb elevated, and wrapped in wet carbolic jute. During the following days there was high fever, the forefinger became gangrenous, the forearm doubtful; the inflammation spread slowly. By the fifth day after his admission the forearm had become gangrenous as far as its middle; high fever; for some days there had been circumscribed reddened portions of skin on both legs. On the following day these portions, which on the right side had spread as far as the thigh, were in parts blue and covered with bluish vesicles; high fever; delirium. Died on the evening of the sixth day after his admission. On post-mortem examination the right forearm was swollen and of a dirty greenish colour, the epidermis being raised in vesicles. On making an incision into it a foul ichor was seen, which soaked through the whole muscular tissue. The right leg was in a similar condition as far as the upper part of the thigh. Lungs full of blood. Bronchi contain œdematous fluid. Spleen moderately swollen; kidneys pale; liver soft; mucous membrane of the intestine swollen, reddened, in parts with hæmorrhages into it and covered with mucus. In the transverse colon a cylindrical epithelioma about the size of half a crown; parenchymatous inflammation of the heart, liver, and kidneys.

"Twelve hours before death I incised places on the right and left limbs where there were no vesicles, after very careful disinfection of the parts, and inoculated the turbid reddish fluid which escaped on P. F. G. Everywhere there grew a pure cultivation of streptococcus pyogenes. In this case the streptococcus was only cultivated on F. P. G. If any one has doubt whether it is sufficiently certain that the streptococcus was streptococcus pyogenes, I would refer him to the following case, which is quite analogous, and in which the identity was fully established by parallel cultivations :—"

It will be sufficient to give a short abstract of this case. The patient, aged 40, had slightly torn his finger eight or ten days before admission: this became covered with a scab, beneath which was pus. Three days later a red spot appeared on the back of his forearm, which was painful, and quickly increased. Patient had shivering and felt ill; the whole of the lower part of the forearm became affected with a tense inflammatory swelling without suppuration. This

became gangrenous, and was incised. The phlegmon spread to the upper arm, and patient got worse. A sort of pustular eruption appeared on the side of the chest, and also on the inner side of both thighs. The gangrene spread to the axilla, and patient died six days after admission. Post-mortem appearances similar to the other case.

Cultivations were made from the fluid which flowed from the first incisions, and gave pure cultivation of streptococcus pyogenes, as was ascertained by comparisons in mode of growth and effect on animals with the streptococcus pyogenes obtained from abscesses. Sections of the tissue were also made, and the cocci were found diffusely infiltrating the tissue as Ogston has described.

"We have here two cases of progressive gangrenous inflammation, the first with metastases, of similar character, and both with severe septic constitutional symptoms. Although at first I was but little inclined to accept Ogston's view, that septicæmia was only caused by micrococcus poisoning, because I believed that in such a special case as this specific organisms must be at work, yet these investigations have altered my opinion in favour of Ogston's. He has observed similar cases. He describes the disease under the name of 'erysipelatoid wound gangrene,' and has likewise found that all these cases were caused by streptococcus (although he has only shown this microscopically, and not by means of cultivations). Unfortunately Ogston classes this disease with erysipelas, and calls it the most intense and dangerous form of erysipelas. As this streptococcus is so frequently found in harmless abscesses, many might doubt the accuracy of the results of cultivation. Is it possible that this organism, because it can grow readily, has accidentally developed, while the real nosogenic microbe will perhaps not grow at all on the soil employed? But the result of the microscopical examination of the tissue, as seen in Ogston's and my cases, speaks too strongly against this idea. Where one finds, scattered in the tissue attacked but still alive, coccus lying beside coccus, and chain beside chain, in the manner described above, while no other microbe, as far at any rate as the microscope shows, can be detected, one must lay the blame on the streptococcus. If it be asked whether other pyogenic cocci can cause progressive gangrene, I am not able to reply, as I have only had the opportunity of investigating these two cases of this rare disease. However, Ogston describes, under the name 'sloughing inflammation or inflammatory mortification,' a similar extensive invasion by

staphylococci, which can lead to suppuration, and also to gangrene of fingers and portions of the skin, and even to death by infection of the system. Ogston also obtained similar gangrene of the skin by injection of staphylococcus into animals . . . ."

c. Spreading Emphysematous Gangrene.

"Franz Fust, mason, aged 21. A falling tree struck the leg of this strong, healthy man to-day, fracturing it at the junction of the upper and middle third, the tibia obliquely, the fibula in the middle. At a corresponding point there is perforation of the skin in the calf, while in front it is intact. Patient was admitted six hours after the injury with a markedly swollen extremity, which at the side above the joint crepitated, either from blood or air. An incision was made in front of the bones. Drainage and washing with carbolic acid as thoroughly as possible. On the following day, May 20, 1881, the swelling had not diminished; the patient was peculiarly quiet. Wound washed out with carbolic acid. May 21, 1881—Marked putrefaction in the wound, with slight swelling; washed out with 10 per cent. chloride of zinc solution. May 22, 1881—On account of more advanced putrefaction amputation through the lower third of the femur. The muscular tissue of the leg (several of the deeper strata are normal) has become converted into a peculiar, reddish-brown, soft mass, containing air bubbles. May 24, 1881—The stump has been similarly attacked: patient sinking, complains of his right hip; is slightly jaundiced. Died of acute (*foudroyant*) sepsis at 2 o'clock in the afternoon. Post-mortem examination made after barely twenty-four hours. Very putrid corpse, with general putrefactive emphysema. Apart from old peribronchial caseous deposits, are pretty extensive fatty embolism, there is nothing abnormal. Spleen large and dark; pulp semifluid."

Rosenbach examined the extremity immediately after the amputation, and found very characteristic organisms, long and short rods of considerable thickness, frequently with a large spore at one end of the shorter rods (fig. 41, Plate VIII.). These were present in large numbers. Only after careful searching could he find a few cocci. He failed to cultivate the bacillus.

He narrates a second case of a similar nature where the affection began in the axilla after removal of axillary glands. The gangrene here extended over the back and down the arm, and the patient died on the morning of the third day after the operation. Here also he found microscopically similar organisms in large numbers (fig. 42, Plate VIII.), but these he also failed to cultivate, though he is inclined to attribute this to defective methods. He also saw a very few cocci, which proved on cultivation to be streptococcus pyogenes.

XI.—PYÆMIA.

Rosenbach has only had the opportunity of examining six cases of pyæmia. The following are the cases, with the results of cultivation :—

1. Pyæmia after amputation of the thigh for injury; putrefaction and suppuration of the stump. Infective thrombosis of the crural vein; abscesses in the muscle around it, close to the stump. Endocarditis verrucosa et ulcerosa micrococcea of the aortic valves; infarcts and embolic abscesses in the kidneys. Suppurative fibrinous pleuritis. Swelling of the spleen; œdema of the lungs. Death.

From the blood of the patient streptococcus pyogenes was cultivated during life, in some instances also staphylococcus pyogenes aureus, but by far the most numerous was streptococcus: thus of 8 tubes inoculated at various times from the blood by stroking the surface or puncturing it, growth occurred in 7; in 4 of these pure cultivations of streptococcus pyogenes; in 2 streptococcus pyogenes, along with staphylococcus pyogenes aureus; and in 1 staphylococcus pyogenes aureus alone.

2. Pyæmia after injury of the diploë by a sword-cut (*Schlägerhieb*). Retention of the purulent secretion by stitches. Small deposits in the diploë, from which a purulent thrombophlebitis extended into the transverse sinus; here there was suppuration of the thrombus and periphlebitic abscess formation. Purulent metastases in the lungs with suppurative pleuritis. Death.

The pleura was tapped during life, and cultivations were made from the pus, resulting in copious growth of streptococcus pyogenes.

3. Intense primary infection with streptococcus of a stump, after amputation through the thigh. Infective thrombophlebitis of the crural, saphenous and hypogastric veins, with suppuration of the thrombi and metastases in the pleuræ, joints, kidneys, and the sheaths of the tendons (extensor hallucis). Death.

Streptococci were found in the metastases in the kidneys, in the thrombi in the crural vein, and in the other suppurations that were examined.

4. Compound fracture of the upper arm, and extensive contusion of the soft parts. In spite of amputation there was development of metastases in the lungs, pleura, kidneys, and pericardium. Death.

The pleura was opened during life, and a thin fluid with thick flakes in it was evacuated. Cultivations from this, taken when the opening was made, showed large numbers of streptococcus

pyogenes. Later cultivations made from the pleura showed a majority of the staphylococcus pyogenes aureus.

5. Erysipelas and pyæmia after removal of recurrent carcinoma of the mamma. Infective thrombosis of brachial vein. Embolism of the pulmonary arteries, infarcts and abscesses in the lungs. Suppurative pleuritis, suppurative gonitis, fatty degeneration of the cardiac muscle, parenchymatous nephritis. Death.

During life the knee was punctured with antiseptic precautions, and the pus inoculated into F. P. A. Pure cultivations of streptococcus pyogenes were obtained.

6. Whitlow on the middle finger, inflammation of the forearm, probably metastases in the lungs, metastasic abscesses on the dorsum of the foot, in the right axilla, left thigh, among the glutæal muscles, and in the calf. Recovery.

Cultivations were made from the abscess on the dorsum of the foot, and pure cultivations of staphylococcus pyogenes aureus obtained. No streptococci were found.

Thus of the six cases of metastatic pyæmia examined, streptococcus pyogenes was found five times, partly in the blood and partly in the metastatic deposits during life; twice combined with staphylococcus pyogenes aureus, but in larger numbers than the latter. In one case, which recovered, staphylococcus pyogenes aureus alone was found.

Most of the writers on pyæmia have found micrococci in the blood and in the metastases, and Ogston in his latest work asserts that pyæmia, septicæmia, and septicopyæmia are only symptoms of "micrococcus poisoning." He holds that pyæmia, even the most acute forms, differs from simple inflammation only gradually and quantitively. He holds that there is no such disease as septicæmia and pyæmia *per se*; they are simply secondary phenomena, dependent on local centres of micrococcus growth. These diseases are only the expression of the malignant influences which come from this centre, and which would in every case disappear if we were able to remove or to cure the local affection. Having called attention to Ogston's views, Rosenbach proceeds as follows:—

"As these local deposits are formed of the pyogenic cocci, one might suppose that Ogston looks on these and their ptomaines as the cause of the general disease, pyæmia. But I consider that it is going too far to look on the constitutional affection in pyæmia as only secondary, and to hold that it will stand or fall with the local deposits.

"In further discussing the question of the micro-organisms of pyæmia I must divide the cases into two chief groups, according to the plan adopted by most authors. (These two groups, however, may be combined in the same case, indeed they generally are so.) The first group is represented by cases which occur in connection with larger or smaller local affections, always, however, of considerable size, such as a suppurating joint (knee, hip, &c.), a large suppurating contused wound of the soft parts, a suppurating compound fracture, an abscess unopened or imperfectly opened, &c. The main point in these cases is that the general symptoms are maintained by local deposits, which are continuously supplying the body with morbid materials. Ogston's description applies to this group. Here we see the patients pine away and die with hectic fever, and often without any other noteworthy symptom. The second group consists of cases in which there is not necessarily anything worthy the name of a local centre. The infective material often enters through a small puncture, or scratch, or boil, &c. The main point in these cases is that a general pyæmic affection follows a single, often very transitory infection, without the presence of a permanent local centre.

"We will first consider the first group, and ask whether in such cases the general pyæmic disease is produced by a definite specific micro-organism, with regard to which we must conclude that during the course of the suppuration it has taken up its abode in the wound accidentally, or whether we must explain the pyæmic symptoms simply as the result of the action of the ordinary pyogenic microbes? My observations point to the latter as the correct explanation.

"One is accustomed to look on an acute abscess as a trivial disease, one indeed which, from a therapeutic point of view, is particularly favourable. The abscess is opened; the inflammation, pain, fever, and general disturbance pass away; hence the conclusion—the cocci of pus are of a very harmless nature. Nevertheless, the abscess is not the true disease, but only the result of the true affection—a circumscribed invasion of cocci—which has been going on previously for a longer or shorter time. When, in connection with the abscess, pain, inflammation, fever and other general symptoms exist, they are to a great extent the result of ptomaine diffusion and absorption; against the rapid

further spread of the cocci the infiltration of the tissue early provides a very certain and sufficient barrier. Under these circumstances the abscess is a very harmless affection, the healing of which is only dependent on its opening. But let us suppose that from the first there has been a very extensive coccus invasion, or an invasion with a continuous supply, or an invasion in very loose tissue or in the serous cavities, invasions in fact which spread so rapidly that the protecting infiltration cannot keep pace with them; let us suppose, in short, that from some reason or other the protective barrier is not sufficient, or, what indeed is often the case, that it becomes less efficient as time goes on, then every one will admit that the prognosis of the disease has become very grave. If we may here refer to experiments on animals, Ogston's and my experiments, as well as those of Krause, show that the cocci of pus are by no means harmless bodies, but have a severe pathogenic action on the animal body. But apart from these experimental observations, I believe that no practical surgeon will deny that an acute suppuration may be of itself ultimately dangerous, and cause death after a longer or shorter time. There are even fatal cases in connection with suppurations where the deposit has never been opened, and where, therefore, no specific microbe of pyæmia could have developed secondarily. But more frequent than these are the observations with regard to severe and fatal suppurations published in recent times, and in these cases the aseptic treatment excludes the entrance of germs from without. Granted, then, that there exist such severe febrile diseases, which are nothing else than infections with the ordinary microbes of pus, the question arises, What name shall we apply to these affections? I believe that it is just for these cases that the name pyæmia is more especially suitable, and I think that most surgeons have reckoned them under the heading of pyæmia. Whether this be done, or whether these cases are termed 'secondary fever, exhaustion,' &c., at all events another form of pyæmia, of a much more definite type, the true metastatic pyæmia, must be distinguished from them. This disease, unfortunately so frequent in pre-antiseptic times, and occurring in connection with all kinds of wounds and injuries, includes for the most part also the cases of the second group.

"These cases not unfrequently arise from very small wounds

or centres, and steadily get worse even after the local centre has been removed or has cicatrized. One also reads how in pre-antiseptic times pyæmia occurred in connection with small injuries treated as out-patient cases, with whitlows, &c., in unhealthy hospitals; and I also mention a case (No. 2) where pyæmia caused the death of the patient, occurring after a small sword-cut wound which healed during the course of the disease. It seems to me that we can only explain such a degree of infectivity, and such a continuous spread of the disease in these cases, by supposing the existence of a microbe which has the property of penetrating into living tissue, growing in it without hindrance, more especially growing in the blood, on the inner walls of veins, on the valves of the heart, and in the thrombi, and thus leading by metastases to new deposits, new local inflammations, from whence again further constitutional infection occurs. We must now ask whether the ordinary cocci of pus can be held to be sufficient to produce these forms of pyæmia, or whether we must here assume the existence of a special specific microbe for metastatic pyæmia, or at least for the very infective forms of that disease. However great the probabilities in favour of the latter view may be, the cultivations which I have made have given the result that the specific microbe, even of the infective metastatic pyæmia, is very probably no other than the streptococcus pyogenes, in other words the same pyogenic coccus which is frequently seen as the cause of ordinary acute abscesses. Although Pasteur and Doléris also came to the same result in regard to puerperal fever, and Ogston in regard to pyæmia, nevertheless this view may seem to many of my readers *a priori* doubtful. I must, however, beg them to turn back to Case 5, paragraph VII. (p. 413), and to the two cases described under progressive gangrene. In these cases the result of the cultivation experiments scarcely leave any doubt that the nosogenic microbe is in fact identical with streptococcus pyogenes, and thus we learn that under unknown conditions in man this organism can take on such pernicious, parasitic, and diablastic properties that it does not stand behind the bacillus anthracis in respect to these qualities. After all there is no contradiction in the view that one and the same infective material may cause as a rule simple local abscesses, but may now and then produce more severe general affections which may go on progressively

and end in death. I might with Ogston point out that on the contrary these conditions find numerous analogies in all the infective diseases. Do not the mild cases of small-pox, with the formation of two or three pustules in all, and with scarcely noticeable constitutional symptoms, depend on the same infective material as causes the most acute cases of hæmorrhagic small-pox? Do we not ascribe the very mild cases of typhoid fever, scarlatina, and diphtheria to the same causes as the severe cases? But I will also take my analogies from the human infective diseases. As is well known we may have very mild cases of anthrax, in which the local affection exists without affection of the constitution, and which recover spontaneously. At times the case is somewhat more severe, there is more inflammatory swelling in the neighbourhood, and there may be lymphangitis and swelling of the glands, but this may also get well spontaneously, or after the application of disinfectants, Lister dressing or cauterization. In other instances, on the contrary, the local affection spreads more rapidly, and is quickly followed by constitutional symptoms, and these cases have, as is well known, a very bad prognosis.

"In the case of anthrax we have learnt by the researches of Pasteur and Koch that the poison can be attenuated, and this may possibly explain the different course of the cases. Whether in the case of the cocci of pus a similar attenuation is possible, or whether the receptivity of different individuals varies to such a great degree, or whether, as Ogston thinks, the number of the organisms plays the most important role, or whether other factors come into play, these are points which we must leave further investigations to explain.

"I will not however here assert that there may not be other quite different infective materials which come into play, more especially in the cases of very infective pyæmia. Davaine has experimentally obtained an increase in the virulence of septic poison by continuously inoculating from one individual to another of the same species. We know from Koch and Gaffky's investigations that the increase in virulence thus obtained arises from the acclimatization (*Einbürgerung*) of specific, very infective organisms. A similar condition may also be suspected in cases where we see very infective forms of pyæmia spreading from man to man. Several of these cases of virulent pyæmia which I have

had the opportunity of observing occurred in connection with infection from dead bodies. Unfortunately these cases occurred before I had commenced these investigations on pathogenic microbes. . . . I must further add that not only the streptococcus but also other pyogenic cocci, especially staphylococcus, may be the cause of metastatic pyæmia. Although the first has been observed in much the greater proportion of cases by all investigators, yet in the small amount of material at my disposal there occurred one case of typical metastatic pyæmia (Case 6; this case recovered) which was caused by micrococcus pyogenes aureus. My view at present is, however, that streptococcus occasions by far the largest number of the malignant forms of metastatic pyæmia, being most fitted for this action by its parasitic and diablastic properties, and that probably in the case of staphylococcus a much greater quantity must pass directly into the blood from the primary focus, as, for example, in cases of severe osteomyelitis with metastases, in which the pus, full of cocci, is pressed directly in large quantities into the veins of the medulla."

XII.—FINGER-ERYSIPELOID, ERYSIPELAS CHRONICUM, ERYTHEMA MIGRANS.

"By this name we understand a special disease which indeed is of little importance, because it is very harmless, but yet demands attention because one might at its commencement confuse it with more severe infective diseases. It occurs in people who have to do with animal matters, such as in butchers, leather-dressers, and cooks; in the latter case, as appears to me, more especially where they have to skin game. From a small wound, generally on the finger (a wound is necessary), a bluish brown red infiltration extends spreading with a sharp margin exactly like erysipelas. The parts attacked remain for some days swollen and red, are somewhat itchy, and burn; but finally they become pale, while the margin spreads. Thus the affection may extend from the tip of a finger over the whole finger, indeed over the back of the hand as far as the carpus, and may attack the neighbouring fingers. As a rule the affection comes to an end over the metacarpus in one or two weeks. The general state of the patient is not disturbed, and there is no fever. I

have very seldom seen this affection anywhere but in the hands, only once in the face. After I had for some time in vain tried to find the microbe causing this disease in the fluids of the affected tissues I at length succeeded in cultivating it in the same way that Fehleisen did the erysipelas organism. The microbe grows on F. P. A. in peculiar, very delicate and beautiful colonies—so small that in order to make them visible in drawings I had to magnify them three to four times. Microscopically the cultures consist of cocci not very small, but of very irregular appearance (fig. 43, Plate VIII.). At the commencement of my holiday I inoculated my upper arm with them in three places. Around each of the punctures there developed a brownish circle with burning and itching, and this increased to about the size of a sixpence, and then became pale and disappeared."

II.—ON THE ETIOLOGY OF ACUTE PURULENT INFLAMMATIONS (OSTEOMYELITIS, FURUNCLE, AND WHITLOW).*

By Dr. GARRÈ,

Assistant in Prof. Socin's Private Laboratory in Basel.

Passing over Garrè's remarks on acute osteomyelitis, in which he also found the staphylococcus pyogenes aureus, we must pay great attention to his observations on the production of inflammation in the human subject by means of the cocci of pus, as these are of the greatest importance, and supply to some extent the link in the chain of evidence which is missing in Rosenbach's work. He examined 48 cases of acute abscess by means of cultivation, and found the staphylococcus pyogenes aureus in 13 cases, the staphylococcus pyogenes albus in 18 cases, the two combined in 14 cases, and streptococcus pyogenes in 3 cases. All the last 3 cases were very severe inflammations, so that he is inclined to agree with Rosenbach that streptococcus is the more malignant form. He was unable to find a difference in any respect between the yellow cocci found in osteomyelitis and those found in acute abscess, and hence he comes to the conclusion "that osteomyelitis is not due to a specific micro-organism (*i.e.*, an organism producing only this and no other affection), but that, in common with a number of inflammatory processes, it is caused by the pyogenic staphylococci (perhaps also in exceptional cases by streptococci).

"One might hesitate to accept the above conclusion till undoubted proof of it has been furnished by experimental means. As we know the paths by which the microparasites enter the body in the case of furuncles, whitlows, and phlegmons, *i.e.*, in the first case with the greatest probability by the ducts of the

* *Fortschritte der Medecin*, vol. iii., No. 6, 1885.

cutaneous glands, in the latter through an injury of the epidermis, one ought to be able, if the foregoing suppositions are correct, to produce experimentally in man both whitlow and furuncle with cocci cultivated from osteomyelitis.

"Starting from this idea I made the following inoculations on myself in default of any other person:—

"I. On June 6th, 1883, after previous disinfection of the skin I made a small wound reaching as deep as the corium, without drawing blood, on the outer border of the root of the nail of the little finger of my left hand. Into this wound I inoculated by means of a previously heated platinum needle a small quantity (as much as adhered to the needle when it was plunged once into the cultivation) of a pure cultivation of staphylococcus pyogenes aureus obtained by cultivation on March 3rd, 1883, from the blood of a patient suffering from acute osteomyelitis. The small wound was protected from impurities for the first 24 hours by wrapping a little charpie around the finger. A slight burning pain remained for a few hours; an insignificant inflammatory redness (traumatic) developed around the wound, but had disappeared after 18 to 24 hours and no further effect followed.

"II. On June 13th, 1883, I introduced with similar precautions three needlefuls of staphylococcus pyogenes aureus from the same source as in the former experiment into three small wounds (inoculation wounds) at a corresponding part of the ring finger of the left hand. The pain and inflammatory redness were on this occasion more intense and lasted longer. On the second day a subepidermal suppuration developed, which had spread around the fold of the nail and already reached its other border. 32 hours after the infection I inoculated agar jelly from this pus and obtained the characteristic yellow cultivation which preserved the same macroscopic and microscopic characters during the next two generations and corresponded in every respect to staphylococcus pyogenes aureus.

"III. On June 17th, 1883, a pure cultivation of the third generation from the pus of the same case of osteomyelitis containing staphylococcus pyogenes aureus was used in the following manner. After washing my left forearm with distilled water I took the whole of the cultivation from the test tube and applied it by rubbing over the region of the supinators in the same manner that one rubs an ointment into the skin. The cultiva-

tion had reached the end of its growth on agar jelly and was 4 to 7 mm. broad and about 5 ctm. long.

"For control a small quantity of sterilized agar jelly was rubbed over the right forearm. It must be noted that nothing pathological could be seen on the skin of either forearm, more especially there was no injury of the epidermis however small, no acne, &c.

"As the immediate result, the effects of the mechanical injury (hyperæmia with a sensation of warmth) became apparent on both sides but had disappeared again in less than an hour. Six hours later (at 4 P.M.) there was a burning sensation over the part in which the inunction of micro-organisms was made, and this steadily became more intense and disagreeable. It was quite analogous to the sensation after touching the skin with nettles. Redness and turgescence of the part was also evident. In the course of the evening little pustules about the size of a pin's head developed around the bases of a number of the hairs. On the following morning (June 18th, 1883) the pustules were already the size of lentils, distinctly purulent and surrounded by circles of inflammation. Their contents were inoculated on gelatine, and a number of cover-glass specimens were prepared.

"The sensation of burning had now given place to a sharp shooting pain which affected the whole of the forearm. The skin was considerably infiltrated and every touch was painful. More than twenty of these pustules had already appeared, each of them forming the apex of a conical inflammatory mass. The affair began to be unpleasant, and hence I attempted to cut the process short by pricking each of the pustules and washing them with 1 per 1,000 corrosive sublimate solution.

"But

> 'Die ich rief, die Geister
> Werd' ich nun nicht los.'

"The classical attributes of acute inflammation increased greatly in intensity, and in four days we had the typical picture of an enormous carbuncle, the periphery of which was surrounded by a ring of isolated furuncles. Pain, sleeplessness, fever, and swelling of the axillary glands were the accompaniments. On the sixth or seventh day the process had reached its height. From more than twenty circular openings pus escaped, and the well-known

necrotic plugs and portions of connective tissue separated, aided by warm compresses soaked with sublimate. On the eighth day the pus pressed out of the depth of the wound was inoculated on nutrient jelly and some dry preparations were made.

"These, as well as the inoculations made on 18th June, 1883, gave on agar agar and nutrient jelly the characteristic yellow growths of staphylococcus pyogenes aureus without contamination with any other micro-organism, as shown by plate cultivations and microscopic examination.

"In the cover-glass preparations, cocci of the typical size and form were found scattered among the pus cells in twos, in fours, and in grape-like clusters. In the pus taken on 18th June, 1883, these cocci were present in extraordinarily large numbers, and seemed, to judge by their arrangement, to be undergoing the most active multiplication.

"With the exception of some neighbouring furuncles the suppuration had come to an end in three weeks, and at the present time only seventeen scars indicate the place of infection.

"From these experiments it is evident *that furuncle (carbuncle) and whitlow are infective diseases, that they can be caused by the same coccus as is found in acute osteomyelitis, and that the yellow producing cocci found in these affections are identical.*

"Although Pasteur,* and many after him have found the 'microbe du pus' in furuncles and whitlow, and have, without further proof, regarded it as the causal agent, this conclusion was not satisfactory, and could not be regarded as such till (to use Bergmann's words †) 'experiment had decided the matter, and the attempt had been made to excite whitlow, and the other disturbances in question, in healthy skin and cellular tissue by means of the supposed noxious agents.'

"The final proof that panaritium as well as carbuncle owe their origin to nothing but the parasite inoculated, is furnished by the subsequent isolation of the coccus in question in pure cultivations from the inflammatory products of the processes artificially excited.

"With reference to the first experiment, in which the result was negative, Krause's experiments on animals fully explain and

* Duclaux, *Ferments et Maladies*, 1882.

† *Die Gruppirung der Wundkrankheiten*, Antrittsrede in Berlin, 1883.

confirm it, for in them inoculations of small quantities into rabbits were always without result.

"I am well aware that in the second experiment a true whitlow was not produced. Nevertheless in this affection there are so many gradations from the slight suppuration to the inflammation of the sheaths of the tendons, gradations which are for the most part dependent on the situation and depth of the seat of inoculation, that to have produced a cutaneous suppuration is quite sufficient for purposes of demonstration. For in this case there was only an endermal implantation of the virus, while the experiments on animals have sufficiently demonstrated that a subdermal inoculation is followed by an inflammation, which can scarcely be separated from the severe forms of whitlow if one does not put the question of the localization of the disease in the front. The matter is similar with regard to acute osteomyelitis, which being an affection of bone in contrast to an inflammation of the skin, has of consequence great differences in its clinical picture, but must on account of its general characters be reckoned among the phlegmonous inflammations.

"While the mode of infection in whitlow and phlegmon scarcely requires further discussion, the matter is different with regard to furuncle. In opposition to a recent work,* according to which it is necessary for the production of furuncle that the 'septic poison' must reach the capillaries of the glands of the skin, and in opposition to the widely spread view that the uninjured skin is an absolute protection against infection, I must here point out that in the foregoing experiment the inunction of the cocci was done on skin which was quite intact, nevertheless the violent reaction followed, and the organisms penetrated into the lymph channels (swelling of the glands). And if the sceptic still thinks that there was a minute injury of the epidermis which might have escaped the naked eye examination, I would point out that the sensory nerves would have betrayed the existence of such a place during the inunction much more promptly than the eye. Besides, how is one to explain the simultaneous appearance of more than twenty separate points of infection?

"Further, the method of the application in this experiment cannot be looked on as anything out of the common with reference

* Sanitätsrath, Dr. Rupprecht, "Ueber Furuncle und Carbuncle," *Deutsche Medicin. Wochenschrift*, May, 1883.

to the natural mode of origin of furuncles. For how else are we to explain the predilection of furuncles for the neck, the loins, the axilla, &c., than by supposing that the closely applied parts of the dress bring about a similar inunction of infective germs present over these parts, the result of this inunction being rendered correspondingly certain by the presence of the sweat.

"It seems to me that in furuncle the infection finds its way through the duct of the cutaneous glands. We have no grounds for assuming that the secretory organs of the skin behave differently towards infective germs than do those of the mucous membrane. I would only recall the mode of infection with anthrax after feeding animals on the spores.* And in the case of cholera and typhoid fever one can also hardly look on the existence of an epithelial defect in the intestine as a *sine qua non*."

* *Mittheilungen aus dem Kaiserlichen Gesundheitsamte*, vol. ii. Experimentelle Studien, &c., by Dr. Koch, Dr. Gaffky and Dr. Loeffler.

ON

DIPHTHERIA IN MAN.

By Dr. F. LOEFFLER.

ABSTRACTED BY

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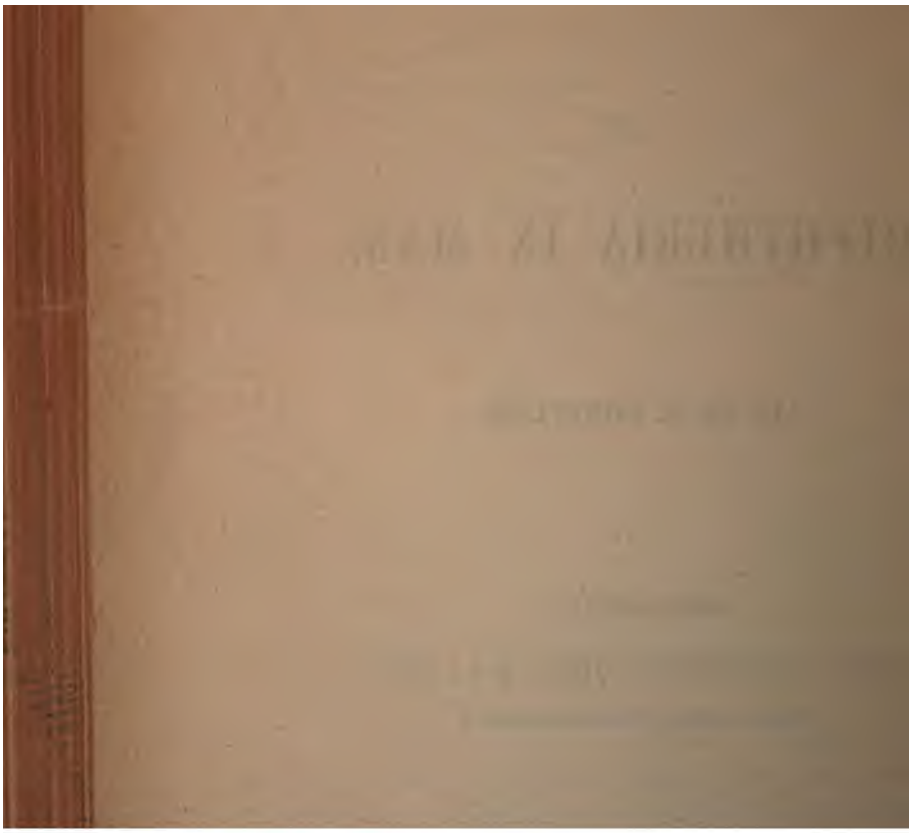

INVESTIGATIONS AS TO THE SIGNIFICANCE OF MICRO-ORGANISMS IN THE PRODUCTION OF DIPHTHERIA IN MAN.*

By Dr. FRIEDRICH LOEFFLER.

Loeffler distinguishes between diphtheria and diphtheritis. The former he defines as a "characteristic and distinct disease, due to a specific *Ens morbi*, like measles and small-pox, which has continued constant for centuries, and occurs epidemically"; by the latter term, diphtheritis, he understands exclusively "a definite pathologico-anatomical form of tissue-change, which occurs in diphtheria along with other forms of tissue-change."†

Since Bretonneau sixty years ago published his classical work on diphtheria, many investigators have endeavoured in vain to clear up the ætiology of this disease. The difficulty of this lies in the nature of the disease itself. The individual cases vary greatly in character according to the age of the patients, the malignity of the epidemic, the stage of the disease, and above all the numerous complications which are wont to ensue during the progress of cases of diphtheria. Besides, other affections of the throat present appearances which cannot be distinguished from those produced by the virus of true diphtheria. The only pathognomonic distinguishing mark is the ætiological agent, the nature of which has to be ascertained from the investigation of individual cases. A further difficulty in the investigation of the ætiology arises from the fact that the specific virus being, as we are obliged to suppose, of the nature of a living organized body, and the diseased surfaces being ulcerated or exposed surfaces, there is unavoidably an immense number of organisms present in the affected parts, and hence

* *Mittheilungen aus dem K. Gesundheitsamte*, Berlin, vol. ii., 1884.

† Loeffler included scarlatinal diphtheria in his investigations.—T. W. H.

the inherent difficulty of recognizing and isolating the specific organism of diphtheria. Pathological material less exposed to admixture might no doubt be obtained from less exposed surfaces than the throat, vagina, &c., and even from internal organs, but the throat has generally been selected because there is undoubted evidence that the morbid products in that situation are infective and therefore contain the diphtheritic virus, while this has not been definitely proved with regard to the juices and tissues of diphtheritic patients.

Virchow was the first to differentiate pathological alterations of the mucous membrane as catarrhal, fibrinous, and diphtheritic, and the artificial production of changes similar to those in diphtheria was established by Bretonneau himself, by injections of olive oil and tincture of cantharides into the trachea of a dog, and by Delafond by the use of chlorine, corrosive sublimate, arsenic, ammonia, &c.

But Weigert* first established experimentally the conditions for producing the various forms of pathological alterations of the mucous membrane with the following results, viz., that catarrhal affections arise from irritation which produces morbid changes in the epithelium without completely destroying it; if the epithelium be destroyed, but the mucous membrane remain intact, the condition of the croupous pseudo-diphtheritic form is produced, whereas in true diphtheria (in the anatomical sense), which implies destruction of the tissue elements of the mucous membrane itself, the metamorphosed tissues are converted into a thick substance like coagulated fibrin.

The first to recognize diphtheria as due to a vegetable organism was Laycock,† but his opinion that the disease was due to the presence of *Oidium albicans* was disproved by T. Hillier,‡ who showed that it is not that organism, but the *leptothrix buccalis* which is found in the false membrane, and that the latter has no significance in diphtheria.

Numerous investigators continued to work on this subject up to the year 1879, when the Commission of the Royal Medical and Chirurgical Society published its report,§ in which, while

* Virchow's *Archiv*, Bd. 70 and 72.

† *Med. Times and Gazette*, May 29, 1858.

‡ *Med. Times and Gazette*, Jan., 1859.

§ Royal Medical and Chirurgical Society's "Report of the Committee on Membranous Croup and Diphtheria," London, 1879.

admitting the presence of micrococci in the membrane of tracheal diphtheria, it did not venture to draw any general and distinct conclusion as to the relationship of these organisms to the disease.

The net result of all the preceding researches, and of the works of numerous succeeding investigators up till quite recently, may be at once stated to be a failure to discover any specific microbe. All the more recent investigators have found bacteria, but the constancy of their presence has been disputed. The specific characters of the organisms, and their relations to the tissues in which they were found, have been equally disputed. The methods of investigation employed were insufficient, and consisted in the use of impure material from the tonsils, &c., for cultures, without any special separation of the different organisms from one another after the manner employed by Koch in his plate cultivations. Of course no satisfactory conclusions could be drawn from inoculations with the cultivations of impure material, and typical diphtheria was never, in Loeffler's opinion, produced in any animal by the various methods employed up to his time.

Loeffler was thus induced to apply the more accurate methods of Koch to the elucidation of the questions as to the significance of the various organisms found in diphtheria, and as to their efficacy in propagating the disease in animals.

Among the difficulties of this investigation, one of the most important to take note of is, that considering the extraordinary rapidity with which the false membrane is produced (three such having been known to grow and be cast off within twenty-four hours), the specific organism present in the epithelium at the outset of the attack may easily be absent at a later stage; further, the effect of agents employed to destroy the poison must be borne in mind, as well as the changes likely to be produced in the organisms during the continuance of the disease or after death, and the difficulty of fixing the locality where the specific bacteria exist, whether in the centre of the morbid products or, like the coccus of erysipelas, exclusively at the edge of the affected part.

As Loeffler did not obtain satisfactory results with any of the methods of staining microscopic sections in vogue, he found it necessary to increase the concentration of the weak methylene blue solution employed by Koch for staining tubercle bacilli. He obtained the most intense and rapid staining by adding 30 c.cm.

of a concentrated alcoholic solution of methylene blue to 100 c.cm. of the solution of potash used by Koch (1 in 10,000 water). This stronger solution has no advantages for staining the tubercle bacillus, but it has for other bacteria. In order to stain most other bacteria in sections, it is sufficient to keep them only a few minutes in the strong solution. After removal from it they are placed in a ½ per cent. solution of acetic acid, and moved about in it for a few seconds to remove excess of stain from the tissues, and get a good nuclear stain. They are then dehydrated in absolute alcohol, afterwards placed in cedar oil, and finally mounted in Canada balsam. Loeffler found that though other solutions would stain cover-glass preparations satisfactorily, the above was the best for sections.

Twenty-seven cases in all were investigated, of which five were scarlatinal diphtheria, twelve are stated to be "diphtheria," five are classed as "typical," and one as "exquisite" diphtheria. But with regard to four, it is not directly stated what the character of the disease was. Indeed, one case is only stated to have died "with pneumonic symptoms on the third day": there was false membrane in the trachea, but the fauces were free. All the cases seem to have been fatal. After consideration of the results of the examination of the whole twenty-seven cases, it is impossible to show any characteristics common to them all, but they may be divided into two classes according to the organism which is present in largest numbers.

In the first class chain-forming micrococci (streptococci) play a prominent part. The pathological anatomy of these cases is distinct from that of the second class. The characteristics of the first class were, loss of epithelium on the surface of the affected mucous membrane, and loss of tissue substance; no false membrane; on the denuded surface of the mucous membrane, and also through its substance, were long chains of rather large micrococci (Plate VI., fig. 20). These organisms were also found in the lymphatics from whence they penetrated into every part of the body, and in various internal organs. As they spread they cause necrosis of the tissues; the masses of organisms being surrounded by a narrow unstained border, and further away by an inflammatory ring (see Plate VI., fig. 21).

Chain-forming micrococci, morphologically identical with those found in diphtheria, are present in various other diseases which

are accompanied by lesions of the mucous membrane, *e.g.*, variola, typhoid and puerperal fever, &c.; and a photograph of the latter is given by Koch in the first volume of the *Mittheilungen*, which represents the micrococci of diphtheria satisfactorily.

As the occurrence of these micrococci is regarded as entirely accidental in variola, &c., there is no reason to suppose that they are not accidental complications of diphtheria likewise.

In the second class of cases the bacilli first described by Klebs are present. These bacilli occur exclusively in those typical cases which are characterized by a thick false membrane extending over the mucous membrane of the fauces, larynx, and trachea, the mucous membrane being traversed by enormously dilated and over-filled vessels. Below the masses of bacteria of different kinds which cover the surface, among which may be the streptococcus before referred to, Klebs' bacilli are found arranged in little groups. They become intensely stained with methylene blue. In one case almost the whole false membrane consisted of colonies of this peculiar bacterium. They have been found also in the alveoli of the lungs and in the liver, but Loeffler is of opinion that their presence there was due to post-mortem changes. He never found them in the other organs, or in the typical hæmorrhagic broncho-pneumonia which accompanies diphtheria.

Klebs' bacilli were not found in all typical cases, so that if they are the specific cause of diphtheria, we can only account for their absence by the assumption that they were eliminated before the patient's death.

So far, therefore, as the investigations into the pathological anatomy go, they fail to give any conclusive information with the means of staining available at present; and there only remains for trial the method of pure cultivation and subsequent inoculation of animals, in order to ascertain if an infective power analogous to diphtheria in man can be set up by either of these two organisms.

a. Pure Cultivations of the Chain-forming Micrococcus.

Koch's nutrient jelly (see footnote p. 46) was exclusively used as the culture medium. Minute quantities of the material from the tonsils were diffused through the fluid jelly, which was then

poured out on sterilized plates of glass and allowed to solidify. The bacteria were separated from one another and developed as isolated pure colonies. Cultivation from portions of the internal organs is still simpler, the only precaution necessary being the exclusion of micro-organisms from without. For this purpose the organ is first placed in a 5 per cent. solution of carbolic acid, and moved about in it for about ten minutes. This suffices to kill all bacilli, micrococci, &c., which may have fallen on the specimen; and in order to destroy any spores it is placed in 1 per cent. solution of corrosive sublimate for five minutes. It is then removed and laid on clean blotting-paper. When dry it is cut partly through with a knife previously heated to redness, and then the two pieces are torn asunder, so that surfaces may be thus secured which have not been touched by any instrument. In this way absolutely pure material, free from extraneous organisms, was obtained and in five different cases Loeffler got pure cultivations of the chain-forming micrococcus from tonsils, heart, liver, kidney, and spleen; two of the cases being scarlatinal diphtheria, and three typical diphtheria.

The chain-forming micrococcus grows rather slowly in the jelly—not until the third day are the minute translucent spots of a light grey colour seen in the line of inoculation. If a colony on the surface be examined with a low power, it appears as a roundish, grey, finely punctated spot, which terminates at the sides in minute wavy lines. After staining with methylene blue these wavy lines are seen to consist of delicate chains of pretty large diplococci, each of which seems slightly compressed in the direction of the long axis of the chain. Viewed by reflected light the cultures appear to consist of small white dots, which are indistinguishable from the colonies of the erysipelas micrococci. These micrococci grow admirably in meat infusion, to which 1 per cent. pepton, ½ per cent. common salt, and 1 per cent. grape-sugar have been added. If kept in the incubator at 37° C. (= 98·6° F.) little delicate flocculi will be noticed after twenty-four hours in the otherwise clear fluid. Under a high power these are seen to consist of long, frequently intertwined chains, composed of as many as 100 links. The addition of 1 per cent. agar agar to the culture medium will keep it solid at the temperature of the body. Blood serum answers still better as the solidifying material, the cocci growing very

luxuriantly on it; they produce a thin, dull skin, not unlike that made by cholestearine. If we add to the blood-serum one-fourth of its volume of the neutral peptonized meat infusion with salt and grape-sugar, a distinct coating of grey, whitish cocci will be seen after incubation at 37° C. (= 98·6° F.) for one to two days. The cocci grow on potato also, but so slowly that they cannot be noticed macroscopically for some eight days.

If the cultures be examined on the cover-glass, some of the links of the chains will often be seen to be much larger than the others, and a fine line indicating commencing division may be seen on each of these larger cocci. Sometimes the entire chain consists of these large cocci.

The result of a very large number of inoculations made by Loeffler on mice, dogs, rabbits, guinea-pigs, birds, and monkeys with the streptococcus is, that it must be regarded as being only accidentally present in diphtheria, and when present it gives rise to complications partly local and partly general in character. As the result of these experiments, and of comparative experiments made with Fehleisen's erysipelas micrococci, Loeffler found that the effects produced by these two organisms were identical. The cocci found in scarlatinal diphtheria, in typical diphtheria, and in erysipelas correspond in their form, mode of growth, and effect on animals, and hence the conclusion is justified that these cocci, if not identical, are at any rate very nearly allied. Loeffler is not at present inclined to regard them as identical, because Fehleisen, in his researches on the erysipelas micrococci, never found them in the blood-vessels.

The following arguments prove that the streptococci cannot be regarded as the specific cause of diphtheria: (1) when inoculated on the lower animals they never produced a disease even resembling diphtheria; (2) they are only found in a limited number of cases of human diphtheria; (3) they are found on and in affected parts along with the bacillus which will be next considered; (4) they are found in internal organs in other diseases in which lesions of the epithelium of the mucous membrane occur. But it is not impossible that these micrococci may cause a disease resembling diphtheria, if they should penetrate into the fauces, and thence along the lymphatics of the trachea into the lung. One of Loeffler's cases supports this opinion.

b. Pure Cultivation of the Bacilli.

The nutrient jelly was found by Loeffler to be unsuitable for the cultivation of these bacteria, and he was obliged to employ coagulated blood serum which can be kept at the temperature of the body and which is well adapted for the purpose.

In order to obtain pure cultivations of these bacilli, Loeffler took a minute portion of a false membrane containing them in addition to other micro-organisms, and diffused this in 5 c.cm. sterilized distilled water. A needle dipped in this liquid was then stroked over the surface of blood serum contained in small flat glasses, and these glasses were then placed in an incubator at the body temperature. On the following day numerous small, separate colonies were evident on the blood serum; most of these proved to consist of micrococci, but some were composed of the bacilli in question. On the third day these two kinds of colonies could be distinguished with the naked eye; the micrococcus-colonies were small and transparent, the bacillus-colonies larger, whitish, and opaque. To get these bacilli perfectly pure a small portion of one of the colonies was diffused in 10 c.cm. sterilized distilled water, and of this as much as could be taken up in the loop of a platinum wire was sown on fresh serum. In this way pure cultivations were obtained from several cases.

The best cultivating medium, and the one used exclusively by Loeffler in his experiments, consists of 3 parts of calf's or lamb's blood serum, to which is added 1 part of neutralized veal infusion, containing 1 per cent. pepton, 1 per cent. grape-sugar and ½ per cent. common salt. This material is sterilized and solidified in the same manner as is done by Koch in the preparation of blood serum for cultivations of tubercle bacilli.

Material for cultivation was selected from four typical cases in children aged 5, 6, 8 and 9 years, the membrane being taken on the second day of the disease before any treatment had been begun. The false membrane, which was in all cases firmly adherent, was seized with forceps, and a portion removed and cut with the freezing microtome. The sections were hardened in alcohol, and treated like other hardened material. In all four cases the characteristic bacilli could be seen after staining in the solution of methylene blue and potash. On the surface were numerous

micrococci, and below that, in the part of the membrane rich in cells, were groups of the bacilli (Plate VI., fig. 22*b*), and then followed the broad fibrinous zone containing few cells and no bacteria. It was from the deeper part that the material was taken for cultivation in all the cases, and that identical organisms were obtained from all the patients was proved both by their morphological and their biological characteristics. It is worthy of remark that in one case out of twenty children and ten grown-up people, Loeffler procured by cultivation a bacillus apparently identical with the diphtheritic one from the material adhering to the teeth of a healthy child.

The diphtheritic bacilli are non-motile, and are very quickly and deeply stained with methylene blue; some of them are straight, and some slightly curved. They vary considerably in length, being on an average about the length of the tubercle bacillus, but they are twice as thick. The longer ones are composed of several members, and where these are connected there is frequently a slight, knotty enlargement. In not a few individuals one terminal member, or both, may appear slightly thickened (Plate VI., fig. 23). Frequently the pole of the bacillus is more deeply stained than its substance. This is distinctly shown when blue preparations are treated with iodine solution, as the body of the bacillus is easily decolourized, while the poles remain deep blue; this gives some of them the appearance of a dumb-bell. Even after the cultivations were kept for a week in the incubator spores were never seen by Loeffler, such as are distinguished in other bacteria by the impossibility of staining them, and by their brighter appearance on central illumination. Exposure to a temperature of 60° C. (=140° F.) for half an hour destroys the bacilli without exception, whether they have the nucleus-like pole or not (Loeffler does not consider this thickening to be due to the presence of a spore), behaving in this respect like all sporeless bacteria. As to the length of life of these bacilli, it seems to be about three months. They require for their development a temperature above 20° C. (=68° F.), and this explains the failure of the attempts at cultivation on the nutrient jelly. In order to test whether these bacilli will grow in this material, Loeffler inoculated a number of tubes, and found that while at the ordinary temperature the track of the inoculating needle continued unchanged, so soon as the temperature reached 20° to

22° C. (=68° to 71° F.), at which the 5 per cent. jelly becomes soft (not quite fluid), the bacteria began to grow, and could be seen as small, round, white dots. When such colonies were examined on the cover-glass many of the bacilli were found to be curiously shaped, some dilated like bottles, others resembling sausages, others looking like large cocci, reminding one of the appearance of the involution forms of anthrax bacilli growing under unfavourable conditions. After being transferred to the serum mixture and placed in the incubator these anomalous forms disappeared. Potato is not suitable for growing this organism.

Inoculation experiments were made on mice, rats, guinea-pigs, rabbits, monkeys, pigeons, hens and smaller birds, and the behaviour of the bacilli towards the various species is a matter of great interest. The modes of infection tried consisted of subcutaneous inoculation, inoculation on the wounded or unharmed mucous membrane and inhalation.

When inoculations were made through the skin or mucous membrane the following results were obtained: rats and mice enjoy complete immunity, while guinea-pigs fall easy victims; their death appears to be due not to the spreading of the bacillus throughout the body, but to a poison produced at the seat of inoculation, which causes an alteration in the walls of the blood-vessels leading to hemorrhages throughout the body. Small birds, canaries, finches, green finches, &c., become infected as certainly as guinea-pigs, but more rapidly. Pigeons also are susceptible, and hens behave very like them, but hens and pigeons are not nearly so susceptible as the small birds and cannot be infected through an uninjured mucous membrane. Rabbits do not give such uniform results as the preceding; it would appear from the experiments of Loeffler that death is due in their case to the mechanical effects of the false membrane caused by infection, and that a chemical poison is not developed in the blood, as in the case of guinea-pigs. From the monstrous forms sometimes assumed by the bacilli (as when cultivated in gelatine), it seems as if the rabbit's body does not in all parts offer a very suitable culture-medium for the organism. One monkey had an erosion at the seat of inoculation on the palate, but did not contract diphtheria. The other became very ill, but had no fever; there was extensive œdematous infiltration under the arm, where it was

inoculated, with enlargement of the axillary glands; a necrotic piece of skin was thrown off after about a week, and the animal recovered.

When infection was attempted without breach of continuity of the skin or mucous membrane, pigeons and hens were unaffected by material introduced even down into the interior of the larynx. Hens, pigeons, rabbits and guinea-pigs were unaffected by a spray containing the virus, also by remaining in a bedding of hay and straw which had been exposed to the spray. Repeated attempts were made to infect through the uninjured mucous membrane of the conjunctiva, fauces, and larynx, but always in vain. Infection of rabbits through the uninjured vaginal mucous membrane could not be produced, but guinea-pigs were successfully infected by the introduction of virus into the vagina, under precautions which practically exclude the idea of the mucous membrane being wounded and presenting any breach of surface.

In reply to the crucial question whether the bacilli, which, when inoculated, produced death in numerous instances with phenomena undoubtedly similar to diphtheria, be the specific cause of the disease or not, it is impossible to give an affirmative answer. The following facts are evidence in favour of its specific character:—They have been found in a large number (13) of typical cases of diphtheria, with fibrinous exudation on the fauces, and in a constantly recurring arrangement: they lie in the oldest part of the membrane, and penetrate deeper than any other bacteria: cultivations of these organisms introduced beneath the skin of guinea-pigs and small birds kill them, producing whitish or hæmorrhagic exudations at the point of inoculation, and extensive œdema of the subcutaneous tissues, the internal organs are not affected, as is the case with man: introduced through a wound of the trachea in rabbits, fowls and pigeons, the poison produces a false membrane, and also if placed on the scarified connective tissue of rabbits, and on the entrance of the dilated vagina of guinea-pigs: in addition to the formation of false membrane, there has been observed the characteristic, serious alteration of the vascular walls, which shows itself by bloody œdema, hæmorrhage into the tissue of the lymphatic glands, and effusion into the pleural cavity. The bacilli have therefore the same effect as the diphtheritic virus. They also have the property, in common with that virus, that

they kill young animals generally more easily and quickly than old ones.

On the other hand, the following arguments may be urged against their specific character:—

1. The bacilli were not present in a number of undoubted cases of diphtheria.

2. They were not found in the false membrane in rabbits and fowls, arranged in the same typical manner as in man.

3. When applied to the uninjured mucous membrane of the fauces, respiratory passages, eyes and vagina, no effect was produced in several animals otherwise susceptible to their action.

4. Animals which survived inoculation showed no paralytic symptoms.

5. A bacterium is sometimes found in the saliva of the healthy child which morphologically and physiologically is indistinguishable from the bacillus of diphtheria.

The strict proof that these bacilli are the cause of diphtheria is therefore not at present obtained, but the possibility of their being so is not excluded. In cases where they were not discovered, they might have been eliminated, and their absence in the diphtheritic membranes in guinea-pigs is probably due to this cause.

As to the third objection, too great weight must not be attached to it, as it is not yet known whether or not the diphtheritic virus attacks man without a previous lesion of the mucous membrane; and further, in the throats of the animals experimented on, there are no hollows corresponding to those on the tonsils, in which the virus can lie and develop.

In regard to the fourth point, (the absence of paralytic symptoms in the animals experimented on,) it must be remembered that the number of animals which survived the inoculations was comparatively small, and that 11 per cent. is the highest percentage in man in which paralytic symptoms have appeared.

Finally, as to the fifth point, it is conceivable that at a time when there were numerous cases of diphtheria about, the virus of the disease might be present in the throat of a child without producing any morbid effect.

As to the practical conclusions to be drawn from the exhaustive and lengthy investigations of Loeffler, although some links are wanting in the demonstration, still certain points have been

established of great value both for public health and for therapeutics.

It is a matter of the first importance that it is rendered almost certain that, in the case of man and of the pigeon, the first serious alterations are produced at the point of entry of the poison, that is, in the great majority of cases, in the *primæ viæ;* that this is the case with the calf is certain.* At this point the multiplication of the poison takes place, and from thence it is transmitted inwards and outwards. Hence the important indication for *isolating* the patient; and further, for *destroying all secretions and excretions emanating from the patient,* especially from the mouth and throat, and also all objects infected by the same. As in the act of coughing infective material may be expelled from the mouth, set free in the air, and deposited on the floor, walls, &c., it is evident that the place occupied by the patient must be carefully disinfected. As superficial lesions form an important predisposing cause, the indication is that all means should be taken to protect existing lesions and prevent others occurring.

Certain important conclusions may also be drawn for therapeutics; in the first place the earliest possible disinfection of the infected parts and removal of the morbid products are indicated. Even if it be assumed that the bacillus found in the false membrane is not the specific cause of the disease, still, from the experience gained in the long series of experiments here dealt with, it is evident that the earliest possible removal of these morbid products is called for, as there is contained in them, in large numbers, a bacterium which produces a chemical poison, extraordinarily deleterious to many species of animals.

* The second part of Loeffler's paper is taken up with the description of diphtheria in pigeons and calves, in which other forms of bacilli are present, which are in all probability the cause of the disease.—T. W. H.

ON

ACTINOMYCOSIS IN MAN.

By JAMES ISRAËL,

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ABSTRACTED BY

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CLINICAL CONTRIBUTIONS ON THE SUBJECT OF ACTINOMYCOSIS IN MAN.

[It may be well, before proceeding to discuss the clinical details in Israël's work, to state in a few words what is known about this disease.* In June, 1877, Bollinger† described, as a new fungoid disease in cattle, a peculiar affection of the jaws of cattle, which up till that time had been looked on by some as scrofulous disease, by others as osteosarcoma. According to his description, the affection consists in the development of a whitish tumour, which starts from the posterior part of the alveolus, or from the spongy part of the bone, distends the bone and bursts through it, generally outwards, destroying all the tissues in its course. The substance of this irregular nodulated tumour is for the most part soft and juicy, and shows on section a large number of yellow, abscess-like points. Microscopically the tumour consists of granulation tissue with minute abscesses here and there, in which lie peculiar yellowish bodies about the size of a hemp seed. These bodies are also found in similar tumours in the tongue, pharynx, larynx, and mucous membrane of the stomach, and in the neighbouring lymphatic glands. When examined more minutely they present a gland-like mulberry appearance, consisting of closely interwoven threads and club-shaped bodies (see fig. 11). Harz suggested that they should be called actinomyces (ray fungus), and hence the disease is termed actinomycosis.

Variations are found in, and different opinions are held as to the characters and mode of development of these bodies, but we need not discuss them here. All the masses consist of three

* See Ponfick, *Die Actinomykose des Menschen.*

† "Ueber eine neue Pilzkrankheit beim Rinde," *Centralbl. für die Med. Wissensch.*, 1877, No. 27.

essential parts. In the centre, forming a sort of nucleus, is a mass of closely interwoven threads. From this nucleus a multitude of threads shoot out in a radiating manner; this middle layer constituting the greater part of the nodule. These threads, often after dichotomous division, swell out at their ends, giving rise to the club-shaped bodies which form the outer part of the mass, and give it its irregular mulberry appearance.

Fig. 11.

A mass of actinomyces, showing the ray-arrangement, the club-shaped bodies, and a thread of mycelium extending beyond the mass, and after division expanding to form clubbed ends.

Attempts have been made by several observers to cultivate this fungus outside the body, but although some say that increase has taken place, this has not occurred to such an extent as to enable any one to classify the organism, or to gain any accurate idea of its life-history, and mode of development.

Attempts have also been made to infect animals with portions of the tumour containing the actinomyces, or with the actinomyces itself, and in some cases this has apparently been successful. Ponfick's results are as follows: rabbits and dogs are not susceptible to the disease in whatever way the inoculation is performed, whether into the anterior chamber of the eye, subcutaneously into the abdomen, or by injection into the veins. On the other hand, he has succeeded in infecting calves by introducing portions of the tumours into the subcutaneous tissues, into the abdominal cavity, or into the veins. After inoculation into the abdominal cavity the animal is apt to die of general peritonitis, but if that does not occur, actinomycotic nodules develop.

In three cases the calves lived after this inoculation, and the result in each case was as follows: the first animal died in 26 days from exhaustion and lobular pneumonia, and there was found on post-mortem examination several patches of peritonitis enclosing the remains of the tissue introduced, and also a development of several independent nodules in the neighbourhood of the stomach and urinary bladder. In the second case the animal died suddenly after 60 days, while an injection of fragments of an actinomycotic tumour was being made into the jugular vein. Various false membranes were found in the abdominal cavity, and scattered through them were 63 large and small nodules containing the characteristic masses of actinomyces. The third animal was first inoculated in the abdominal cavity, 7 days later portions of a fresh tumour were introduced under the skin in the region of the left lower jaw, but inflammation followed this, and the portions introduced were evacuated with the pus; 99 days after the first operation portions of a recent tumour were introduced under the skin on the right side of the neck. The animal was bled to death 7 months (210 days) after the first operation. On post-mortem examination the following conditions were found: numerous (21) large and many small fleshy actinomycotic nodules in the omentum, in the false membranes resulting from the primary peritonitis, and in the serous coats of most of the abdominal organs; several large and many small nodules in the subcutaneous and intermuscular tissue of the right side of the neck; numerous large and small nodules in both lungs.

It is worthy of note that Ponfick was unable to produce any effect by introducing the isolated masses of actinomyces; it was necessary to introduce them embedded in portions of the tumour.—T. W. H.]

Actinomycosis in man was first recognized and described by Israël in 1877,* and subsequently by Ponfick.† It is a specific infective disease, due to the action of a fungus (actinomyces),

* This work mainly treats of the clinical aspect of this disease, unforeseen difficulties having prevented the completion of the ætiological investigations of the author.—T. W. H.

† *Die Actinomykose des Menschen, eine neue Infectionskrankheit*, von Prof. Dr. E. Ponfick.

is very malignant in character and is identical in man with the disease known in cattle. Three paths have been ascertained by which the microbe successfully invades the human body:

(1) The mouth and pharynx,
(2) The respiratory passages,
(3) The digestive tract.

For descriptive purposes the cases of the disease may be conveniently grouped as affecting—

(1) The head and neck,
(2) The chest,
(3) The abdomen.

There are, however, some anomalous cases which can with difficulty be assigned to any of the above groups, either as to the source of invasion or localization.

Israël has collated in all 38 cases,* of which 29 have already been published—6 by himself, and 23 by others. Of the total cases, 9 are published in his work, already named, for the first time; 7 are cases observed by himself, and the particulars of 2 have been supplied by friends.

It is needless to detail the name of each of the patients, and the journal in which each case was reported, as is done in full by Israël. The following scheme showed how he has grouped the cases:—

GROUP I.—*Invasion through the Mouth and Pharynx.*

(*a*) Localization in the lower jaw,
1 case.

(*b*) Localization on the edge of the lower jaw, in the submaxillary and submental regions,
9 cases.

(*c*) Localization in the neck,
2 cases.

(*d*) Localization in the periosteum of the upper jaw,
1 case.

(*e*) Localization in the region of the cheeks,
5 cases.

* See also J. Wolff, "Ueber einen Fall von Aktinomykose" (*Breslauer ærztl. Zeitschr.*, 1884, pp. 284—6); and Lorenzo Magnussen, *Beiträge zur Diagnostik und Casuistik der Aktinomykose*, Inaug. Dissert., Kiel, 1885; also Aufrecht, "Ein Fall von Aktinomykosis Hominis," *Patholog. Mittheilungen*, Fasc. ii. p. 50, Magdeburg, 1883.—T. W. E.

GROUP II.—*Invasion through the Respiratory Passages.*

(*a*) Localization on the bronchial mucous membrane,
1 case.

(*b*) Primary localization in the parenchyma of the lung; extension to the chest wall,
3 cases.

(*c*) Primary localization in the parenchyma of the lungs; metastases,
5 cases.

GROUP III.—*Immigration from the Intestinal Tract.*

(*a*) Localization on the mucous membrane,
1 case.

(*b*) Intestinal affection with extension to the peritoneum and abdominal wall; metastases,
6 cases.

GROUP IV.—*Cases in which the Source of Invasion was Uncertain.*

(*a*) Probable immigration from the respiratory passages,
2 cases.

(*b*) Probable immigration from the œsophagus,
1 case.

(*c*) Probable immigration from the intestine,
2 cases.

Group I.—(*a*) Seat of disease in the interior of the mandibula.

The following interesting and instructive case is not only characteristic of the disease, but is the first and only one in which a central origin is established for a case of actinomycosis of the lower jaw. The case is reported by Dr. J. Israël:—

Frau H., ætat. 46, noticed, two months before coming under observation, a slight tenderness on the external side of the right lower jaw. The cause was supposed to be a small swelling at the junction between the gum and the buccal mucous membrane, which, however, on repeated puncture and incision, neither discharged pus nor disappeared.

Status, Jan. 29, 1884.—Externally no swelling is visible, but a thickening of the lower edge of the right lower jaw can be felt, midway between the angle and the symphisis. All the teeth in the upper jaw are absent; the three anterior molars and the canine tooth are absent in the lower jaw on the right side, and the atrophied alveolar processes are smoothly covered with mucous membrane.

The successive steps in the migration of the tumour are well illustrated in one case as follows: at first the tumour was situated on the alveolar process close to carious teeth; later on it was found close to the edge of the jaw, but quite separate from it; subsequently the submaxillary region was found quite free, but a large abscess had formed in the carotid fossa, below the hyoid bone, and after this had been opened and had healed another abscess formed close above the clavicle.

It is but seldom that the contents of an actinomycotic abscess connected with the mouth or pharynx are fœtid, though it is common enough to meet with this in other regions. But in the last case referred to this was present, and in addition there were two other peculiarities, viz., the rapid development of the abscess, and the preponderance of the fluid contents as compared with the granulation tissue.

The starting point has also been associated with an attack of pharyngitis and tonsilitis.

It is not a little remarkable that no trace of the migration of an actinomycotic tumour can usually be detected between the point of its indubitable origin and the point where it has come to light. This curious fact is well illustrated in a case of Israël's, in which the tumour, first noticed in August in the submaxillary region between the spina mentalis and the angle of the jaw, had descended by December 1st to the level of the thyroid cartilage. A knowledge of this fact, that an actinomycotic mass may thus traverse a considerable distance without leaving any evidence of its passage *en route*, will prove of great service. It throws a light on the pathogenesis of many cases in which the tumour is met with at a distance from any of the orifices or canals of the body which are familiar as the points of entry of microbes.

That the consumption of pork is not an essential cause of actinomycosis is shown by the case of a Jewish teacher, who suffered from an attack in the cheek, and had never partaken of any article of food excluded by the ritual of his church.

In some cases there occur small breaches of the cuticle, affording an outlet from time to time of small quantities of the broken down tissues of the tumour. The openings soon close spontaneously, but the swelling shortly recurs, the closed orifice again becomes prominent, discharge takes place and again ceases.

Pathology of Actinomycosis of the Face and Neck.

Although there is no doubt that greater experience will add new features to the disease as at present recognized, still it will be advantageous, seeing the existing ignorance of the disease, to give a sketch of it as pourtrayed by our present knowledge.

The first point of interest is the point of entrance of the microbe. In the cases, so far considered, this has undoubtedly been the cavity of the mouth and pharynx. This is proved not merely by anamnestic evidence, but by the topography of the tumour, the course of the affection, and the operative and post-mortem examination. In such cases we either find on examination morbid alterations with loss of tissue, which open a way for the microbe into the tissues, or else the patient assures us that such conditions existed before and at the commencement of his suffering. Among such predisposing morbid conditions must be reckoned, in the first place, caries of the teeth; and in the second, injuries and fistulæ of the jaw; and lastly, inflammatory processes of the pharynx and tonsils.

With regard to the topographic relations of actinomycosis of the face and neck, it appears that with reference to the point of origin they may be divided into those which commence—

(1) In morbid affections and lesions of the lower jaw and teeth;

(2) In morbid affections and lesions of the upper jaw and teeth.

Probably it will be found that there are other points of origin, *e.g.*, the tonsils.

Invasion from the lower jaw is the most frequent, probably because the lower teeth are most frequently carious. In cases of this class the tumour is found either central in the mandibula, or close to the edge of the lower jaw, or in the submaxillary or submental region, or finally it may be found lower down in the neck, in the fossa carotidea, or in or near the median line. It is rare to find the tumour in man situated centrally in the bone. It is equally rare for it to pass through the masseter muscle. The paramandibular tumour is usually found either as a circumscribed, or more frequently diffuse hard swelling, close to the lower jaw, most frequently surrounding the angle; it is not uncommon to find it affecting the ascending ramus for some distance; and a band may often be felt stretching from the lower part of the swelling down the neck.

The tumours which are found in the submaxillary region either have no detectable connection with the lower jaw, or a cord-like process may be felt extending towards the jaw. The same is true of tumours situated lower down in the neck. The patient can generally state that such tumours situated low down in the neck, and quite isolated from the lower jaw, were at first close to the jaw, and gradually migrated, the direction being usually downwards and inwards, the path of the migration being at first traceable by a cord or band to be felt reaching from the upper part of the tumour to the jaw. This band, according to the statement of a number of patients, disappears in time, so that no connection can be perceived between the tumour and the jaw. These statements correspond with the objective evidence obtainable on examination of various patients.

Wherever the point of origin may be, actinomycotic tumours at first feel hard, and do not affect the skin. Their course in strictly typical cases is never an acute inflammatory one, but slow and torpid, extending usually over several months, and scarcely exciting any attention by reason of painfulness. Pain is usually not felt until after some time, and after changes have taken place in the tumour. It is only quite exceptionally that any general symptoms are present in the class of cases under consideration. Secondary changes may occur consisting at first of false or true fluctuation in the tumour, according to the amount of fluid it contains. This is usually but small, the tumour consisting mainly of granulation tissue in various stages of fatty degeneration. The fluid is in most cases thin, seropurulent and odourless, and contains the characteristic granules. In only one case were the contents fœtid, and here also they were exceptionally fluid. If the process goes on undisturbed discharge will take place through the skin; and though the openings may in rare cases close, they most frequently continue as fistulæ with a slight discharge. There is another course which these cases may pursue, viz., they may shrink and become hardened through displacement of the granulation tissue by cicatricial-like connective tissue, constituting a sort of spontaneous healing. But this retrogressive process will not be found in the whole of the tumour, but only in its oldest parts. Thus, as in cases of schirrus and many syphilitic processes, a cicatricial shrinking may be found on one side, and on the other a pathological cell-

proliferation. Within the region of cicatricial shrinking there may be found here and there islets of granulation tissue containing fungi. This tissue may soften and form pus, and lead to the formation of fistulæ, undermining the indurated tissues. This combined process of shrinking of the oldest parts (those nearest the jaw) and peripheric formation of fresh actinomycotic granulation tissue is the cause of the apparent descent of the tumour from the jaw into the neck. The hard connecting bands which may often be felt extending between the angle of the jaw and a tumour situated below in the regio submaxillaris or mediana colli are the product of the retrogressive formation occurring in the affected tract.

The formation of granulation tissue scarcely ever becomes large enough, or assumes a sufficiently circumscribed prominence above the surrounding healthy tissues to attract attention as a distinct tumour, as in the case of a sarcoma or lipoma. The swelling is diffuse and not very prominent, as distinguished from the corresponding affection in cattle, and clinically gives the impression of chronic inflammation. In cattle the tissue-formation is so great as to suggest a sarcomatous tumour.

Sometimes a slight degree of trismus is noticed, but chiefly where inflammatory symptoms have been prominent, where pain has been felt throughout, or where there has been initial fever.

Actinomycotic disease of the lower maxillary bone itself is rare. In cases where peri- or parosteal suppuration occurs the bone may be deprived of periosteum and become roughened; but a specific actinomycotic central disease of the bone, such as occurs in cattle, was only once observed by Israël, and in that case it was of but limited extent.

Experience of invasion from the upper jaw is less extensive than of that originating in the lower. When the affection has commenced in the last upper molars it involves the soft parts of the side of the face, the cheeks and jaws, and the neighbourhood of the temple and malar bone. It extends either by passing through the muscles of mastication (thereby causing inflammation of the muscles, leading to trismus, and ending in induration and connective tissue degeneration), or by creeping under the mucous membranes from the gum to the cheek as far as the anterior edge of the masseter muscles, without penetrating them. In the latter case the path made by the progressive morbid change

can be detected by the cord or band, which can be felt extending from the alveolar process of the upper jaw to the cheek, and which limits the full opening of the mouth by its rigidity.

When the process has become visible it has already, seeing the slowness of its progress, made considerable alterations in the deeply seated soft parts, the masseter muscles, and the buccal and retromaxillary fatty tissue. The progress of the disease when affecting the upper jaw differs essentially from what is observed in the lower jaw, as in the latter case the disease shows itself much more directly at the surface before it has effected serious mischief deeper down. This fact is of great importance in the treatment and prognosis of cases of each class.

The forms under which the process is observed on the surface of the cheeks, jaws, and temples are various. Sometimes we find diffuse, flat, hard infiltrations; at other times circumscribed knobs and nodules, from the size of a cherry-stone to that of a lentil, which at first are hard, then fluctuating, and ultimately form true abscesses. According to the age of the tumour the skin covering it will be found intact or involved in the morbid process, of a bluish or livid colour, diminished in thickness or already broken through. After the softened contents have been discharged openings remain with thin, undermined edges, and these discharge a scanty seropurulent fluid containing fungus-granules, and lead by tortuous paths, seldom passable by a probe, towards the alveolar process of the upper jaw.

But this passage from the upper jaw to the face is by no means the most serious result. Owing to the immediate connection of the alveolar process of the upper jaw with the temporal bone and the base of the skull, it is evident that the disease may pass on to attack the latter. If this should happen, then the disease may descend along the bones, and in front of the spinal column into the mediastinum; work its way through the base of the skull and attack the brain; and wander through the muscular tissue of the neck after destroying the connections of the vertebræ.

From these observations it will be seen that the *prognosis* is much more serious when the actinomycotic process has its origin in the upper than in the lower jaw. All the cases of the latter kind observed have been of a benign character. Healing was effected either by assisting the elimination of the fungi by

incision, or by scraping out the granulation tissue which contains them. In one case part of the jaw had to be chiselled away in order to remove the mass, in another resection of one half of the jaw was performed. In most cases there will be no necessity for resorting to such severe measures, as the bone, except in very rare cases, will be found eroded only on the surface, if at all. Still cases of this kind may assume the most malignant character.

The danger arising from actinomycosis depends chiefly on the locality where the disease is situated, the importance of the organs affected, and the greater or less facility for getting at it for operation. But further, the fungi themselves do not appear to have always the same degree of malignity, and do not in all cases exhibit that energy which in the more malignant forms produces the astounding and extensive destruction of tissue. Whether or not this malignity is simply proportional to the power of multiplication of the fungus, or whether the forms present in these cases are of more malignant character, is still undecided.

Group II.—*Primary Development of the Mycosis in the Respiratory Tract.*

(*a*) Localization in the bronchial mucous membrane (bronchitis actinomycotica).

Only one case has been observed in which the fungus has developed on the surface of the mucous membrane, and in the catarrhal secretion of the respiratory tract, without apparently any deeper lesion of the parenchyma. This was the more remarkable as the case referred to had been going on for seven years. In this case another peculiarity was observed, viz., putrid decomposition of the sputum under the influence of the actinomyces. The sputum presented the characters of ordinary putrid bronchitis, in which the actinomyces appeared as an accidental concomitant; but this new form of bronchitis exhibited special characters, distinguishing it from ordinary putrid bronchitis. For whilst in the latter the sputum is very copious, thin, separates into three layers, and contains the well-known yellow masses in which the specific morbific agents, the leptothrix pulmonalis and needles of fatty acids are found, in the former

case the sputum was scanty, separated into two layers, and exhibited, when fresh, no microbes except actinomyces. From this it would appear that the opinion formerly expressed by J. Israël as to the power of actinomyces under certain circumstances of causing putrid decomposition of albuminoid bodies, is well-founded.

(*b*) Localization in the pulmonary parenchyma, with extension to the pleura, and the peri- and prævertebral tissues.

Several remarkable features were present in a case of this character, which terminated fatally, and of which the following is a *résumé* :—

A man, aged 20, who had suffered much from caries of the teeth, had had a dental fistula, and had complained repeatedly of burning pains in the thorax during the summer of 1882, was attacked in Dec., 1882, with left exsudative pleuritis. After five to six days he improved, and after Dec. 29th he was able to do a little work. About Jan. 7th he again became feverish, and was obliged to keep his bed. While the exsudation was collecting afresh a solid swelling appeared above the lowest rib on the left side: this gradually softened and on March 25th (ten weeks afterwards) opened spontaneously and discharged stinking pus mixed with actinomycotic granules. Notwithstanding free opening with the knife, the morbid process extended to the left side of the back, in the form of œdematous infiltration, and here, without any distinct inflammatory symptoms, it led to extensive purulent breaking down of the subcutaneous tissues, from which pus, similar to that which was evacuated spontaneously at first was discharged. Symptoms of pulmonary mischief, which had shown themselves at the outset in cough and specific expectoration containing microbes, ultimately became more marked. Inflammatory condition of the pericardium ensued. The patient became exhausted from the very profuse suppuration and hectic fever, marantic thrombosis of the vena crural. dext., and after attacks of fainting, with cessation of diaphragmatic breathing, the patient died May 14th, after five months illness.

From the full details of this case the following noteworthy points may be selected :—

(1) Pulmonary actinomycosis may begin unnoticed, run a chronic course, and lead to cicatricial contraction of the lung.

(2) The characteristic sputa are muco-purulent masses, consisting of a convolution of mucous threads, including actinomycotic granules. The sputum may become bloody, and at the same time thick, translucent, and similar to pneumonic sputum.

(3) Pulmonary actinomycosis may lead to inflammation of the pericardium and pleura. The pleural exsudation may be absorbed.

(4) The actinomycosis may proceed from the lung to the chest-wall, to which the lung is adherent; the tumour then produced

in the chest-wall is at first hard and elastic, and later on softens down and suppurates.

(5) The purulent contents of the thoracic abscess may, before opening, have an offensive smell, without any other organisms, except actinomyces, being discoverable in it.

Both Weigert and J. Israël are of opinion (Case No. 21) that the actinomyces may reach the lung by swallowing or aspiration, and after adhesion of the pleural surfaces, be thus transported to the peripleural tissues. From this case it appears also that so long as the disease is limited to the pulmonary parenchyma, its existence may escape notice entirely, and that the extension of the actinomycosis from the lungs to the peripleural tissues may lead to attacks of pain, very similar to intercostal neuralgia.

Pathology and Diagnosis of Group II.: Primary Actinomycosis of the Lung.

The available material for a discussion of this group is small, and possibly more extended knowledge may lead to a modification of some of the opinions here expressed.

Primary actinomycosis of the lungs appears in two very distinct forms:—

(1) As a superficial catarrhal affection of the respiratory passages;

(2) As a destructive disease of the pulmonary parenchyma itself.

There is only one clinical history, without post-mortem, of the first type. In this case there was chronic diffuse bronchial catarrh, without any detectable alteration of the pulmonary parenchyma. There was fœtid, scanty, stringy secretion, which on standing became divided into an upper mucous layer (the more abundant), and a less abundant lower layer of sediment, in which besides pus corpuscles and pulmonary epithelium there was an abundance of actinomycotic granules. Notwithstanding that the disease had existed for years, and that there had been frequent intercurrent short attacks of fever, there was no evidence that the general constitution of the patient had suffered.

As there was no post-mortem in this unique case, there is no material on which to found a description of the pathological anatomy of catarrhal, superficial actinomycosis of the respiratory

tract. But a general idea of the state of matters may be gained from an analogous case recorded by Chiari, in which the intestinal mucous membrane was superficially affected. It is not known whether this superficial form passes into the parenchymatous form.

The parenchymatous form of primary actinomycosis of the lung is both clinically and anatomically very distinct. The fungi inhaled into the lung do not remain in the larger bronchi, but pass on into the finer branchi and alveoli. Here they cause the formation of peribronchitic or pneumonic patches of various sizes, sometimes not larger than a millet seed, by producing in the alveoli a chronic inflammatory proliferation of round cells, which soon undergo fatty degeneration. This causes the small hepatized masses to assume a yellowish-white colour.

With increasing age this newly formed cell-growth undergoes a necrobiotic metamorphosis with multiplication of the groups of actinomyces. More or less considerable quantities of pus corpuscles are also to be found in the mass, and, not rarely, slight capillary hæmorrhages. Thus there are formed in the compact masses cavities filled with a soft material consisting of pus-cells, fat, granules, free particles of fat, blood corpuscles, crystals of hæmatin, and actinomyces; cylindrical epithelium is also not infrequently found in it. If the cavities increase in size, so as to lie very close to one another, they become at length continuous, through the breaking down of the intervening septa. In the neighbourhood of this chronic, inflammatory mass, there ensues a reactive, diffuse affection of the portion of the lung involved, by the hypertrophy of the connective tissue, which leads to compression of the alveoli, disappearance of the alveolar epithelium, and finally to the conversion of the pulmonary tissue into a thick mass of connective tissue, rich in elastic fibres, and not easy to cut through, varying in colour, according to the proportion of pigment, from light grey through slate-colour to blue-black. The pathological cavities are separated from this ground substance by a thin wall of granulation tissue, which, when the affection is of long standing and the cavities are large, is speckled brown-yellow, of a softish consistency, and when immersed in water shows a shreddy appearance, and contains the characteristic granules of the ray fungus (actinomyces) in its depressions and sinuosities.

Such is the anatomical condition during *the first stage*, while the disease is confined to the lung, the organ primarily attacked. So far as regards the symptomatology of this stage, it is unfortunately very obscure. In the majority of cases attention is first attracted by the symptoms which follow the first stage, the latter being for the most part latent. The disease usually has not an acute commencement. The inflammatory effects of the invasion of the specific microbe develop so gradually that scarcely one of the patients could tell when his malady commenced. The first symptom which points to a pulmonary affection is a scanty expectoration occurring with but slight cough, both so inconsiderable that the less intelligent or less educated patient pays no attention to them. Hence medical assistance is rarely sought during this stage. The scanty sputum further appears quite harmless, and yet it can suffice for making a correct diagnosis. It is whitish, consists of a mass of fine muco-purulent threads from the smallest bronchi; between these threads the characteristic actinomycotic granules can be seen with the naked eye, if it be carefully spread out with a needle on a glass plate, or a black dish. Microscopically, besides pus corpuscles, there are found alveolar and cylindrical epithelium. If the specific microbe should not be found in the sputum, it is quite possible that percussion of the thorax may give a negative result, considering the small size of many of the infiltrated patches and cavities, especially when they are not near the surface. But if, as has always been found in cases of long standing, these masses are surrounded by a considerable area of lung, rendered dense by growth of connective tissue, then it is often enough possible to determine the seat of the disease both by percussion and by auscultation. And this determination is of the greatest importance for distinguishing actinomycosis pulmonum from tuberculosis pulmonum, with which it has many points of resemblance. The apex of the lungs, which is most usually attacked by tuberculosis, escapes actinomycosis, while the latter disease attacks the parts of the lungs from the clavicle downwards, and especially the posterior and lateral portions. In all the cases so far examined the apices of the lungs have been found free from actinomyces.

Attention to this point is all the more important, as the further course of the disease has a strong resemblance to chronic

phthisis pulmonum. The patient has a slight cough, sometimes has night sweats (probably in consequence of hectic febrile conditions), he grows paler and short of breath. Should hæmoptysis ensue, as is sometimes the case, the similarity becomes still greater. Still this resemblance is really only superficial. For in the first place neither a long-continued hæmoptysis, nor a considerable pulmonary hæmorrhage, has been observed in actinomycosis pulmonum. Further, when there is sanguinolent sputum, it is more like the rusty sputum of pneumonia than the hæmoptysis of tuberculosis. Just as in patients suffering from circumscribed, chronic tubercular affection of the lungs, so in cases of pulmonary actinomycosis, in which only a limited portion of the organ is affected, the disease may run its course without the patient feeling ill, losing much of his power for work, or suffering in appetite, digestion, or sleep.

This uncertainty of diagnosis suggests the necessity of examination of the sputum for both actinomyces and tubercle bacillus.

More characteristic symptoms are not noticed until *the second stage* of the disease, when it extends beyond the lungs. This takes place partly by spread of the disease to the parts in the immediate neighbourhood, partly by metastatic migration of the fungi to distant organs. In the first case the infection is preceded by simple adhesive processes of greater or less extent, or by pleural exudation, or by both simultaneously. As the disease goes on there may be retraction of the chest-wall, and this may be of great diagnostic value. For should there occur a retraction of the chest-wall at the same time with an attack of acute pleuritis (the latter being a frequent occurrence), it is evident that there must have been some chronic affection before the acute one appeared.

The specific actinomycotic infection of neighbouring parts, through immediate continuity of structure, removes all doubt as to the nature of the case. After the affected lung has become adherent to neighbouring parts, the actinomycotic process extends from one of the pulmonary masses in various directions, and the general character of the clinical history depends largely on the direction it may take.

Three principal directions are deserving of attention.

(1) It extends most frequently to the peripleural tissues of the chest-wall, frequently involving the prævertebral tissues, more

frequently posteriorly and laterally than anteriorly. If it starts from the lower part of the lung, it may creep behind the costal insertion of the diaphragm downwards towards the posterior abdominal wall, spread there in the retroperitoneal tissue, and from thence attack the ileo-psoas and quadratus lumborum muscles.

(2) It may proceed from the base of the lung through the diaphragm into the abdominal cavity. If adhesions have not previously formed between the diaphragm and the liver or spleen, there will ensue a diffuse peritonitis, or else a subphrenic abscess above the liver or spleen. But if adhesions already exist, abscesses may be caused by direct extension in either of these great abdominal organs.

(3) The further propagation may be towards the anterior mediastinum and the pericardium. After penetrating the parietal fold, the actinomycotic granulations fill the pericardial cavity as a gelatinous mass, permeated with masses of fungi.

The reactive proliferation of connective tissue in the lungs causes the progress of the disease to be slower, and checks the spread of the disease. The progress of the disease through cellular tissue is not limited by this proliferation, and hence in the latter far greater progress may be made in a short time than in the lungs after a chronic course. No matter where the primary seat of the lung affection may be, the mycotic degeneration, when once it has passed beyond the lungs, usually assumes such great dimensions that the affection of the parenchymatous organ assumes a secondary importance, and the disease acquires a new physiognomy, owing to the phlegmonous superficial affection of the chest-wall and back. The disease may have extended widely in the deeper layers before it breaks through the surface, and it will usually come to light latest along the backbone, near the thoracic or dorsal vertebra, in consequence of the greater depth of tissue to be traversed.

This period, in which the disease has extended beyond the lungs and reached the skin, is called by J. Israël the *second stage*. The symptoms occurring during this stage generally cause the patient to seek medical assistance for the first time. Sometimes it is accompanied by acute, serous pleuritis of short duration. But even apart from this the patient has the appearance of serious deterioration of health. His pallor is striking,

and may assume a waxen character. His ability to work is diminished; some patients take to bed; all complain of an extraordinary feeling of weakness. Many indicate the seat of the disease by leaning slightly to one side, whilst others support the chest while coughing, manifestly in order to limit the painful movements of the thorax. Sometimes they complain of dull feelings of weight and pressure in the back, less frequently of severe pain, very like intercostal neuralgia.

The condition of the body's temperature during this stage is various. Some of the cases have a high, remittent temperature, others a low, hectic fever, a third set progress apparently without any essential elevation of temperature, although the occurrence of frequent night sweats suggests that the condition is febrile, though not continuously so. Sometimes the regular course of the temperature is disturbed by irregular, intercurrent rigors, which no doubt are attributable to metastasis of micro-organisms.

As the disease approaches the surface the symptoms become more marked and characteristic. The regions where it first breaks through the skin, which is either the chest or the neighbourhood of the dorsal or lumbar vertebræ, at first show a diffuse swelling without alteration in the colour of the skin. It looks like anasarca, but palpation proves that the supposed infiltration must be much denser than that of anasarca; it is in fact due to the formation of soft granulation tissue beneath the skin. This swelling may extend over the whole side or back of the thorax, and ultimately a discoloured livid elevation is formed, which on puncture gives no fluid, or at most a drop which, if secured, decides the nature of the case. If the opening take place spontaneously there will be a discharge either of abundant thick muco-purulent stinking material, or else of a scanty, thin, odourless fluid, mixed with the specific fungus elements.

The actinomycotic tissue degeneration passes through two stages: (1) that of granulation, which produces elastic, pseudo-fluctuating swellings of a torpid character; (2) necrobiotic, purulent breaking down, leading to formation of abscesses or ulcers. The period occupied by either stage varies too much to enable any probable limit to be stated.

A considerable area of the body may be undermined and permeated by sinuous passages filled with purulent fluid or

granulation tissue. The latter has a peculiar appearance; it is full of the specific microbe, yellow and red in colour, and is undergoing destruction and suppuration. It easily breaks down under the finger, but is not easy to scrape away. There is no distinct line of demarcation from the healthy tissue.

This last stage, in which the surface is broken down, is the *third stage*, and the peculiarities are so marked that diagnosis of the nature of the disease can hardly be difficult. If the sputum contains the specific microbe, or if there is evidence of contraction of the lung, the lung has probably been the starting point of the disease. Failing either of these distinctive signs, there remains, as essential means of diagnosis, the exploratory puncture. The discovery of metastatic masses of actinomyces in the subcutaneous and muscular tissues is another certain means of diagnosis.

As the disease may take a fatal turn at any time from the occurrence of acute incidental symptoms, its duration can only be approximately given. If it proceed uninterruptedly, its course may be very prolonged; from the commencement of the first characteristic symptoms till death there may intervene from five to twenty months. How long it may exist in the lungs without any evident symptoms is unknown, but from one case it would appear that the disease may last two to three years, and then terminate fatally.

GROUP III.—*Primary Actinomycosis of the Intestinal Tract.*

(*a*) Superficial lesion.

Very characteristic appearances were found in the intestine of a patient of Chiari's, who after two years' suffering died at the age of 34 of progressive paralysis, with general marasmus. This case is exceptional as being undoubtedly one of primary intestinal infection. The mucous membrane of the intestine was everywhere, except at its commencement, covered with a whitish material consisting of patches, some of which were roundish, some longish in shape, about 1 sq. ctm. in area and 5 mm. thick. They were raised above the surface in the centre, and were covered with yellow and brown granules. They could not be separated without removal of some of the subjacent tissue at the same time. Some of the characteristic actinomycotic

granules were calcified; the normal rays of the fungus penetrated into Lieberkühn's glands, which were quite filled with them.

Not a trace of actinomyces was to be found in the mouth or pharynx, or in any of the very carious teeth. The white coating found on the intestine consisted of the mycelium of the fungus, as first described by J. Israël.*

(*b*) Intestinal actinomycosis, with extension of the disease to the peritoneum and abdominal wall.

In a case reported by Dr. Blaschko the intestinal infection originated in the stomach, and in consequence apparently of an adhesion between the abdominal wall and the infected portion of the stomach, the disease extended to the former and a fistula was established, without inflammation or abscess, in the abdominal cavity. The patient rallied from his first illness, and was able to go to his work for two years, when suddenly the disease for the first time assumed the character of general metastatic mycotic infection, with special implication of the joints, and with intermittent pyæmic febrile attacks.

The infection, in a fatal case of Middledorpf's, undoubtedly originated in the intestines, which were perforated in two places —in the anterior wall of the rectum, and in the small intestine, the former being the earlier lesion. From this the disease spread behind the peritoneum towards the sacrum and ilium, and caused perforation in the right hypogastrium.

Zemann reports three cases, two males and a female, all of which terminated fatally.

Pathology and Diagnosis of Group III.: Primary Actinomycosis of the Intestinal Tract.

The intestines may be infected in three ways by actinomyces.

(1) Primarily, by mycotic invasion from the interior of the intestine;

(2) Secondarily, through mycotic embolism of the intestinal vessels;

(3) By direct extension of the actinomycotic process from neighbouring parts to the intestines.

Cases of primary actinomycosis of the intestines may be divided into two classes, just as in the case of the pulmonary

* In 1878.—T. W. H.

affections, viz.: (1) non-destructive superficial disease of the mucous membrane; and (2) destructive parenchymatous progressive actinomycosis.

The parenchymatous form has been observed in the small intestine, the cæcum, and the rectum, either as solitary or multiple masses, and to some extent as a superficial affection. At first the infective masses are small nodules the size of a lentil or pea, situated in the submucous tissue, and sometimes in the mucous membrane itself. When they undergo softening ulceration occurs at the centre, and they become ulcers with undermined edges, the base reaching at times down even to the muscular layer. When ulceration has occurred it may be very difficult to recognize the mycotic nature of the disease except by examination of the neighbouring parts which may have been attacked. The case is still more difficult when cicatrisation has taken place. Tuberculosis and actinomycosis may both occur in the intestine along with cicatrisation of certain portions, and it may be very difficult to recognize the primary affection.

So long as the intestinal affection continues localized a certain *diagnosis* is impossible, except in the case of the rectum. In some patients it may continue a long time without any symptoms, in others the intestinal catarrh which it causes has no characteristic features. The disease may readily extend from the peritoneum to the abdominal wall, or to the abdominal cavity, leading to the formation in the latter of encapsuled abscesses, which, undergoing softening and causing further inflammatory and adhesive processes, lead to great increase in the affected parts. Perforation of the bladder, intestine, or abdominal wall may arise from these intraperitoneal infective masses.

The course of these cases may be very chronic, with little or no elevation of temperature, similar to one class of cases of pulmonary actinomycosis. But just as there are cases of the pulmonary affection which differ from these, in presenting the characters of protracted pyæmia, so in intestinal actinomycosis there are cases in which there occur irregular rigors with high intermittent temperature, the body becoming charged with innumerable actinomycotic emboli, after a stage of an indolent, torpid character has been passed through.

The only means of ascertaining certainly the existence of

intestinal actinomycosis before perforation of the skin, bladder, or intestine, is by exploratory puncture and aspiration. Too small a needle must not be used, so that the mycotic granules may traverse it. A positive find will decide the case, but a negative one will not exclude actinomycosis. The stools and urine should always be carefully examined as to the presence of the specific fungus, whenever there is reason to suspect the existence of the disease.

Group IV.—*Cases of Actinomycosis with Unknown Points of Origin.*

J. Israël has collected five cases,* all of which terminated fatally, and which, though all distinctly actinomycotic, cannot be classed under any of the preceding groups. His intelligent criticism of the symptoms and anatomical conditions of these cases, with a view to ascertain the seat of the primary lesion, will be of great value and interest to any one investigating this disease. These cases present a very great variety of symptoms, and many points of interest, but offer no features calling for notice here.

Ætiology.

No substantial addition has been made to our knowledge of this serious and interesting disease since J. Israël proved in his first work on the subject that the ray fungus (actinomyces) is its cause. It is still unknown where and how the micro-organism is introduced into the body, and what is still more remarkable, the existence of the microbe outside the body has never been ascertained. There is no doubt that the microbe may be introduced through the mouth and the respiratory passages, and there is strong evidence that it has also penetrated from the digestive tract. It has further been shown that the points of entry from the mouth into the tissues may be decayed teeth, dental fistulæ, or wounds caused in extracting teeth; and invasion through the tonsils and pharynx has been shown to be probable. The introduction of the fungus with the food in cases of primary intestinal actinomycosis has also been established

* Patients of v. Langenbeck, Mosdorf, and Birch-Hirschfeld, Ponfick (two cases), and Zemann.

by exhaustive criticism of the history and symptoms of such cases.

Although it is certain that several animals, as well as man, are subject to this disease, no ætiological connection between the disease in each has been established. J. Israël* has proved by experiment that human actinomycosis may be imparted to the rabbit, but both he and Johne obtained negative results in trying to infect a calf by injecting actinomycotic material taken from a man into the jugular vein. On the other hand, infection of man from animals has not so far been demonstrated. Not one of the 38† cases reported was of a person engaged in the management of cattle, such as butchers, slaughterers, farmers, &c., which is rather opposed to the assumption of facility of infection by contact. J. Israël is satisfied that in several of the cases infection by eating pork must be excluded, as the patients were strictly orthodox Jews, and he considers that the extreme care exercised in examining all cattle intended for food, and in rejecting all which have the smallest blemish, makes infection by eating beef no less unlikely.

Hence it would appear that man and animals must be infected from some third and common source, either by vegetables or by water. Unless the spores of the fungus are more capable of resisting the action of water than the mature fungus, water must be excluded. But Jensen‡ has traced an endemic of actinomycosis in Seeland to eating rye grown on newly cultivated soil reclaimed from the sea. Johne's discovery of fungi strongly resembling actinomyces, in grains of rye stuck in the tonsils of pigs, also supports the view that vegetable food may be the source of infection.

The fungi which hitherto, except in König's case, have been found in and upon the carious teeth and tonsils of actinomycotic patients, have been always described as leptothrix. But the accuracy of this description has never been experimentally proved. Undoubtedly many forms may be seen in a preparation of actinomyces which cannot be distinguished from leptothrix, and

* *Centralblatt f. d. med. Wissenschaft*, 1883, No. 27.

† One patient not included in the above (Stelzner, Jahresber. f. Nat. u. Heilkunde, Dresden, 1882-83), who had suffered much from decayed teeth, and contracted actinomycosis, was engaged in managing animals, several of whom had glandular affections.

‡ *Tidskrift f. Veterinaer*, xiii., 1883.

similarly in masses of microbes, distinctly consisting of leptothrix, from the teeth, tonsils, &c., there are many forms to be found very like actinomyces.

It is therefore quite possible that among the micro-organisms denominated leptothrix early forms of actinomyces are to be found at times, which only become typically developed within the body. In what follows Israël sketches the characters of actinomyces from his own observations.* There is great variety in the macroscopic appearance of the actinomyces; sometimes the granules are barely visible, sometimes they are 2 mm. in diameter; sometimes they are smooth, sometimes mulberry-like; sometimes colourless, sago-like; sometimes white, bright yellow or sæpia-brown, yellowish-green to dark green, or speckled. The variations in the microscopic appearances are more important. The most characteristic appearances are the globular masses the surface of which is closely covered with palisade-like rows of the clubbed refracting bodies, while the interior consists of a close network of fine filaments, which are directed radially towards the periphery in undulating curves or spirals, and ultimately after more or less frequent dichotomous branching expand at their ends and form the clubbed bodies.

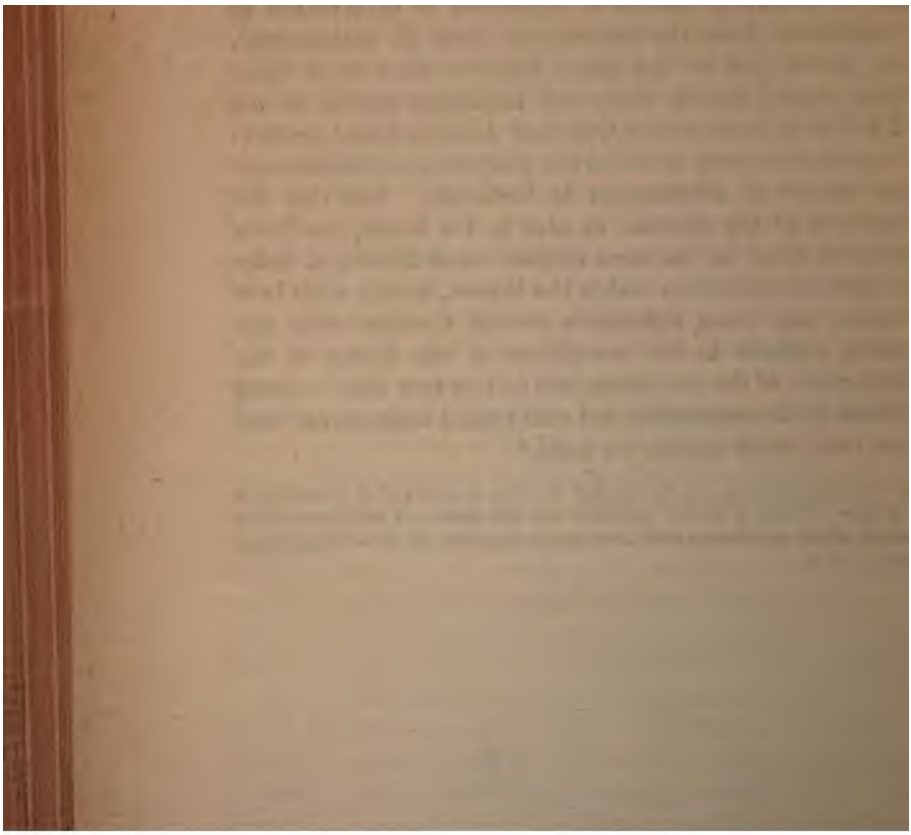
The first variation from this quasi-schematic type is produced by vegetative alterations in these clubbed extremities, partly through budding, leading to hand or fan-like structures, and partly by transverse segmentation, a change which Israël, as the result of repeated very careful observations, maintains to be correct in spite of the objections urged by Ponfick. Rudimentary development of the clubbed bodies leads to other variations, these bodies often appearing little broader than the filaments of which they form the extremities. The size may be diminished so that in extreme cases no clubbing is visible at all, but merely a greater power of reflecting light at the extremity, and in some cases the termination of the filaments offers no distinguishing character from the filaments themselves. Some colonies have many, some very few, and some no clubbed bodies at all, but consist entirely of mycelium arranged radially towards the periphery. The latter stage of development corresponds exactly to the streptothrix of Ferd. Cohn.

* For drawings see J. Israël's "Neue Beobachtungen auf dem Gebiete d. Mykosen d. Menschen," Virchow's *Archiv*, Bd. 74, Tafel ii.—v.

In other forms it is impossible to recognize any arrangement to which the term ray fungus (actinomyces) is applicable, but their similarity with leptothrix of the mouth is most striking, and J. Israël considers that when found in the deposits on the teeth they could not be recognized as actinomyces.

The fact that true dichotomy sometimes occurs in leptothrix, according to J. Israël, renders it impossible to differentiate at times leptothrix from the streptothrix form of actinomyces. J. Israël states that he has found forms in colonies of leptothrix from dental mucus which had analogous growth to the clubbed bodies of actinomyces, but they have no radial arrangement; and he lays great stress on the morphological resemblance in other points of actinomyces to leptothrix. But that the characteristics of the microbe as seen in the mouth should be different from those of the same microbe when developed under entirely different conditions within the tissues, is only what is to be expected, and these differences do not therefore offer any insuperable obstacle to the acceptance of the theory of the original identity of the two forms, and in this may also be found the solution of the remarkable fact that typical actinomyces have never yet been found outside the body.*

* Very little consequence can be attached to these morphological resemblances between groups of what is termed leptothrix and the masses of actinomyces, and they certainly afford no evidence that these micro-organisms are in any way related to each other.—ED.



RECENT PAPERS

ON DISINFECTION.

ABSTRACTED BY

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I.—ON DISINFECTION.

By Dr. R. KOCH,

(*Mittheilungen aus dem K. Gesundheitsamte*, vol. i.)

There has up to the present time been no accurate knowledge as to the mode of action of disinfectants, or as to their real utility. Nor is this surprising, considering how little still is known of the nature of the contagia which disinfectants are required to destroy.

It cannot yet be regarded as proved that all infective matters are organised, and even in those instances where the existence of an organised contagium may with more or less probability be assumed, it is still possible that the respective micro-organisms may be very differently constituted, and, therefore, very differently affected by the same reagent.

Hence, for strict accuracy, it would be necessary to test a disinfectant separately against each and every form of virus which it is called upon to destroy, and under the same conditions as in actual practice. Thus if sulphurous acid were to be tested with reference to the disinfection of rooms, it would be requisite to experiment on rooms which were infected by patients suffering from scarlet fever, diphtheria, &c., respectively; and, after applying sulphurous acid, to prove that the infective matters have been rendered harmless. Such proof is hard to obtain. Further outbreaks of the same illness may happen in the room, and so show that the contagium still remains active, but its destruction cannot be inferred from the non-appearance of the disease. Direct proof of the value of disinfection can only be obtained where the disease can be readily and certainly imparted to animals, but at present this is scarcely practicable for any of the known infectious diseases, and it is very questionable whether it will ever be so for all, or even for the greater number. Other means must, there-

fore, be found of forming an approximately accurate estimate of the value of disinfection.

All those who have studied this problem have commenced with the assumption that the contagia have the closest similarity to ferments, and that since the former are not available for experiment the latter may without hesitation be taken as their representatives in testing disinfectants. The influence of the ever-progressing study of the organised, living ferments or micro-organisms has been so strong that almost without exception this species of ferment alone has been employed. No notice has, however, been taken of the fact that the different forms of micro-organisms have not always the same relation to disinfectants; that some, having no special protective covering, are readily acted upon, while others are enclosed in a stout envelope as "resting-spores," and resist destructive influences in an almost incredible way.

Until it is proved that all contagia are micro-organisms, it seems very one-sided to test disinfectants by means of micro-organisms only, to the neglect of the non-organised ferments.

Experiment has shown the uselessness of seeking for universal disinfectants, which will serve for all conditions under which disinfection is required. We shall far sooner learn how to disinfect with certainty in all cases if the various disinfectants are employed each in its own place and with due regard to its chemical and physical properties. The problem should be subdivided more systematically than has hitherto been done; clothing, linen, bedding, &c., must be dealt with quite differently from compact articles, and one disinfectant may be the best for sick-rooms, while another is most suitable for ships or railway carriages. The examination of a disinfectant must take into account the conditions under which it is to be employed in practice.

Although the estimation of the value of a disinfectant by observation of its effect upon micro-organisms cannot be conclusive, this method has incontestable advantages, and is so simple that in every case the inquiry should begin in this way.

It seems to be universally agreed that a true disinfectant must have the power of destroying all living micro-organisms and

their spores, and not merely that of checking their growth. The most resistent bodies at present known are the resting-spores of *bacilli*, a group which comprises many pathogenic organisms, including those of anthrax, leprosy, typhoid fever (according to Eberth), artificial septicæmia of mice, and many others. All these possess spores, which resemble the resting-spores of other bacilli in their resistent properties. It is very probable that many pathogenic bacteria at present unknown will be found to belong to this group. If the view that ordinary malaria is due to a bacillus is confirmed, it may be assumed that all malarial diseases belong also to this category. Spore-forms may be suspected in diseases, such as small-pox, the contagia of which long retain their virulence even in the dry state.

It is therefore essential that a disinfectant which is to be employed against contagia of unknown and possibly highly resistent properties shall be able to destroy the spores of bacilli.

In the earlier experiments the tests of successful disinfection generally trusted to were loss of motion in the bacteria, prevention of the development of smell, deodorization of a putrefying material, &c. These, however, were found to be untrustworthy criteria, and it has of late been recognized that loss of vitality in the bacteria is the only reliable test.

The plan adopted in almost all recent experiments has been to take putrescible liquids, such as infusion of tobacco or of meat, and after a sufficient growth of bacteria has occurred, to expose a portion of the infusion, or articles impregnated with it, to the action of the disinfectant. A small quantity is then transferred to a suitable liquid cultivating medium, protected from accidental contamination by aërial germs by means of a plug of cotton wool. If the cultivation fluid becomes cloudy, growth of bacteria, and hence imperfect disinfection, are inferred, while if it remains clear the disinfection is considered to have been complete.

There are several weighty objections to this method. In the first place, the experiment is made with an uncertain mixture of many species of bacteria, and it is not ascertained which varieties are affected by the reagent and which are not. It is also a matter of chance whether spores are present or not, and thus contradictory results may be obtained. Further, the sources of error which attend "pure cultivation" apply here

with greater force. If a given species, say a bacillus, is being cultivated alone, the appearance of micrococci among the bacilli shows that accidental impurities have gained access to the cultivation, and that it is no longer pure, so that no importance can be attached to the result. If, however, we are experimenting, as in the above method, with a mixture of unknown varieties of bacteria, it is impossible to determine, when growth occurs in the test fluid, whether the bacteria were introduced accidentally or developed from those acted on by the disinfectant. Moreover, cloudiness of the cultivation fluid may be due merely to precipitates, and, on the other hand, a clear fluid may be found to contain bacteria when examined under the microscope.

Dr. Koch, therefore, abandoned this method, and arranged his experiments upon the following principles. Pure cultivations were obtained of species of bacteria which are not liable to occur in accidental impurities derived from the air, and which at the same time possess striking and characteristic peculiarities. Solid cultivating material was employed in place of liquid, in order to avoid the necessity of complex precautions. Small quantities of the respective pure cultivations having been exposed to the action of disinfectants in the manner to be described later on, were transferred to solid nutrient jelly or potato, and the resulting growth, if any, compared with that occurring in a control experiment, which was made in every instance. By these means such complete evidence is obtained as to the vitality of the respective bacteria, before and after disinfection, that errors are entirely excluded.

Micrococcus prodigiosus and the bacteria of blue pus were selected as representing those bacteria which form no spores and are readily destroyed by disinfectants. Both produce such characteristic growths upon potato that confusion with other bacteria is impossible. For example, the cut surface of a potato is inoculated with these bacteria, and after development has occurred a thin slice is dried; a portion of this is then exposed to the action of a disinfectant. It is then laid upon the cut surface of a boiled potato, and there appears a rapidly growing and spreading red colony of *micrococcus prodigiosus*, or a light brown colony of the bacteria of blue pus. The growth occurs not at a point, as in the case of accidental contamination, but all around the fragment, and can only be due to the

fact that the disinfectant has failed to destroy the bacteria in question. If, on the other hand, the disinfected fragment of potato yields no such growth, while in a control experiment a similar fragment, not disinfected, gives rise to copious growth when placed upon the surface of a boiled potato, it is demonstrated to a certainty that the bacteria have been effectually destroyed.

The bacilli of anthrax from the spleen of animals just killed, and other pathogenic bacteria, were also employed as samples of micro-organisms without spores. Anthrax spores were chiefly used to represent the spore forms. It was desirable to test the disinfectants directly upon pathogenic bacteria, and this species had the further advantage of being readily recognized by its peculiar mode of growth when cultivated upon nutrient jelly, and also by the results of inoculation experiments upon animals. Other spores, such as those of the hay bacillus and potato bacillus, and those which occur in ordinary garden earth, were also occasionally employed.

It is necessary to guard against carrying to the nutrient material too much of the disinfectant along with the sample of bacteria tested, for otherwise the disinfectant might hinder their development, though it had not killed them. To avoid this Koch takes as small a sample as possible, and employs a relatively large cultivation area, so that the trace of disinfectant is so much diluted as to lose any power of checking the growth of the bacteria. In the experiments with anthrax spores, for instance, the material employed consisted of short pieces of silk thread soaked in some cultivating fluid containing the spores, and then dried. In doubtful cases the disinfectant was washed off by means of sterilized distilled water, alcohol or other indifferent liquid, or recourse was had to inoculation of animals.

Under certain circumstances complete disinfection, *i.e.*, destruction of all organisms, may be impossible, or it may be only necessary to check for a time the growth of the organism, as for instance in dealing with large quantities of water, such as trade effluents or sewage. It is therefore necessary to test substances with regard to their power of hindering development, as well as with regard to their disinfecting power. As a rule the reagents best adapted for this purpose will be those which are found to be the best disinfectants, suitably diluted.

A complete examination of a reagent with regard to its disinfectant action must therefore include the following points:—

Its power of destroying micro-organisms and their spores. Of this the destruction of the spores of bacilli may be accepted as complete evidence.

Its action upon less resistent micro-organisms, such as spores of fungi, torulæ, dried and moist bacteria.

Its power of checking the growth of micro-organisms in cultivating media.

The degree of concentration and length of time required in each case; the influence of the solvent, of temperature, and of preparatory treatment, such as moistening the object to be disinfected.

The diffusion of gaseous disinfectants in closed spaces; and the effect of combinations of several disinfectants.

To apply this programme to all disinfectants would involve many years' labour. The following experiments were designed to give a reliable preliminary estimate of the value of the various disinfectants, and only the reagents recently advocated, and those which seemed worthy of it, were subjected to further investigation.

CARBOLIC ACID.

1. *Carbolic Acid in Aqueous Solution.*

a. Action on Spores.

Anthrax spores dried upon silk threads were placed in carbolic solutions of various strengths, and kept in corked bottles. A thread was removed from time to time, and planted on the nutrient jelly. The following table shows the results:—

Strength of carbolic solution.	Days of exposure of anthrax spores.
2 per cent.	1, 3, 5*
5 „	1, 3
1 „	1, 2, 3, 4, 5, 7, 15
2 „	1, 2, 3*, 4*, 5*, 7*
3 „	1 ,2*, 3*, 4*, 5*, 7
4 „	1*, 2*, 3, 4
5 „	1*, 2, 3, 4

The plain figures in the second column signify that the spores

removed from the disinfectant solution on those days developed on the nutrient jelly as actively as in the control experiments, and had therefore not been affected by their exposure to the action of the carbolic solution. The figures bearing an asterisk signify that the spores transferred to the jelly on those days yielded scanty or retarded growths, less active than those of the control experiments. The doubly underlined figures indicate complete sterilisation—that is, that the spores removed from the carbolic solution on the corresponding days yielded no growth whatever upon the jelly.

Thus 1 per cent. and 2 per cent. failed to destroy anthrax spores within a week; 3 per cent. took seven days, 4 per cent. three days, and even 5 per cent. required more than one day.

These results were most unexpected, since it is customary to regard a 2 per cent. solution of carbolic acid in water as able to destroy all germs in a few seconds or minutes. The surgeon washes his hands, and cleanses his instruments in such a solution,* and believes that he has thereby rendered them free from living organisms, and that they may then with safety be brought into contact with open wounds. We now see, however, that beyond the mere mechanical effect of washing, such precautions are of no avail whatever in the case of organisms as resistent as anthrax spores.

For practical purposes a disinfectant should not require much longer than twenty-four hours, as it loses power by volatilization, dilution, or chemical changes. Measured by this standard even the 5 per cent. solution of carbolic acid is unreliable, although protected against the above causes of deterioration. Complete disinfection is much more difficult to attain in the case of complex fluids in which carbolic acid causes precipitates, and possibly forms less active compounds, or if the objects to be disinfected can only be brought into contact with the disinfectant for a short time. For such purposes not less than a 10 per cent. solution would be necessary, and it then becomes a question whether its cost and destructive properties do not render it liable to be superseded by other reagents to be described later on.

* This is hardly correct. A 5 per cent. solution is generally used for these purposes.—ED.

b. Action on Bacteria destitute of Spores.

Bacilli anthracis. Bacilli from the spleen of a mouse dead of anthrax, in which spores are not formed, were dried upon silk threads, and subjected to experiment in the same manner as the anthrax spores, but with very different result. Two minutes exposure to a 1 per cent. carbolic solution killed the bacilli. It was also found that blood from an animal dead of anthrax, mixed with an equal volume of 1 per cent. carbolic solution and allowed to stand for a short time, did not infect an animal into which it was injected; but if the strength of the carbolic solution were only 0·5 per cent. anthrax ensued.

Carbolic acid cannot therefore be regarded as a reliable disinfectant, since it has not the power of destroying resting-spores, under the conditions of practical disinfection. In exceptional cases, where spore forms are known to be absent, it might be employed with advantage.

c. Inhibition of Growth of Bacteria.

Threads charged with anthrax spores were placed in a series of seven vessels, each containing 10 ccm. of fresh clear blood serum; and to these were added respectively 1, 2, 4, 6, 8, 10, and 15 drops of a 2 per cent. carbolic solution. They were protected from evaporation and pollution by dust, by means of a cover. In the first four the growth was as active as in the control experiments; in the fifth it was delayed, but ultimately equally vigorous; in the sixth there was only a scanty growth of short and crooked threads; in the seventh there was no growth whatever, but the spores still grew vigorously, after 72 hours' immersion, when transferred to fresh nutrient jelly.

These experiments were repeated with similar quantities of fluid, the blood serum, however, being replaced by a solution containing 1 per cent. of peptone, and ½ per cent. of meat extract. Five drops of 2 per cent. carbolic solution had no effect, ten drops caused marked retardation, and twenty drops entirely prevented the growth.

Hence one part of pure carbolic acid in 850 parts of the cultivating fluid completely prevents the growth of anthrax bacilli, while one part in 1,250 causes marked diminution of activity.

These proportions apply only to carbolic acid and anthrax bacilli. It was observed that in some instances aërial organisms, accidentally introduced, developed freely in carbolised media in which anthrax bacilli remained quiescent.

Jalan de la Croix found that carbolic acid in the proportion of 1 to 400 or 1 to 500 was sufficient to prevent the growth of aërial organisms in meat infusion.

2. *Carbolic Acid Vapour.*

Schotte and Gaertner had shown that to destroy putrefactive organisms in dry objects, 15 grms. of carbolic acid vapour to the cubic metre are required, so that disinfection of rooms by this method is impracticable. It remained to be seen how far a higher temperature, or longer exposure at ordinary temperature, would modify these results.

1. Earth, containing spores of bacilli, was kept in air saturated with carbolic vapour at the ordinary temperature for 45 days, without diminution of the vitality of the spores at the end of that time.

2. Earth, containing spores, was placed in a flask through which by means of an aspirator air saturated with carbolic vapour at 20° C. was drawn. The temperature within the flask could be varied at will. Exposure to the vapour at a temperature of 55° C. for half an hour had no apparent effect upon the vitality of the spores, but after three hours exposure there was but little growth upon the jelly. Two hours exposure at 75° C. was followed by sparse growth. It has been already seen that at ordinary temperatures (15° to 20° C.) there was no effect even after 45 days' exposure.

We may conclude that the disinfection would be complete at 75° C. in five or six hours, but it would take several hours to raise the temperature of the interior of large objects above 50° C., even if the disinfecting chamber were at 100° C., so that the total duration would not be less than eight or ten hours. For practical purposes disinfection by heat and carbolic vapour must not occupy more than two hours.

For the present the investigation was not carried beyond this point, but it is evident that disinfection by means of carbolic

vapour and dry heat is scarcely practicable in the case of large objects, though with high temperatures it may be applicable to smaller articles. Carbolic vapour with *moist* heat promises good results.

The increased activity of carbolic vapour at higher temperatures suggested the possibility that the same might prove to be the case with other volatile reagents, which at ordinary temperatures have but slight power of disinfection. The following table gives the results of experiments made to test this point:—

Vapour, generated at 20° C., of	Temperature in disinfecting flask.	Duration of exposure of earth-spores in hours.	Subsequent cultivation on nutrient jelly.
Carbolic Acid	55° C.	½	Luxuriant growth.
"	55	1½	Many colonies of bacilli.
"	55	3	Few colonies.
"	75	2	Very few colonies.
Bisulphide of Carbon	50	½	Luxuriant growth.
" "	50	1	Luxuriant growth.
" "	50	3	Very few colonies.
" "	80	½	Few colonies.
" "	80	1	Very few colonies.
" "	80	2	No growth.
Benzol	67	½	Luxuriant growth.
"	67	1	Luxuriant growth.
"	67	2	Luxuriant growth.
Crude Wood-spirit	70	3	Luxuriant growth.

Bisulphide of carbon, therefore, like carbolic acid, gains in power at higher temperatures.

3. *Carbolic Acid in Combination.*

Several compounds containing carbolic acid were tested as to their power of destroying anthrax spores, but all proved to be less powerful than the pure acid. The experiments were performed in the manner already described.

		Days of exposure of anthrax spores.		
Carbolate of Soda, 5 per cent. in water			1*, 10*	
Sulpho-carbolate of Soda, 5 per cent. in water		1, 10		
Sulpho-carbolate of Zinc, 5 per cent. in water			1*, 2*	5
Crude Wood-spirit	undiluted	1, 2	5*	20
Crude Pyroligneous acid	undiluted	1,		1/2
Wood-tar	undiluted	1, 20		
Coal-tar	undiluted	1, 20		

(The signs have been previously explained, under the first table.)

Undiluted crude pyroligneous acid appears to be equivalent in power to a 5 per cent. solution of carbolic acid. Of the carbolic compounds the sulpho-carbolate of zinc stands next to carbolic acid as a disinfectant.

"Disinfection" by carbolic acid often consists of washing with a 1 per cent. or 2 per cent. solution, or free application of milk of lime containing 2 per cent. of carbolic acid. Solutions of this strength could have no effect upon spores of bacilli, but in order to ascertain whether stronger solutions would give the desired results, the conditions of practical disinfection were imitated experimentally by placing threads impregnated with anthrax spores in the crevices of a rough board. 5 per cent. solutions of carbolic acid, in water and milk of lime, were daily poured freely over boards so prepared, but after seven and even ten repetitions of this process development occurred at various parts of the threads when transferred to nutrient jelly. Thus, though many spores were destroyed, the result could not be regarded as a true disinfection.

4. *Carbolic Acid dissolved in Oil and in Alcohol.*

Anthrax spores were found to be absolutely unaffected by lying for 110 days—upwards of three months—in a 5 per cent. solution of carbolic acid in oil, and equally so by 70 days' exposure to a 5 per cent. solution in alcohol.

Even the sensitive anthrax bacilli were not more affected by 5 per cent. solution of carbolic acid in oil than by pure oil* :—

	Days of Exposure of Bacilli anthracis
Carbolic acid, 5 per cent. in olive oil	1, 2, 3*, 4*, 6
Carbolic acid, 1 per cent. in olive oil	1, 2, 3*, 4*, 6
Pure olive oil	1, 2, 3, 4*, 6

These results are utterly contradictory to all former experience and belief, but there is no possibility of error in the experiments, as they were repeated several times, and always with concordant results. Moreover, the same holds good as regards thymol and salicylic acid. It might be supposed that the enveloping membrane of the spores is impervious to carbolic acid unless softened by

* The table shows a slight difference upon the third day of exposure.—B. A. W.

imbibition of water, but the action of carbolic and other vapours upon dry spores tells against this view.

If carbolic oil comes into contact with substances containing water—for instance, the tissues of the human body—doubtless part of it will be taken up and exert some antiseptic action. In all other cases, as when it is sought to disinfect dry objects, such as instruments, silk, catgut, &c., by means of carbolic oil, there is absolutely no effect, even upon the least resistent micro-organism, beyond that due to the oil itself.

Bearing in mind that carbolic oil is absolutely inert, and that a spray of 2 per cent., or even 5 per cent., carbolic solution can have no appreciable effect upon spores in the brief time occupied by a surgical operation, and, further, that in order to prevent bacterial growth the carbolic acid must be present in the proportion of 1 to 400, it cannot any longer be a matter for surprise that, in spite of the most scrupulous antiseptic precautions, bacteria are so often found under Listerian dressings.*

SULPHUROUS ACID.

In the following experiments sulphurous acid was generated by burning sulphur, and the percentage volume of the gas determined by analysis.

I. *Experiments conducted in an almost air-tight chamber of 390 litres capacity.*

Experiment 1. Percentage volume of sulphurous acid at the beginning of the experiment, 0·986; after $3\frac{1}{2}$ hours, 0·930.

Test Organisms.		Minutes of Exposure.
Micrococci contained in the blood of a guinea-pig, dried upon threads..	α Dry ...	1, 2*, 5*, 10*, 15*, 20, 30
	β Moist...	1*, 2, 5

Experiment 2. Percentage volume of sulphurous acid at the

* This statement is somewhat misleading. Judging from Dr. Koch's experiments with carbolic acid one would certainly expect to find bacilli in wounds treated antiseptically, because the spores would not be killed, while one would equally expect micrococci to be absent, because they are readily destroyed. But exactly the reverse is the case: bacilli are never present, unless indeed there has been very great carelessness, while micrococci are frequently found. The explanation of this fact is given in my paper in the *Path. Trans.*, vol. xxx., and in my Antiseptic Treatment of Wounds. As an antiseptic in wound treatment carbolic acid is much more satisfactory than would be supposed from Dr. Koch's experiments.—Ed.

beginning of the experiment, 1·0; after 24 hours, 0·75; after 72 hours, 0·54.

	Minutes of Exposure.
Anthrax bacilli (dried upon silk thread)	8, 10, 12*, 15*, 20*, 30, 40
Anthrax spores (dried upon strips of filter-paper)	Unaffected by 72 hours' exposure.
Spores of potato bacillus (dried upon strips of filter-paper)	

Experiment 3. Percentage volume of sulphurous acid at beginning of experiment, 6·13; after 24 hours, 4·88; after 72 hours, 4·47; after 96 hours, 3·3.

Results:—Anthrax spores, earth spores, and spores of the hay bacillus were unaffected by 96 hours' exposure, in the dry state.

Experiment 6. Percentage volume of sulphurous acid at beginning, 0·120; after 24 hours, 0·119; after 48 hours, 0·100.

Test Organisms.	Results. Hours of Exposure.
Micrococcus prodigiosus (A)	4, 20, 28, 48
„ „ (B)	4, 20*, 28*, 48*
Bacteria of blue pus (A)	4, 20, 28, 48
„ „ (B)	4, 20*, 28*, 48*

In this and the following experiment one sample of each variety (A) was exposed upon the under surface of a slice of potato, the other (B) upon the upper surface.

Experiment 7. Percentage volume of sulphurous acid at commencement, 0·84; after 24 hours, 0·55; after 48 hours, 0·302. Excess of moisture was present.

Test Organisms.	Results. Hours of Exposure.
Anthrax bacilli (upon silk threads)	1, 2, 4, 24, 48
Micrococcus prodigiosus (A)	1, 2, 4, 24, 48
„ „ (B)	1, 2, 4*, 24, 48
Bacteria of blue pus (A)	1, 2, 4, 24, 48
(B)	1, 2*, 4, 24, 48
Anthrax spores	1, 2, 4, 24, 48
Earth spores	1, 2, 4, 24, 48

Experiment 9. 26 grammes of sulphur were burnt. The percentage volume of sulphurous acid shortly afterwards was 4·66; after 6 hours, 3·16; after 24 hours, 3·28.

The atmosphere in the disinfecting chamber was saturated with moisture.

The test objects were samples of anthrax spores and garden earth, some of each (C) having been previously kept for 24 hours in an atmosphere saturated with moisture, while the others (D) were placed in distilled water for the same period.

They were then exposed to the sulphurous acid vapour for 24 hours.

All the samples of anthrax spores were destroyed. Of the earth spores, those which had been kept in water were unaffected, but those kept in moist air gave a scanty and retarded growth upon gelatine.

Experiment 10. Percentage volume of sulphurous acid shortly after kindling the sulphur, 5·44; after 3 hours, 5·3.

The conditions and test objects were the same as in the last experiment, except that the soaked samples (D) were exposed (in a watch glass) without removing them from water.

The results were upon the whole less favourable than in Experiment 9. Both samples of earth spores were unaffected. Of the anthrax spores, sample D was destroyed, but sample C retained full vitality.

II. *Experiments in an ordinary room.*

Experiment 4. Percentage volume 1 hour after setting fire to the sulphur, 2·89; after 24 hours, 0·02; after 48 hours (end of experiment), 0·01.

The test objects were cultivations of *Micrococcus prodigiosus*, pink yeast, and bacteria of blue pus. All these, though exposed freely, retained their vitality after 48 hours' exposure.

Experiment 5. Percentage volume of sulphurous acid half an hour after setting fire to the sulphur, 3·12; after 2 hours, 1·25; after 22 hours, 0·015.

The test organisms, viz., dried cultivations of *Micrococcus prodigiosus* and of the bacteria of blue pus, were unaffected by 50 hours' exposure.

Experiment 11. 3,690 grammes of sulphur were burnt in a room, and the percentage volume of the sulphurous acid produced was calculated to be 10·56.* About an hour later, however, it was only 4·05, and after $3\frac{1}{3}$ hours 1·8.

* The common practice of allowing 1 lb. of sulphur per 1,000 cubic feet would give theoretically a percentage volume of about 1·1.—B. A. W.

The air of the room was saturated with moisture.

Samples of anthrax spores and earth spores (some dry, some previously moistened as in Experiments 9 and 10) were placed in various parts of the room—*e.g.*, in the centre, in corners, in the pocket of a coat, in crevices between boards, &c., &c.

After 24 hours' exposure to sulphurous acid as above, *one* sample of anthrax spores (which had previously been soaked in distilled water) was so far affected as to grow more slowly than the rest when planted upon nutrient jelly; but all the rest, whether dry or moist, whether exposed freely or hidden in crevices, grew just as vigorously as those employed in the control experiment, which had not been exposed to sulphurous acid at all.

These results show that for purposes of practical disinfection sulphurous acid is useless.

In the comparatively dry state (Experiments 2, 3, 6) spores of bacilli, and even *Micrococcus prodigiosus* and the bacteria of blue pus, survived protracted exposure, although (Experiments 1, 2) anthrax bacilli were quickly destroyed if exposed directly to the action of the gas, in very thin layers—as, for instance, upon silk threads. The addition of excess of moisture (Experiments 1, 7) gave slightly better results.

Preparatory moistening (Experiments 9, 10) was found to favour the action of sulphurous acid upon spores in a manner which it is not easy to explain. Dr. Koch suggests that there may be, outside the proper spore-membrane, a mucoid covering which is capable of being softened by water or certain saline solutions, but not by water containing sulphurous acid. Thus a solution of sulphurous acid being unable to penetrate this covering would have no effect upon the spores unless they were already thoroughly moist. Even here, however, under conditions far more favourable than occur in practice, there was no complete disinfection of spores of bacilli. In Experiments 4, 5, 11 the conditions were more similar to those of actual practice. The results show how rapidly the gas disappears when it is generated in an ordinary room. In Experiment 11, where the initial proportion of sulphurous acid was large, it failed almost completely to kill even those spores which were freely exposed after being thoroughly moistened.

Sulphurous acid in aqueous solution.—11·4 per cent. by weight

(4,000 per cent. by volume) killed anthrax spores in two days; 5·7 per cent. took five days.

Sulphurous acid in combination with a base.—A solution of sulphite of lime, containing 90 grms. of sulphurous acid per litre, took 15 days to kill anthrax spores.

CHLORIDE OF ZINC.

Zinc chloride has ranked as a disinfectant of such potency as to be considered effective even in 0·1 per cent. watery solution; but the following results prove it to be practically worthless:—

Sporeless micro-organisms.—1 per cent. watery solution failed to destroy *Micrococcus prodigiosus* in 48 hours, although after 16 hours' exposure the cultivation was somewhat scanty.[1]

Spores of bacilli.—5 per cent. watery solution had not at the end of a month in any way affected anthrax spores. Preliminary soaking in water made no difference in the result.

Checking development—0·5 per cent. had no effect in retarding the development of anthrax spores in blood serum.

Since the three principal disinfectants (carbolic acid, sulphurous acid, and zinc chloride) hitherto in common use prove to be unreliable, it becomes necessary to seek for others, and with this object a long series of experiments was instituted, the results of which are given in the following table. Anthrax spores (dried upon silk threads) were chosen as test objects, for the reasons already stated. The experiments are in many cases to be regarded merely as preliminary to further investigations; for instance, salicylic acid and thymol ought to be further tested in aqueous solution, it having been found in the course of the inquiry that certain reagents which possess some power in aqueous solution lose it when dissolved in alcohol or oil.

Reagent.	Solvent.	Strength.	Result. Days of Exposure.		
Distilled water	—	pure	90		
Alcohol	—	absolute	110		
„	water	50 per cent.	110		
„	„	33 per cent.	110		
Ether	—	pure	5	8*	30
Acetone	—	„	2	5*	
Glycerine	—	„	110		
Butyric acid	—	„	5		
Oil	—	„	90		
Bisulphide of carbon	—	„	20		
Chloroform	—	„	100		
Benzol	—	„	20		
Petroleum ether	—	„	5		
Oil of turpentine	—	„		1*	5
Chlorine	water	?			1
Bromine	„	2 per cent.			1
Iodine	„	?			1
Hydrochloric acid	„	2 per cent.	5		10
Ammonia	„	?	10		
Ammonic chloride	„	5 per cent.	25		
Sodic chloride	„	Concentrated.	40		
Calcic chloride	„	Concentrated.	40		
Baric chloride	„	5 per cent.	100		
Ferric chloride	„	5 per cent.		2*	6
Potassic bromide	„	5 per cent.	25		
Potassic iodide	„	5 per cent.	80		
Mercuric chloride	„	1 per cent.			1
Arsenic	„	0·1 per cent.	6		10
Lime water	„	?	10	15*	20*
Chloride of lime	„	5 per cent.		1*	2* 5
Sulphuric acid	„	1 per cent.	3	10*	20*
Zinc sulphate	„	5 per cent.	1	5*	10*
Cupric sulphate	„	5 per cent.	1	5*	10*
Ferrous sulphate	„	5 per cent.	6		
Aluminic sulphate	„	5 per cent.	12		
Alum	„	4 per cent.	12		
Potassic chromate	„	5 per cent.	2		
Potassic bichromate	„	5 per cent.	2		
Chrome alum	„	5 per cent.	2		
Chromic acid	„	1 per cent.	2		
Potassic permanganate	„	5 per cent.			1
„ „	„	1 per cent.	2		
Potassic chlorate	„	5 per cent.	6		
Osmic acid	„	1 per cent.			1
Boracic acid	„	5 per cent.	2	6*	10*
Borax	„	5 per cent.	15		
Sulphuretted hydrogen	„	?	1	5*	
Ammonic sulphide	„	?	2		5
Oil of mustard	„	?	5	10*	
Formic acid	„	Sp. Gr. 1·120	2		4
Acetic acid	„	5 per cent.	5		
Potassic acetate	„	Concentrated.	10		
Plumbic acetate	„	5 per cent.	12		
Potash soap	„	2 per cent.	12		
Lactic acid	„	5 per cent.	5		

Reagent.	Solvent.	Strength.	Result. Days of Exposure.			
Tannin	water	5 per cent.	10			
Trimethylamine	"	5 per cent.	12			
Chloro-picrin	"	5 per cent.	2			6
Benzoic acid	"	Concentrated	70			
Sodic benzoate	"	5 per cent.	10			
Cinnamic acid	†	2 per cent.	10			
Indol	water	Satd. Soln.	80			
Skatol	"	Satd. Soln.	80			
Leucin	"	0·5 per cent.	10			
Quinine	††	2 per cent.		1*	5*	
"	‡	1 per cent.	5			10
Iodine	alcohol	1 per cent.		1*	2*	
Valerianic acid	ether	5 per cent.	5			
Palmitic acid	"	5 per cent.	5			
Stearic acid	"	5 per cent.	5			
Oleic acid	"	5 per cent.	5			
Xylol	alcohol	5 per cent.	90			
Thymol	"	5 per cent.	15			
Salicylic acid	"	5 per cent.	15			
"	oil	2 per cent.	80			
Animal oil	alcohol	5 per cent.	12			
Essential oil of peppermint	"	5 per cent.	12			

† 60 parts water and 40 alcohol. ‡ Water with hydrochloric acid.
†† 40 parts water and 60 alcohol.

According to Nägeli's theory, germs ought to lose their vitality after lying for a few days in water, but both in distilled water and in drinking water anthrax spores remained for months with unimpaired activity, as determined by cultivation upon gelatine and inoculation of mice.

Bisulphide of carbon, ether, chloroform, benzol, petroleum-ether, and oil of turpentine were tried in the hope that they might act upon the contents of the spores, which must be regarded as containing much fat and but little water. It is noteworthy that ether and oil of turpentine, both ozonizing agents, are the most active of this group. Further experiments showed that the vapour of oil of turpentine failed to affect earth spores in 60 days, and ten days' exposure of anthrax spores to water containing a few drops of oil of turpentine, with frequent shaking, gave a similar negative result. Possibly in some other form, as for instance in conjunction with dry or moist heat, this reagent may prove useful.

It is surprising to find that among reagents which have been regarded as destructive to organic life, some prove to be inert as regards anthrax spores, *e.g.*, hydrochloric acid (2 per cent.),

sulphuric acid (1 per cent.), and concentrated solutions of calcic chloride and sodic chloride; while others, including boracic acid, borax, potassic chlorate, benzoic acid, sodic benzoate, quinine, and almost all metallic salts, have but slight effect.

A good disinfectant must be rapid as well as certain in its action. In no case ought more than 24 hours to be allowed for the complete destruction of all germs, and in many cases only a few minutes exposure can be obtained.

Few out of the long list of substances tried comply with these conditions. Except chlorine, bromine, and iodine, only mercuric chloride, osmic acid, and potassic permanganate (5 per cent.) destroyed anthrax spores within 24 hours. Since a 5 per cent. solution of permanganate is inadmissible for disinfection in bulk, and osmic acid is out of the question, we have left only mercuric chloride and iodine, bromine, and chlorine.

By another series of experiments it was sought to determine roughly the effect of various reagents in checking the development of anthrax bacilli. This question has a direct bearing upon many important problems of hygiene, as, for example, the preservation of food substances. The experiments were conducted in the same manner as those with carbolic acid, already described. The results have, however, a more limited significance than those relating to the destruction of anthrax spores, since anthrax bacilli differ markedly from other bacilli in their relation to disinfectants, as has been seen in the case of carbolic acid. Still it may fairly be concluded that any reagent which fails when so tested will probably fail to check the growth of other pathogenic bacteria, and will certainly fail in the case of the less sensitive bacteria of decomposition.

Again, the results vary according to the composition of the cultivating medium chosen. In all the present experiments with anthrax bacilli either blood serum or peptonized solution of meat extract was used. This point is most important, but has received little or no attention hitherto. As an instance, Davaine found that a highly dilute solution of blood containing anthrax bacilli was still infective when inoculated upon animals, but that upon the addition of iodine it became harmless. The iodine doubtless destroyed the bacilli, and it was assumed from this that when introduced into the system it must be an infal-

lible safeguard against anthrax. But in these experiments the blood was so dilute as to be comparable to water, whereas in the human body the iodine immediately enters into combination with the alkalies contained in quantity by the blood. If the experiment is repeated, using blood serum instead of water, the results are very different; iodine in the proportion of 1 to 7,000 has no effect, and retardation of growth upon gelatine only begins when the proportion reaches 1 to 5,000. Hence to check, in any measure whatever, the progress of anthrax in man, it would be necessary that there should be not less than 12 grammes of iodine constantly in circulation, which is impossible.

Chlorine, bromine, and osmic acid also were found to have less power of preventing the growth of anthrax bacilli in blood serum or peptonised meat extract than might be anticipated from their destructive action upon anthrax spores.

Reagent.	PARTS PER MILLION †		
	Development of Anthrax Spores.		
	Impeded.	Arrested.	
Allyl alcohol	6	12	
Oil of mustard	3	30	
Oil of peppermint	30	?	
Thymol	12½	?	
Oil of turpentine	13	?	
Oil of cloves	200	?	
Potassic arsenite	10	100	
Chromic acid	100	200	
Picric acid	100	*	* 200 insufficient
Hydrocyanic acid	25	125	
Carbolic acid	300	1,200	
Boracic acid	800	1,250	
Borax	500	1,430	
Hydrochloric acid	400	590	
Salicylic acid	300	666	
Benzoic acid	500	?	
Camphor	400	*	* 800 insufficient
Eucalyptol	400	*	* 1,000 insufficient
Quinine	1,200	1,600	
Chloral hydrate	1,000	*	* 2,500 insufficient
Cinnamic acid	*	?	* 1,000 insufficient
Potassic chlorate	4,000	?	
Acetic acid	4,000	?	
Crude pyroligneous acid	4,000	?	
Sodic benzoate	5,000	?	
Alcohol	10,000	80,000	
Acetone	*	?	* 20,000 insufficient

† For convenience of comparison the proportions are here given upon a constant basis, viz., as parts per million.

Reagent.	PARTS PER MILLION. Development of Anthrax Spores.		
	Impeded.	Arrested.	
Sodic chloride	15,500	*	*42,000 insufficient
Iodine	200	?	
Bromine	666	?	
Osmic acid	666	?	
Potassic permanganate	330	714	
Mercuric chloride	1	3	
Potash soap	200	1,000	

Mercuric chloride heads the list, since even in the enormous dilution of 3 parts per million it entirely arrests the development of anthrax bacilli.

Allyl alcohol also has extraordinary power in extreme dilution, and oil of mustard is even more active. The excessive volatility of these bodies renders exact determinations difficult, but in air-tight vessels still more minute proportions than those here given would probably suffice for the preservation of articles of food.

Quinine impedes the development of anthrax bacilli at a dilution of 1,200 parts per million, and entirely prevents it at 1,600 parts per million. These figures agree fairly well with the results obtained by Moczutkowsky, who found that 2,000 parts per million of quinine was necessary in order to destroy the spirochætæ of relapsing fever, and calculated that the dose of quinine required to destroy the spirochætæ in the blood of a man suffering from relapsing fever would be 12 to 16 grms. If quinine were given internally with the intention of acting upon the anthrax bacilli in a case of malignant pustule, it would be necessary to take thirteen times the above dose, since the entire body, and not merely the blood, has to be taken into account. Such calculations serve to show the necessity for caution in advocating the therapeutic use of antiseptics.

If a reagent be intended to restrain bacterial growth in the 5,000 grms. of blood of an adult, it must first be proved that in quantity not exceeding the maximum permissible dose it is able to keep 5,000 grms. of blood, or of cultivating fluid of similar composition, free from bacteria. Having thus established the

possibility of its therapeutic value, it remains to verify this by further observations upon its absorption and loss by excretion, when introduced into the body. Bearing these considerations in mind, there seems little prospect of keeping the whole of the blood free from bacteria, save by antiseptics of exceptional potency.

Potash soap has considerable power in checking the development of anthrax spores, 200 parts per million retarding, and 1,000 parts completely preventing it. Potash, however, has only about one-eighth of this power, while oleic acid (500 parts) and butyric acid (330 parts) are without effect. It is probable, therefore, that some other fatty acid present in the soap is very active.

Some of the substances tried gave precipitates with the cultivation fluid, thus causing loss of important ingredients, and especially of albuminates. In these cases, on account of this loss of nutrient material, the numerical results are not accurate measures of the inhibitory power of the reagent. Thus—

Sulphide of sodium gave no precipitate; 4,000 parts per million had no effect upon growth of anthrax bacilli.

Sulphide of calcium gave a slight precipitate; 2,857 parts per million impeded growth.

Sulphide of potassium gave a heavy precipitate; 500 parts per million impeded growth.

Among the reagents which caused precipitation were chloride of lime, alum, ferrous sulphate, zinc chloride, and plumbic acetate.

The result of both series of experiments is to show that the only real disinfectants (*i.e.*, reagents capable of destroying micro-organisms) available for practical purposes are chlorine, bromine, and corrosive sublimate, while the most potent "antiseptics" (*i.e.*, reagents preventing the growth of micro-organisms) are mercuric chloride, certain ethereal oils, and allyl alcohol. How far these can be practically utilized, and for what particular purposes respectively, are points for future research.

Since sulphurous acid cannot in future be relied upon for the disinfection of rooms, it becomes necessary to return to chlorine and bromine, which have latterly fallen almost completely into disuse. The following experiments seem to show that bromine is, of the two, the more effective.

Anthrax spores and earth spores were suspended a few centimetres above the surface of bromine water, chlorine water and alcoholic solution of iodine respectively, in closed vessels. Some of the spores were taken out at different times and planted on nutrient jelly.

	Days of Exposure. Anthrax Spores.	Earth Spores.
Bromine, 2 per cent. in water	1	1
Chlorine water	1, 2	1, 2, 5
Iodine, 2 per cent. in alcohol	1, 2, 5	1, 2, 5, 10

Moist heat affords the best means of disinfecting movable articles of moderate size, such as bedding, clothing, &c.; but for very large objects, such as railway carriages, other methods must be found. In order to ascertain whether bromine would answer this purpose, anthrax spores were laid upon a smooth board and bromine solution (2 per cent.) was poured over them and allowed to evaporate slowly, which took about half an hour. After the first application the spores yielded a diminished growth upon gelatine, and none after a second. These conditions were, however, more favourable than could occur in practice, and therefore a rough board was substituted, and the bromine water applied in the form of spray, which dried in a few minutes. It was then found that four applications of a concentrated (4 per cent.) solution were necessary before the spores were destroyed. In the proportions used in the experiment six grammes of bromine would be needed for each square metre, or 24 grammes for the (*minimum*) four applications. The internal and external surfaces of a railway carriage amount to about 100 square metres, so that disinfection by this method would be very costly. The spray would have to be applied not less than five times, and each application would cost about five shillings, at the present price of bromine.

Mercuric chloride alone remains, therefore, where heat and gaseous disinfectants are inapplicable. That it has hitherto been

so rarely employed is due partly to its poisonous properties, partly to the unfounded faith in carbolic acid, sulphurous acid, chloride of zinc, &c.

SALTS OF MERCURY.

Further experiments showed that a 1 per 1,000 watery solution of mercuric chloride, mercuric nitrate, or mercuric sulphate destroyed anthrax spores within ten minutes. To ascertain whether simply wetting the material once with the solution would suffice, anthrax spores and earth spores were laid on a rough board, and thoroughly moistened with 1 per 1,000 solutions of the above salts by means of spray. In a few minutes the objects were dry, and were transferred to the nutrient jelly. In every instance the anthrax spores were killed; the earth spores were destroyed by the chloride, but not completely by the sulphate or nitrate.

Anthrax spores which had been exposed for ten minutes to a 1 per 20,000 solution of mercuric chloride and then washed in alcohol, gave no growth upon gelatine, whereas after exposure for an hour to a 1 per 50,000 solution, the growth was as vigorous as in the control experiment. From other experiments it appears that the reagent may produce an effect upon those spores which it does not destroy. Silk threads containing anthrax spores were exposed for ten minutes to 1 per 10,000, 1 per 20,000, and 1 per 50,000 solutions of mercuric chloride, respectively; then washed in alcohol and introduced beneath the skin of mice. All three mice died of anthrax, the first upon the 5th day, the second upon the 4th day, the third upon the 2nd day. The two stronger solutions had, in addition to killing some of the spores, so affected the rest as to retard their development in some unexplained way, but the third mouse died as early as in the control experiment.

In a repetition of this last experiment the solutions were of the same strength, but the spores were left in them for an hour. The first mouse remained alive; the second died between the 3rd and 4th day, and the third died after 40 hours. Here the retardation affected the third mouse also.

Garden earth was completely sterilized by a spray containing 1 part of mercuric chloride in 5,000 parts of water. After a spray of 1 in 10,000, mycelium developed upon the gelatine;

after 1 in 20,000, a few colonies of bacilli; and 1 in 50,000 had no appreciable effect in preventing subsequent growth.

Mercuric chloride is therefore the only known disinfectant which, without any previous moistening or other preparation of the articles to be disinfected, destroys the most resistent organisms in a few minutes by a single application of a highly dilute solution (1 to 1,000 or even 1 to 5,000). With longer exposure it only begins to be unreliable when diluted beyond 1 to 20,000. The sole drawback to its use is its poisonous nature; but owing to its rapidity of action only a quarter or half an hour is needed for disinfection, and the reagent can then be removed by copious washing with water. Minute traces would doubtless remain, but would be absolutely harmless. In certain cases the other and less poisonous salts of mercury may be preferable, but if so the solution must be applied two or three times.

For the disinfection of a ship's bilge only carbolic acid and salts of mercury are suitable, and this instance will serve as an illustration of the advantages of the latter. A 5 per cent. solution of the former must be left for 48 hours, while a 1 to 1,000 solution of mercuric chloride would only require a few minutes. The other salts of mercury would be equally applicable here, as the loss of a few minutes or hours is immaterial. According to American regulations, 100 gallons, say 500 litres, of disinfecting fluid must be thrown in after removal of the bilge water. If carbolic acid be used this would require 25 kilos, which amount of crude acid would cost about thirty shillings. The requisite weight of mercuric chloride or mercuric sulphate would be half a kilo, and would cost 2*s*. 9*d*. or 3*s*.

The proportions indicated by the previous experiments will not hold good unless the whole of the disinfectant is available as such. If liquids containing albumen, or sulphuretted hydrogen, or other bodies forming insoluble compounds with mercury salts, are to be disinfected, enough of the mercuric chloride must be added to leave at least 1 to 5,000 in solution. This may readily be determined by means of bright strips of copper, left in the liquid for half an hour; if the solution contains not less than 1 part of mercuric chloride per 5,000, there will be a distinct formation of amalgam upon the surface of the copper.

In disinfecting liquids by means of mercurial salts it must be remembered that precipitates will form, which if the process is

repeated may accumulate and become dangerous, on account of the mercury which they contain. For this reason salts of mercury are unsuitable for cases in which repeated disinfection is required.*

* The following are some of the more important results of experiments by Schill and Fischer, upon the disinfection of fresh tubercular sputum, containing bacilli and spores (*Mittheilungen aus dem K. Gesundheitsamte*, vol. ii.).

Samples of the sputum were exposed to the action of eight or ten times their volume of the following reagents, which unless otherwise stated were in aqueous solution :—

Reagent.	Strength.	Result. Hours of Exposure.		
Alcohol	Absolute	.....		20 =
Creasote	1 per cent.	.....	20	
Thymol in Alcohol	5 „	.....	18	
Arsenious Acid	1 „	.....	20	
Bromine	1 „	.....	24	
Iodine	0·2 „	.....	20	
Salicylic Acid	Saturated			20 =
Carbolic Acid	1 per cent.	.....	20	
„ „	2 „	.....	20	
„ „	3 „	.....		20 =
„ „	5 „	.....	2	
Acetic Acid	32 „	.....		20 =
Sodic chloride	Saturated	.....	20	
Aniline	Saturated (3·2 per cent.)		2	20 =
Soda	2 per cent.	.....	24	
Potash	10 „	.....	24	

Hot air at 100° C. disinfected only objects of small thickness, spread out and freely exposed for several hours.

A current of steam at 100° C. disinfected in 15 minutes, but should in practice be continued for an hour.

Boiling in water disinfected in 10 minutes.

A 0·2 per cent. solution of mercuric chloride failed to disinfect an equal volume of sputum in 24 hours, owing probably to coagulation.

Absolute alcohol was only effective when in large proportion, not less than 5 volumes to 1 of sputum.

An equal volume of 5 per cent. solution of carbolic acid gave complete disinfection in 24 hours.

A saturated solution (3·2 per cent.) of aniline disinfected one-tenth of its volume of sputum in 24 hours.

Hence the two substances most useful for the disinfection of fresh sputum are carbolic acid (5 per cent. watery solution) and aniline oil (saturated watery solution), and of these carbolic acid is the more satisfactory.

It is noted that in spite of the action of putrefactive organisms, the sputum retained its virulence for six weeks in the moist state, and for three months and upwards if dried.

II.—DISINFECTION BY HOT AIR.

By Dr. R. KOCH and Dr. G. WOLFHÜGEL.

(*Mittheilungen aus dem K. Gesundheitsamte*, vol. i.)

The object of the following experiments was to ascertain whether hot air would maintain its high reputation as a disinfectant when subjected to the crucial tests now at our disposal. No process of disinfection can be accepted as reliable in all cases, until it is shown to have the power of destroying the most resistent micro-organisms within a reasonable time and without seriously injuring the articles requiring disinfection. Specimens of various microbes, including the highly resistent spore-forms of bacilli, were exposed for measured periods to known temperatures, and then transferred to a suitable cultivating medium. The result was in every instance compared with that of a control experiment, similar in every detail save in the exposure to heat.

Two hot-air chambers were employed for the purpose of these experiments. The smaller had a cubic capacity of 6836 cubic metres, the larger 9540 cubic metres. Both were heated by means of coils of copper tubing lining the interior, through which compressed steam was passed, at a temperature regulated by the pressure. The maximum pressure allowed by the strength of the boiler was 5½ to 6 atmospheres, and this gave a temperature of about 140° C. within the hot chamber.

A preliminary experiment showed that the distribution of heat was very unequal, thermometers in different parts of the chamber registering temperatures ranging from 119·7° C. to 131·5° C.

Experiment 1.

Duration, 1½ hours. Initial temperature of the oven 66° C., final 113° C.

Samples of several varieties of micro-organisms were placed in test-tubes, and exposed to the heat of the oven, with the following results.

A. Sporeless bacteria (*Micrococcus prodigiosus*, bacteria of septicæmia of the guinea-pig, and bacteria of septicæmia of mice) were destroyed.

B. Earth spores and other spores of bacilli (anthrax bacillus, hay bacillus, potato bacillus, &c.) were unaffected.

C. Spores of fungi. *Penicillium glaucum* was killed, but *Aspergillus niger* unaffected.

Experiment 2.

Duration, $1\frac{1}{2}$ hours. Initial temperature of oven, 110° C.; final, 118° C. The test objects were exposed as in the previous experiment. The apparatus was not ventilated, and the hot air was saturated with moisture.

A. Sporeless micro-organisms, including *Micrococcus prodigiosus*, *Bacillus Anthracis*, bacteria of septicæmia of mice, pink yeast, and several others, were completely destroyed.

B. Spores of bacilli (including amongst others spores of potato-, hay-, and anthrax bacilli, and garden earth), were unaffected, except that in the case of anthrax the growth was retarded, though abundant.

C. Spores of fungi, including *Penicillium glaucum*, *Aspergillus niger*, and *Botrytis vulgaris*, were completely destroyed

D. The samples of earth spores and spores of hay- and potato- bacilli which had been employed in the first experiment were again subjected to heat, but still without effect.

Tyndall found that hay infusion, which is extremely difficult to sterilise on account of the spores of bacilli contained in it, could be freed from these by repeatedly heating for a short time to a point below 100° C., and it has therefore been suggested that objects to be disinfected should be treated in this way. Hay infusion is, however, not merely a carrier of germs, but also a cultivating medium, so that the spores which were not destroyed by the first heating must sooner or later develop into bacilli, in which state subsequent moderate heating will readily destroy them. Almost all objects requiring disinfection are merely carriers of germs, and not cultivating media, so that if

the spores clinging to such objects withstand the first heating they still remain as spores, and are as little affected by the subsequent heatings as by the first. Even if the objects were moistened, there would still be no pabulum for the micro-organisms.

Experiment 3.

Duration, 1 hour. Initial temperature of oven, 137° C.; final, 143° C. Test objects exposed as before. Anthrax spores after exposure gave a retarded and somewhat scanty growth upon gelatine, but earth spores and spores of hay bacillus were unaffected.

DISINFECTION OF LARGER OBJECTS.

Experiment 4.

Duration, 3 hours. Initial temperature, 142° C.; final, 148° C. The test objects were—

A. A paper parcel, containing a number of open test-tubes, in which were spores of various bacilli, and a maximum thermometer.

B. A roll of blanket, 72 cm. by 36 cm.; samples of garden earth and anthrax blood, and a thermometer, were placed at the centre.

C. A linen bag, containing samples of various fabrics.

The results were as follows :—

A. The thermometer indicated 145° C. All the spores, including anthrax and earth spores, were killed, showing that three hours' exposure to hot air at 140° C. destroys the spores of bacilli.

B. The temperature in the centre of the bundle had not exceeded 70° C., and the samples were unaffected. The interior of the bundle was very moist.

Thermometers were also inserted at intervals of 4 layers of blanket. The temperatures recorded were, 70° C. (at centre), 72° C., [94° C.], [94½° C.], [95° C.], 79½° C., and 93½° C. (beneath 4 layers only). The indices of the 3rd, 4th, and 5th thermometers had only been shaken down to about 95° C.

C. Effect upon certain fabrics :—

Linen	had become	yellow.
White silk	,,	yellow, with loss of lustre.
Red silk	,,	lighter in tint, with loss of lustre.
Cotton-wool	,,	brown, with burnt smell.
Gauze	,,	yellow.
White yarn	,,	yellow, with burnt smell.
Blue cloth	,,	discoloured and faded.
Black cloth	,,	slightly altered in colour and lustre.
Buckskin	,,	lustreless, but unchanged in colour.
Newspaper	,,	brown and brittle.
Jute	,,	darker in colour.
Cotton	,,	brown, easily torn.
White feathers	,,	yellow.
Wash-leather	,,	hard, and discoloured in patches.
Ornamental leather	,,	discoloured and brittle.

Horse-hair was unaltered.

These results show that at the temperature necessary for the destruction of spores of bacilli, almost all fabrics which require disinfection are more or less injured.*

In a repetition of this part of the experiment, the fabrics were directly exposed for three hours to a temperature of 135° to 140° C., the effect upon them being in all respects the same as before.

Experiment 5.

Duration, 3 hours. Initial temperature of oven, 123° C.; final, 149° C.

Thermometers, together with samples of garden earth, anthrax spores, *Micrococcus prodigiosus*, and bacteria of blue pus, were enclosed in each of the following :—

A. A bag containing cloth and linen garments, 25 cm. in least diameter.

B. Two blankets suspended by the centre, and loosely tied together by a cord. Diameter, at level of thermometer, 20 cm.

C. A roll of cotton-wool; diameter 13 cm.

* For more extended observations upon the effects of dry heat upon the colour, texture, and strength of fabrics, see Ransom (*Brit. Med. Journal*, Sept. 6, 1873), De Chaumont (*Lancet*, Dec. 11, 1875), and Vallin (Traité des Désinfectants, 1882).—B. A. W.

D. A coat and a woollen shirt, rolled lengthways, the coat outside; diameter 18 cm.

E. A blanket, very dry, rolled up into a bundle 75 cm. long and 12 cm. in diameter.

F. A bundle of tow in a linen bag; 27 cm. in least diameter.

G. A larger bundle of tow, bound with cord; 50 cm. in least diameter.

H. An open vessel, in which the test objects were exposed to the full heat of the chamber.

Results.—The temperatures attained were respectively, 121° C., 113° C., 111° C., 90° C., 83° C., 77° C., 74° C., and 139° C. In every instance *Micrococcus prodigiosus* and the bacteria of blue pus were destroyed, but earth spores and anthrax spores were unaffected, except in the open vessel (H), where the temperature (139° C.) had been sufficient to completely destroy them.

This shows how slowly heat penetrates into the interior of bundles of moderate size, even if only loosely packed. Woollen and other hygroscopic articles offer the greatest resistance.

Experiment 6.

Duration, 3 hours. Initial temperature, 131° C.; final, 147° C.

Garden earth and anthrax spores, with a maximum thermometer, were placed in the centre of each of the following:—

	Temperature attained at centre.	Results.	
		Anthrax Spores.	Earth Spores.
A bag containing soiled linen—two of the articles rather damp—32 by 19 by 15 cm.	79° C.	×	×
A pillow loosely filled with feathers; thickness 24 cm.	100° C.	×	×
A coat, loosely bound with cord, forming a bundle 15 cm. in thickness	—	×*	×
A blanket, suspended loosely by the middle	140° C.	0	0
A horse-hair mattress, 14 cm. thick	133° C.	0	×*

0 No growth.
×* Scanty growth, in isolated colonies.
× Growth as abundant as in the control experiment.

This experiment shows that articles which are compactly

folded into many layers, and are not too small, are not disinfected even by several hours' heating to 140° C. Single blankets hanging loose were completely disinfected, but not if a cord were even loosely tied around them. The low temperature (79° C.) attained in No. 1 shows how deleterious is the influence of moisture upon disinfection by hot air.

Experiment 8.

Duration, 4 hours. Initial temperature, 115° C.; final, 138° C.

A. Two rolls of blankets, one dry and the other moist, each tightly corded and measuring 100 cm. in length by 50 cm. in diameter. Thermometers were placed in series from the centre of each, at intervals of eight layers of blanket.

The dry roll remained dry throughout, and the temperatures ranged from 34·5° C. at the centre to 100° C. eight layers from the surface. The moist roll became dry outside, but remained damp within; the temperatures ranged from 45·8° C. at the centre to 74·4° C. beneath eight layers only.

Samples of anthrax spores and garden earth, placed beneath 12 layers of blanket, retained their vitality in both cases.

B. A roll of flannel, 15 cm. in diameter. About 40 layers.

The thermometer at the centre reached 83° C. *Micrococcus prodigiosus* was killed, but anthrax and earth spores remained unaffected. The centre of the roll was damp.

C. A roll of coarse black cloth, damp, 8 cm. in diameter.

The central thermometer indicated 81° C. Spores of bacilli were unaffected, but *Micrococcus prodigiosus* was killed. The roll was dry externally, but very damp within.

D. Fur, folded lengthways, with the hair outwards, and tied with cord.

Became hard and dry externally, rather moist within. The temperature at the centre reached 86° C.; anthrax and earth spores placed there were unaffected.

E. Earth and anthrax spores freely exposed, in a vessel.

The thermometer indicated 138° C., and all the spores were killed.

Experiment 9.

Duration, 4 hours. Initial temperature, 110° C. (after 30 minutes 130° C.); final, 140° C.

A. Seven thermometers were buried in compressed tow, at distances from the surface varying from 8½ to 32½ cm. The readings, after heating, ranged from 76° C. to 79° C.

B. Sheepskins rolled into a bundle 1 m. long and 1·8 m. in circumference, and corded.

The temperatures recorded were—beneath 1 layer, 96° C.; 3 layers, 84° C.; 5 layers, 74° C.; 7 layers, 74° C.

C. A large roll of canvas, corded, measuring 1 m. in length and 1·15 m. in circumference.

The temperatures recorded were—beneath 20 layers, 86° C.; beneath 80 layers, 72° C.; beneath 380 layers, 23° C.; and beneath 410 layers, 20½° C.

The outer 40 layers were dry and hot; next came hot and moist layers, but beyond 150 layers the heat and moisture constantly diminished, the centre being cool and dry.*

The most important conclusions from these experiments are—

1. Sporeless bacteria are destroyed in 1½ hours by hot air at a temperature slightly exceeding 100° C.

2. Spores of fungi require 1½ hours at 110° to 115° C.

3. Spores of bacilli require 3 hours at 140° C.

4. Heat penetrates so slowly that even for articles of moderate size, such as pillows, 3 to 4 hours' exposure at 140° C. is insufficient.

5. Exposure for 3 hours to 140° C., which is necessary for thorough disinfection, damages most fabrics more or less.

* The maximum duration of these experiments (4 hours) falls considerably short of the limits usually adopted in this country for the disinfection of bulky or compact objects. Such objects are commonly exposed to hot air at 250° or 255° F. for eight or even twelve hours.

Dr. Ransom (*loc. cit.*) experimenting with objects about 5 inches in thickness, viz, horsehair pillows, flock pillows, and folded blanket, found that the internal temperature came within 10° or 20° F. of the temperature of the oven in three or four hours or less, if the objects were dry, but that eight or ten hours' exposure was often required if the objects were at all moist.—B. A. W.

III.—DISINFECTION BY STEAM.

By Drs. KOCH, GAFFKY and LOEFFLER.

(*Mittheilungen aus dem K. Gesundheitsamte*, vol. i.)

The earlier experiments were made with steam under pressure; the later with steam at the atmospheric pressure.

As test objects those micro-organisms were selected which offer the greatest resistance to heat and other destructive agencies, viz., the spores of anthrax and those found in ordinary garden earth. Samples of these, after exposure to steam, were placed upon nutrient jelly, and the resulting growth, if any, compared with that obtained from a similar sample which had not been exposed.

A.—STEAM UNDER PRESSURE.

The apparatus used consisted of a closed iron vessel, 20 cm. in diameter by 40 cm. in height, filled to about one-fifth of its capacity with water, and heated by gas burners.

1. *Preliminary Experiments.*

Flasks of cold water were suspended in the steam, which in 15 minutes reached 120° C., and was maintained at that point for 30 minutes; the water in a flask 4½ cm. in diameter had then reached 102° C., but in one 12 cm. in diameter only 85° C.

A litre flask filled with cold water, and exposed to steam at 127° C., had not reached 65° C. in half an hour, but an hour's exposure to steam at 120° C. raised the temperature to 115° C.

Hence it must not be assumed that bodies exposed to steam readily attain the temperature of the steam, and we must accept

with the greatest reserve the cases in which hay infusion is said to have been heated by steam to a temperature of 100° C. for hours without being sterilized.

The same considerations apply to solid bodies similarly treated. A moist clay ball, 10 cm. in diameter, in the centre of which was the bulb of a maximum thermometer, was exposed to steam, which in 30 minutes reached 120° C., while the index of the thermometer embedded in the clay had not reached its lowest graduation, viz., 65° C.

A roll of coarse black cloth 25 cm. by 8 cm. was exposed to steam, which in 30 minutes reached 120° C. The temperature at the centre of the roll had not then risen to 65° C., but the steam being raised to 126° C., and kept at that temperature for 30 minutes more, the temperature within the cloth rose to 118° C.

2. *Action of Steam (under pressure) upon Spores freely exposed.*

In a series of experiments, the results of which are given in the following table, samples of garden earth, and silk threads impregnated with anthrax spores, were exposed freely to the steam within the closed vessel :—

Temperature of Steam.	Duration of Exposure at given Temperature.	Result.	
		Anthrax Spores.	Earth Spores.
120° C.	30 min.	0	0
110°	60 „	0	0
110°	30 „	0	0
110°	15 „	0	0
110°	10 „	0	0
up to 110°	—	0	0
105°	30 „	0	0
105°	20 „	0	0
105°	10 „	0	0
up to 105°	—	0	+**
100°	30 „	0	+**
100°	20 „	0	+**
100°	10 „	0	+**
up to 100°	—	0	+**
95°	10 „	0	+**
up to 95°	—	+*	+
90°	10 „	+*	+
up to 90°	—	+*	+

0 No growth.
+ Growth as abundant as in the control experiment.
+* Growth delayed, but abundant.
+** Scanty growth, in isolated colonies.

Thus, ten minutes at 95° C. sufficed to destroy anthrax spores, but the spores contained in garden earth required ten minutes' exposure at 105° C.

3. *Disinfection of larger objects.*

A roll of flannel, 25 cm. in length by 15 cm. in breadth, was exposed to steam at 120° C. for 1½ hours. The roll was then found to be damp throughout; a thermometer at the centre indicated 117° C., and both earth spores and anthrax spores which had been placed there were completely sterilised.

The same roll of flannel was exposed for 1 hour to steam at 110° C. The centre thermometer then indicated 96·5° C.; anthrax spores at the centre were found to be killed, but earth spores gave a scanty growth upon nutrient jelly.

The rolls of flannel and cloth (p. 527) were the same that were used in the experiments with hot dry air (p. 524), and the comparative results are given in the following table:—

		Exposure	Temperature of Apparatus.	Temperature of Roll.		Result.
				At centre.	Midway between centre and circumference.	
Hot air	Flannel Roll	4 hours	130°—140° C.	83° C.	92° C.	Anthrax spores and earth spores unaffected.
„	Cloth Roll	4 „	130°—140° C.	81° C.	—	
Steam	Flannel Roll	1½ „	120° C.	117° C.	—	Anthrax spores and earth spores killed.
„	„	1 „	110° C.	96½° C.	100° C.	Anthrax spores killed; earth spores still gave a scanty growth.
„	Cloth Roll	½ „	120°—126° C.	118° C.	—	—

The contrast is very marked. With hot air, four hours' exposure to a temperature of 130° to 140° C. only brought the internal temperature of a small roll of flannel to 83° C., and in no way impaired the vitality of spores of bacilli placed at the centre; while exposure to steam at 120° C. for 1½ hours raised the internal temperature to 117° C., and killed the spores. Even 1 hour at 110° C. sufficed to raise the temperature at the centre to 96½° C. and to destroy anthrax spores placed there.

It is plain, therefore, that steam acts much more powerfully than hot dry air upon spores, and penetrates porous objects more rapidly and deeply.

From the table on p. 527 it will be seen that anthrax spores were killed by steam at 100° C. in ten minutes, and that of the species of spores present in garden earth only one (forming a short thick bacillus when cultivated) survived the same process. Although 30 minutes' exposure still failed to destroy these last, the results make it highly probable that exposure for an hour or more would be sufficient to destroy the most resistent organisms.

Complete disinfection would thus be obtained within a short space of time, and with the further great advantage that since the temperature need not exceed 100° C., only a very simple apparatus, not necessarily steam-tight, would be required.

B.—STEAM AT ATMOSPHERIC PRESSURE.

1. *Preliminary Experiments.*

It was found that the temperature varied from 98·3° C. to 99° C. in different parts of a vessel of boiling water, and from 97° C. to 97·6° C. upon the intensity of the source of heat being diminished, so that ebullition affords no certainty that the temperature of the water has in all parts reached the boiling point.

Steam generated at the bottom of a deep vessel had a temperature of 70° C. to 78° C. 1 cm. above the surface of the boiling water, while in a shallow vessel, in which the steam mixed readily with the air, the temperature at a similar level was 10° C. lower than this.

2. *Disinfection of Spores fully exposed.*

A glass flask was employed, the neck of which was prolonged by means of a glass tube fitted into it. When water was made to boil briskly in the flask, the temperature of the steam, even within a few cm. of the upper end of the tube, reached 100° C.

Anthrax and earth spores were wrapped in filter paper, and suspended 40 cm. above the surface of the boiling water.

Earth spores were completely destroyed in 15 minutes, and

partially so in five minutes, the subsequent growth upon nutrient jelly being both retarded and scanty. Anthrax spores were killed in five minutes.*

The "earth spores" are of the same species of bacilli (*e.g.*, hay bacillus) as those found in hay infusion and solution of extract of meat; and as experience has shown that it may be necessary to boil these latter for hours before they are completely destroyed, we have here an apparent conflict of evidence.

The attempt to sterilize such infusions has, however, always been made either by heating them by steam in a closed vessel, or by partially immersing the test-tubes containing them in boiling water, in an open vessel. In the former case the steam would only very slowly raise the liquid to the boiling point, as has already been shown; and in the latter case the submerged part of the tube would probably attain a temperature of 95° C. to 98° C., while the rest of the tube would only reach 50° C. or 70° C. If, therefore, the interior of the upper part of the test-tube be wetted, as is almost inevitable, by the infusion, any spores clinging to it may escape the full action of the heat for hours or days.

The experiments recorded here show that the spores of bacilli withstand steam at 100° C. for a few minutes only, and any apparent exception is due to the temperature falling short of this point. A few minutes longer may, however, be required when the spores are encased in some dry resistent material, which has to be dissolved or moistened by the steam before the spores are affected. We may thus explain the longer time required for the destruction of earth spores, as compared with anthrax spores.

3. *Disinfection of larger objects.*

Water was boiled in an iron vessel 40 cm. in height by 20 cm. in diameter. Resting on this was a cylinder 20 cm. in diameter and 1½ metres in height, bearing at its upper end a movable cap with a narrow aperture. The temperature was found to be 100° C. in all parts of the interior of the apparatus.

Small packets of earth, each accompanied by a thermometer,

* Schill and Fischer (*loc. cit.*) found that tubercular sputum was disinfected within 15 minutes.

were enclosed in the following objects, which were so placed in the cylinder that steam passed freely around them on all sides:—

1. A roll of canvas, 37 cm. long by 17 cm. in diameter.
2. A tightly bound bundle of tow, 26 cm. by 14 cm.
3. A roll of black cloth, 25 cm. long by 8 cm. in diameter (*vide* p. 528).
4. A roll of flannel, 25 cm. by 15 cm. (*vide* p. 528).

A flask, containing three litres of cold water, was also placed within the cylinder.

In about twenty minutes the temperature of the steam issuing from the opening rose to 100° C., and the experiment was continued for thirty minutes more.

The flask of water had then attained a temperature of 100° C., in marked contrast to the result of the experiment with compressed steam at 127° C., which in thirty minutes only heated the water to 65° C.

All the objects were damp throughout, but dried rapidly upon being unrolled. Each of the thermometers indicated 100° C.

One of the three samples of earth enclosed in the canvas roll yielded a solitary colony of bacilli when transferred to nutrient jelly, showing that the temperature had not, at that particular point, long reached 100° C. All the other packets, including one more deeply placed in the canvas roll, were completely sterilized.

Blue cloth was found to have become lighter in tint, but red silk, jute, and horse-hair were unaltered. Leather became shrivelled, hard, and brittle. Writing paper suffered very little, having merely become dull and slightly yellow.

4. The experiments were continued upon a larger scale in an apparatus of similar construction to the former, but measuring 50 cm. in diameter and 250 cm. in height. Although the precautions against loss of heat were very imperfect, it was found practicable to obtain a temperature of 97° C. and even 98° C. at the outlet by applying 22 gas-burners, but beyond this point an increased number of burners was of no avail.

The following objects were prepared:—

A. A roll of canvas, 50 cm. by 30 cm.; thermometers and packets of earth were placed at the centre and at regular intervals of 30 layers of canvas—at five points in all.

B. A flannel roll, 25 cm. by 15 cm.; a thermometer and a sample of earth were placed in the centre.

In fifty minutes the temperature at the outlet reached 97° C., and the experiment was continued for two hours after this. As before, the bundles were then found to be moist throughout, but dried rapidly upon being unrolled. The central thermometer of the canvas roll indicated 97° C., and that of the flannel roll 99° C.

The spores were killed in the flannel roll, and also in the two outer stations in the canvas roll. There was a scanty growth of short thick bacilli when the three inner samples from the latter were transferred to the nutrient jelly, showing that the heat had not acted long enough upon them to destroy all the spores. There can be no doubt that all would have been destroyed by a current of steam at 100° C.

A still larger roll of canvas, 50 cm. long by 40 cm. in diameter, containing packets of earth and thermometers arranged as before, was exposed to a current of steam for three hours after the thermometer at the outlet had reached 98° C. The central thermometer then stood at 98° C., although covered by 210 layers of canvas tightly rolled and bound with cord. Of the seven samples of earth, the five outer were completely sterilised, but the two inner, including the central specimen, gave a much retarded and very scanty growth of short thick bacilli.

5. Finally, it was sought to compensate for the loss of heat (from imperfect insulation of the apparatus) by generating steam at a higher temperature than 100° C. Instead of pure water, 40 litres of a 25 per cent. solution of sodic chloride were heated by 30 burners. The roll of canvas was prepared and placed as before, with extra thermometers, freely exposed to the steam, below and above it.

Within an hour the outlet thermometer indicated 92° C., in $1\frac{1}{2}$ hours 97° C., and in $1\frac{3}{4}$ hours 99° C.; in $2\frac{1}{2}$ hours it reached 100° C., and remained at that point until the end of the experiment, three hours later.

The temperature below the canvas roll, 70 cm. above the boiling liquid, was 105·3° C.; above the canvas, 102° C. The thermometers enclosed in the roll indicated from without in, 101·5° C., 101·5° C., 100° C., 100° C., 100° C., 100° C., and 100° C. (centre), respectively.

All the spores, even at the centre, were destroyed.

These results leave no room for doubt as to the form of disinfection by heat which should be adopted in the future. The hot air apparatus is complicated and costly, and is untrustworthy when the objects to be disinfected are at all bulky, or folded, or wet. Disinfection by steam under pressure at temperatures above 100° C. is open to the same objections, though to a less degree. In every respect exposure to a current of steam at 100° C. is a far more satisfactory method than either of the above. It is more certain, more simple, more rapid, more economical both in original cost and expense of working, and involves less injury to the articles to be disinfected.*

* Virchow's *Archiv*, Oct., 1885, contains a report by Wolff of experiments with Schimmel's and Bacon's disinfecting apparatus, in which hot air and current steam can be employed together or separately. Electric thermometers were used, to indicate the exact moment at which the internal temperature of bulky objects reached 100° C. The results, which were very favourable, confirmed those of Koch and his colleagues. Wolff found that although steam was by far the more powerful disinfectant, it was advantageous to employ hot air in conjunction with it, in order to avoid injury to fabrics, from condensation. The temperature in the disinfecting chamber ranged up to 155° C.; the steam pressure in the boiler was about 2 or 3 atmospheres. It was found possible to raise the temperature at the centre of a tight roll of 22 blankets (1 m. long by 0·5 m. in least dimension) to 100° C. in 70 minutes in one form of apparatus, and to 104° C. in 60 minutes in another. Moisture greatly retarded the penetration of heat.—B. A. W.

IV.—DISINFECTION BY MEANS OF CHLORINE AND BROMINE.

By Drs. FISCHER and PROSKAUER.

(*Mittheilungen aus dem K. Gesundheitsamte*, vol. ii.)

A.—CHLORINE.

In order to ascertain how far, and under what conditions, chlorine may be regarded as a reliable disinfectant, two series of experiments were undertaken.

A number of typical micro-organisms were exposed to its action in a closed vessel, in which the concentration of chlorine, the degree of humidity of the air, and the duration of exposure could be varied at will.

Having learned by this means the conditions necessary for complete disinfection—that is, for the destruction of all known micro-organisms—the experiments were continued upon a larger scale, under circumstances resembling those which are met with in practical disinfection.

I. Preliminary Experiments.—A wide-mouthed cylindrical jar, containing 21·35 litres, was so arranged that it could be charged with chlorine to any desired degree by means of an aspirator. Test-objects were received in little cups attached to a central rod passing through the cork, for facility of introduction and removal.

Chlorine was generated by the action of binoxide of manganese upon hydrochloric acid; or, when small quantities were required, by employing chlorine-water. The proportion of chlorine present in the air in the jar was determined analytically by passing a measured quantity through a solution of potassic iodide, and estimating the liberated iodine by means of a standard solution of sodic hyposulphite.

The following Table summarises the results of 13 experiments made with this apparatus. The signs +, $+^{x}$, —, indicate that after exposure to chlorine the vitality of the micro-organisms in question was found to be unimpaired, impaired, or destroyed, respectively.

TABLE I.

	Experiment I.	Experiment II.	Experiment III.	Experiment IV.	Experiment V.	Experiment VI.	Experiment VII.	Experiment VIII.	Experiment IX.	Experiment X.	Experiment XI.	Experiment XII.	Experiment XIII.
Percentage volume of chlorine ..... *A*...	44·7	4·6	4·6	4·0	1·0	1·3	3·2	3·5	0·56	0·32	*C* 0·04	*C* 0·06	*C* 0·004
B...	41·3	4·0	3·8	4·0	0·8	1·0	0·6	3·8	0·34	0·14	0·02	0·056	0·003
Humidity of air in flask	Dry.	Saturated.	Same as air of room (*a*)	Same as air of room (*b*)	Same as air of room (*c*)	Saturated.	Saturated.	Saturated.	Saturated.	Saturated.	Saturated.	Same as air of room (*d*)	Saturated.
Humidity of test objects	Desiccated.	Moist	Air-dried.	Air-dried.	Air-dried.	Air-dried.	Air-dried.	Air-dried.	Air-dried.	Air-dried.	Air-dried.	Air-dried.	Air-dried.
	Hours.	Hours.	Hours.	Hours.	Hours.	Hours.	Hours.	Hours.	Hours.	Hours.	Hours.	Hours.	Hours.
	1 3 24	1 3 24	1 3 24	1 3 24	1 3 24	1 3 24	1 3 24	1 3 24	1 3 24	1 3 24	1 3 24	1 3 24	1 3 24
Anthrax spores (*h*)	+ +ˣ —	— — —	— — —	+ˣ — .	+ˣ+ˣ—	— — —	— — .	. — .	— — —	. — —	. — —	. . +ˣ	. . +ˣ
Garden earth (*h*)	+ +ˣ .	— — —	— — —	+ˣ — .	+ˣ —	— — —	+ˣ — .	. — .	— — —	. — —	. — —	. . —	. . +ˣ
„ „ (*i*)	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	+ˣ — —	. — —	. +ˣ —	. . —	. . +ˣ
Bacilli anthracis.....	. . .	. . .	. . .	— — .	. . .	. . .	. . .	. . .	+(*e*). .	. . .	. — —	. . —	. . +
Bacilli of septicæmia of mice	. . .	. . .	. . .	— — .	. . .	. . .	— . .	. — .	+(*e*). .	. — .	. . .	. . .	. . .
Micrococcus tetragenus ...	. — +	— . .	. . .	. . .	. . .	. . .	+ — .	. — .	. . .	. — —	. — —	. . —	. . —
Microc. prodigiosus (*f*)...	+ + +	— — —	+ˣ+ˣ.	+ˣ+ˣ—	+ˣ— —	+ˣ— —	— — .	. — .	— — —	. — —	. — —	. . +	. . +ˣ
Microc. prodigiosus (*g*) ...	+ + +	— — —	+ +ˣ.	+ˣ+ˣ—	+ˣ+ˣ—	+ˣ+ˣ—	+ˣ+ˣ.	. +ˣ.	. . .	. . .	. . .	. . .	. . .
Microc. of erysipelas (*h*)	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. — —	. . .	. . —
Pink yeast (*g*)	+ + +	— — —	+ˣ— —	+ +ˣ.	+ˣ+ˣ—	+ˣ+ˣ—	+ˣ+ˣ.	. +ˣ.	. . .	. . .	. . .	. . .	. . .
„ „ (*f*)	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. — .	+ˣ— —	. — —	. — —	. . +ˣ	. . +ˣ
Bacteria of septicæmia of rabbits.....	. . .	. . .	. . .	— — .	. . .	. . .	. . .	. . .	—(*e*). .	. — —	. . .	. . .	. . .
Orange-coloured sarcinæ (*f*)	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. — .	. . .	. . .	. +ˣ—	. . +ˣ	. . +ˣ
Orange-coloured sarcinæ (*g*)	. . .	. . .	. . .	. . .	. . .	. . .	— — .	. +ˣ.	. . .	. . .	. . .	. . .	. . .
Aspergillus niger	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. — .	— — —	. — —	. — —	. . +ˣ	. . —
Bacteria of fowl-cholera .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . —
Tuberculous sputum.....	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	— — —	. . .	. . .	. . .	. . .

A. At beginning of experiment.
B. At end of experiment.
C. Varying, during first four hours, between.....

a. Highly charged with moisture.
b. Relative humidity, 62 °/₀ at 25° C.
c. „ „ 76 °/₀ at 16° C.
d. Relative humidity, 35 °/₀ at 14·5° C.
e. Desiccated.
f. Dried upon silk threads.
g. Growth upon potato.
h. Cultivated upon gelatine, after chlorine.
i. „ „ potato, „ „

In Experiment I. a highly-concentrated but dry atmosphere of chlorine failed to destroy desiccated micro-organisms which in the second and subsequent experiments readily yielded to a far smaller proportion of chlorine in the presence of moisture.

Experiment II. shows the effect of the presence of moisture, there being complete disinfection within an hour.

In Experiments III., IV., and V. neither the test objects nor the air in the jar were artificially dried or moistened, and the results are seen to vary rather according to the degree of atmospheric humidity than to the proportion of chlorine present.

In Experiments VI., VII., and VIII., the air in the jar was saturated with moisture, the test objects remaining as before. Disinfection was found to be more rapid than in the former cases, even although the proportion of chlorine were less. Experiments IX. and X. afford still stronger evidence of this, and in Experiment IX. desiccated bacilli of anthrax and of septicæmia of mice survived the spores of anthrax.

The results of Experiments VI., VII., VIII., were less favourable as regards cultivations upon potato than as regards similar organisms exposed upon threads. The difference is attributable to the greater depth to which the chlorine had to penetrate in the former case. When, however, the object was thoroughly saturated with moisture, as in Experiment II., or taken in an absolutely fresh state (like sarcinæ in Experiment VII.), chlorine readily destroyed all vitality.

In Experiments IX., X., XI., XIII., progressively smaller proportions of chlorine were employed, the test objects being in each case air-dried, and the air in the flask saturated with moisture at temperatures ranging from 14° to 16° C. The results show that 0·32 per cent. sufficed to destroy all organisms tested within three hours, whereas with 0·04 per cent. earth spores and orange sarcinæ still survived at the end of that period, but were destroyed within twenty-four hours.

0·004 per cent. killed only *Micrococcus tetragenus*, *Aspergillus niger*, and bacteria of fowl-cholera, although it impaired the activity of several others.

Finally, Experiment XII. compared with XI. shows the inferior results obtained with a comparatively dry atmosphere, even

although the proportion of chlorine be somewhat increased. The difference in the effect upon earth spores and anthrax spores is attributed to the formation in the former case of ferric chloride, which is strongly hygroscopic.

In some instances (Experiments IX. and XI.) it was found that, after exposure to chlorine, organisms would still grow upon potato, or, if inoculated, upon animals, but not upon gelatine. This is explained by assuming that some substance clings to the test object after its removal, which is so weakened by diffusion in the former cases as to be inactive, but upon gelatine does not diffuse, and therefore is able to inhibit the growth. This substance may conceivably be hydrochloric acid, or some product of the action of chlorine upon organic matter.

In air of moderate humidity 1 per cent. chlorine would kill all organisms within twenty-four hours, but disinfection under such circumstances would be somewhat uncertain. By artificially increasing the moisture of the air a much smaller proportion of chlorine is needed. If the air is saturated with moisture 0·3 per cent. will completely disinfect in three hours, or 0·04 in twenty-four hours, provided that the objects to be disinfected are not too thick, and are not shielded in any way from the chlorine.

II. Disinfection of Rooms by Means of Chlorine.—Further experiments were undertaken in a cellar of about 28 cubic metres capacity. The floor was asphalted, the walls and ceiling limed. The openings comprised an external window, a door, and two ventilating apertures provided with flaps.

In order to be able to ascertain at all times the composition of the air within the room, glass tubes were carried from the centre, at different levels, into an adjoining room. Similar tubes were carried from the same levels, but beginning close to the window, into the open air.

Chlorine was generated by acting upon bleaching powder by hydrochloric acid, this method being the cheapest, and requiring no heat. The sample of chloride of lime first used was found to yield 23 per cent. of its weight of chlorine, and to require 1 cc. of hydrochloric acid per gramme. 1 kg. of chloride of lime, with 1 litre of hydrochloric acid, was calculated to yield 230 grammes, or 723 cc. of chlorine, and in the room in question six times these quantities were employed, giving theoretically 1·54 per

cent. of chlorine. A loss of even 80 per cent. would thus still leave the requisite 0·3 per cent.

EXPERIMENT I.—A large quantity of water was evaporated in the room for two hours before the experiment began. All the openings were rendered as nearly air-tight as possible. Samples of various micro-organisms were exposed in different parts of the room, some freely, others more or less covered, enclosed in garments, &c.

Chlorine was generated in 14 vessels, 4 of which were placed on the ground, 8 near the ceiling, and 4 at intermediate levels. All the acid being added at once, the reaction was violent, and some of the liquid overflowed.

The following Table shows the proportion of chlorine found by analysis to be present at different levels, and at different times:—

TABLE II.

	Percentage of Chlorine present at end of					Average of first four determinations.
	½ hour.	2 hours.	3½ hours.	4½ hours.	24 hours.	
Near ceiling	0·014	0·13	0·039	0·029	0·00045	0·053
At mid level	0·4	0·223	0·083	0·044	0·00033	0·187
Near floor	1·2	0·28	0·089	0·044	0·00033	0·403
Mean	0·538	0·21	0·070	0·039	0·00037	

There was a distinct smell of chlorine in the room overhead during the experiment.

At the end of twenty-four hours the door was opened. The walls and ceiling were not at all discoloured, nor was painted woodwork. Leather and woollen articles were wet, and discoloured in those parts immediately exposed. Silk and velvet were also discoloured, but white cotton and linen articles were unaffected. In no case did the strength of the fabric appear to be impaired.

The results as regards the micro-organisms may be stated thus:—

1. Those which were sheltered, as, for instance, by lying in the pocket of a coat or beneath a glass plate, were unaffected.

2. Those which had been fully exposed to the action of chlorine were almost all destroyed, viz. :—

2	out of	3	samples of	anthrax spores
2	,,	3	,,	orange-coloured sarcinæ
3	,,	3	,,	pink yeast
3	,,	3	,,	*Bacilli anthracis*
3	,,	3	,,	bacilli of septicæmia of mice
3	,,	3	,,	bacteria of septicæmia of guinea-pigs
3	,,	3	,,	*Micrococcus tetragenus*
19		21		

3. Those which had been wrapped in filter paper, but otherwise unsheltered from the chlorine, had, as a rule, retained their vitality. Only those which had been placed near the window or door were destroyed, viz. :—

2	out of	6	samples of	anthrax spores
1	,,	6	,,	garden earth
2	,,	6	,,	pink yeast
3	,,	6	,,	orange-coloured sarcinæ
3	,,	6	,,	*Aspergillus niger*
2	,,	3	,,	*Micrococcus tetragenus*
13		33		

Although the proportion of chlorine was so much greater below than above (*vide* Table II.), there did not appear to be any corresponding difference in the effect upon objects exposed.

The immediate neighbourhood of the window and of the door appeared to have been favourable to disinfection. Since the distribution of chlorine was fairly equal in horizontal planes, as will be seen in Experiment II., it is suggested that the thinness, and perhaps permeability, of the partition at these points between the warm, moist, chlorinated air of the room and the comparatively cold and dry outer air may have assisted the action of the chlorine by promoting the condensation of moisture, and perhaps also the more active interchange of gases.

Experiment II.—In a second experiment further precautions were adopted, and the result was more favourable.

The chloride of lime used on this occasion yielded only 21 per cent. of chlorine, and each gramme required 1·3 cc. of crude hydrochloric acid for complete decomposition in the cold. 6 kg.

of chloride of lime being taken, the theoretical proportion of chlorine in the room would be 1·41 per cent.

In order to moderate its action, and to allow time for the attendants to leave the room after completing the arrangements, the acid was made to drip slowly into a small vessel, which it had to fill before overflowing into the chloride of lime.

0·5 kg. of chloride of lime was put in each of 12 deep earthen vessels, all of which were placed near the ceiling.

A large quantity of water was evaporated in the room for two hours previously.

Garden earth, anthrax spores, *Micrococcus prodigiosus*, and *Aspergillus niger* were employed as test objects. Some were freely exposed, some wrapped in filter paper, some placed in crevices or folds, or otherwise sheltered.

A faint smell of chlorine was soon perceptible outside the door and window, and later on also in the rooms adjoining and overhead.

TABLE III.

	Percentage of Chlorine present at end of						Average of first five estimations.
	½ hour.	1½ hours.	2½ hours.	3½ hours.	4½ hours.	24 hours.	
Near ceiling	0·958	0·502	0·324	0·178	0·106	Nil.	0·442
At mid level	0·974	0·736	0·447	0·239	0·162	Nil.	0·485
Near floor	1·159	0·653	0·357	0·206	0·145	Nil.	0·461
Mean	1·030	0·630	0·376	0·207	0·137		

The proportion of chlorine found to be present half an hour after the beginning of the experiment, though much greater than in Experiment I.—owing, no doubt, to better arrangements for generating it—was much below the theoretical 1·4 per cent. This is accounted for by the decomposition of the chloride of lime being still unfinished; by absorption of chlorine, and its chemical action; and lastly, by loss from leakage. Near the ceiling there was found to be rather more, but near the floor rather less, chlorine at the window than in the centre of the room.

At the end of twenty-four hours the door was opened. The walls and floor were moist. Several pieces of leather, hangings,

and articles of clothing (silk, cotton, and woollen), which had been exposed, were found to be moist, and for the most part discoloured, and so impaired in texture as to be readily torn.

The effect upon the four varieties of micro-organisms was found to have been as follows:—

1. The "sheltered" samples of each variety, 9 in all, survived, except one sample of *Aspergillus niger*.
2. Of those enclosed in filter-paper, one of each kind was moist, the other air-dried. In every instance the former perished, and the latter survived.
3. Of those more freely exposed, 22 out of 27 were killed, viz.:—

14	out of	15	samples of	earth spores
2	,,	4	,,	anthrax spores
2	,,	4	,,	*Micrococcus prodigiosus*
4	,,	4	,,	*Aspergillus niger*

The results were in some instances contradictory under apparently identical conditions, probably owing to some unexplained variation in the humidity of the air.

Complete disinfection of rooms appears, therefore, to be unattainable by means of chlorine, owing partly to its slight power of penetration into crevices and fabrics, partly to the difficulty of saturating the air with moisture as completely as was done in the flask experiments.

Nevertheless, chlorine may be of great value as a disinfectant in many cases, since it is at least capable of destroying all organisms, even the most resistent, which lie upon the surface. Hangings, &c., may be removed (for disinfection by means of steam), and the surfaces either washed with a solution of mercuric chloride or scraped. A preliminary partial disinfection by means of chlorine would greatly lessen the danger to those employed in a subsequent and more thorough disinfection.

In all cases where a gaseous disinfectant is needed, chlorine is the best at our command, being superior to both sulphurous acid and bromine.

For the disinfection of a room by means of chlorine, the proportion of the gas ought not to be less than that attained in Experiment II., viz., 1·0 per cent. It is difficult to secure this in practice, especially as the leakage will in most cases be greater than that which occurred in the experimental chamber. 0·25 kg. of chloride of lime and 0·35 kg. of hydrochloric acid ought to be allowed for each cubic metre.* The acid must be added gradually, by means of some such contrivance as described in Experiment II., and the vessels holding the reagents—each vessel containing not more than 0·5 kg.† of chloride of lime—should be placed at the upper part of the room, since the gas is heavy, and readily finds its way to the lower parts. The apparatus must be so arranged that all the attendants have time to leave the room before the chlorine begins to be given off. The air in the room must be saturated with moisture as completely as possible, and for this purpose it is well not only to wet the walls, floor, and all available surfaces, but to diffuse moisture by means of steam and spray. Twenty-four hours is suggested for the duration of the process, but probably a much shorter time—say eight hours—would serve equally well, since the chlorine after that period would, as a rule, be too dilute to have any effect.

All hangings, carpets, articles of clothing, &c., should be removed, and disinfected by means of moist heat. Not only does chlorine fail to destroy organisms hidden in them, but such fabrics are liable to be seriously injured by its action.

Metallic surfaces should be smeared with some protective coating, such as varnish or vaseline.

B.—BROMINE.

The experiments were similar to those with chlorine, and the same apparatus was employed.

In the preliminary flask experiments (Table *a*.) the air in the flask was charged with various quantities of bromine, determined in each case by analysis. The test objects were all placed at the same level in the flask on account of the irregular diffusion

* About 15½ lbs. of chloride of lime and 22 lbs. of hydrochloric acid, per 1,000 cubic feet.—B. A. W.

† About a pound.—B. A. W.

of bromine vapour. Specimens were removed at the end of one hour, three hours, and twenty-four hours, and tested as to their vitality.

TABLE *a*.

	Experiment I.			Experiment II.			Experiment III.		Experiment IV.	
Percentage volume of bromine	At beginning, 3·1 %. At end of 3 hours, 2·4 %.			At beginning, 0·14 %. At end of 3 hours, 0·21 %. At end of 24 hours, 0·06 %.			During first 4 hours varying between 0·033 % and 0·029 %. At end of 24 hours, 0·012 %.		During first 4 hours varying between 0·006 % and 0·002 %. At end of 24 hours, 0·0006 %.	
Humidity of air in flask	81 % relative humidity at 16° C.			Saturated.			Saturated.		Saturated.	
Humidity of test objects	Air-dried.			Air-dried.			Air-dried.		Air-dried.	
	Hours.			Hours.			Hours.		Hours.	
	1	3		1	3	24	3	24	3	24
Anthrax spores (*a*)	+x	—	.	—	—	—	+	—	+	—
" " (*b*)	+	+	.	—	—	—	+	—	+	+
Garden earth (*a*)	—	—	.	—	—	—	+x	—	+x	—
" " (*c*)	+x	—	.	+xx	—	—	+x	—	+	+
Tuberculous sputum (*d*)	—	—	.	—	—	—	.	.	.	.
Micrococcus tetragenus (*a*)	.	.	.	.	.	.	—	—	—	—
" " (*b*)	.	.	.	.	.	.	—	—	+	—
Micrococcus of erysipelas (*a*)	.	.	.	.	.	.	—	—	—	—
Bacillus anthracis (*b*)	—	.	.	—	.	.	—	—	+	—
" " (*a*)	.	.	.	.	.	.	—	—	+x	—
Bacillus of septicæmia of mice (*b*)	.	.	.	.	—	.	.	.	.	.
Micrococcus prodigiosus (*c*)	+x	—	.	—	—	—	+x	—	+x	—
Pink yeast (*c*)	+x	+x	.	+x	—	—	—	—	+	+x
Orange-coloured sarcinæ (*c*)	.	.	.	.	.	.	+x	—	+x	+x
Aspergillus niger (*c*)	—	—	.	—	—	—	—	—	+x	—

a. Tested, after exposure to bromine, by cultivation upon gelatine.
b. " " inoculation upon mice.
c. " " cultivation upon potato.
d. " " inoculation upon guinea-pigs.

In Experiment I. the air in the flask was more moist than that of the room at the time of the experiment, but 3 per cent. failed to destroy anthrax spores within three hours.

Experiment II. shows that complete disinfection may be attained within three hours with 0·2 per cent. of bromine in an atmosphere saturated with moisture. 0·03 per cent. destroyed

all the organisms within twenty-four hours, though not within three hours (Experiment III.), while in Experiment IV. many survived even the longer period, the initial percentage of bromine being only 0·006.

These results are closely parallel to those obtained with chlorine, and prove that bromine is able to destroy even the most resistent organisms if in proper concentration (0·2 per cent.) and in presence of sufficient moisture.

DISINFECTION OF ROOMS BY MEANS OF BROMINE.—The same cellar was used as in the previous experiments with chlorine.

The bromine was diffused by means of Frank's process, *i.e.*, exposure of small pieces of porous earth (*Kieselguhr*) which had previously been allowed to absorb liquid bromine. The bromine was found in these experiments to evaporate completely within two or two and a half hours.

EXPERIMENT I.—One kg. of bromine was used, being equivalent to 0·5 per cent. by volume, if the vapour were diffused through the room. Each piece of *Kieselguhr* held 100 cc. of bromine, and all were exposed in shallow vessels near the ceiling.

Several determinations of the amount of free bromine were made during the experiment, with the following results :—

TABLE *b*.

	Percentage of Bromine present at end of				Average of the first three determinations.
	½ hour.	1¾ hours.	3½ hours.	24 hours.	
Ceiling	0·009	0·009	0·018	0·00036	0·012
Mid level	0·027	0·032	0·023	0·00056	0·027
Floor	0·042	0·036	0·027	0·00056	0·035
Mean	0·026	0·026	0·023	0·0005	

The slowness of the evolution of bromine accounts for the first and second determinations giving results so nearly equal.

The proportion of bromine was in every case far below 0·2 per cent., and complete disinfection was therefore not to be looked for.

At the end of twenty-four hours the room was opened. The bromine was found to have attacked the metallic surfaces, and

to have stained wood and paper. Woollen, cotton, and linen fabrics were very moist, discoloured, and readily torn.

The action of the vapour upon the various micro-organisms employed as test objects was very irregular.

Out of	18	samples of	anthrax spores	5	were	destroyed.
,,	15	,,	garden earth	10	,,	,,
,,	18	,,	pink yeast	6	,,	,,
,,	18	,,	orange-coloured sarcinæ	9	,,	,,
,,	15	,,	*Aspergillus niger*	11	,,	,,
,,	3	,,	*Bacilli anthracis*	3	,,	,,
,,	6	,,	*Micrococcus tetragenus*	3	,,	,,

Proximity to the sources of the bromine, and to the upper part of the window, seemed favourable to disinfection, no doubt owing to the greater concentration of the vapour there at first, and, in the case of the window, to the moisture which condensed upon it.

Those of the organisms which had previously been kept for twenty-four (or, still better, forty-eight) hours, in an atmosphere saturated with moisture, gave better results than those which were air-dried.

Experiment II.—The same quantity of bromine was employed, and in the same way, but the preliminary evaporation of water in the room only took place for one hour instead of two.

During the experiment the windows became dry. The proportions of bromine found were as follows:—

Table C.

	Percentage of Bromine present at end of						
	1 hour.	2 hours.	3 hours.	4 hours.	5 hours.	8 hours.	24 hours.
Ceiling	0·0668	0·0278	0·0446	0·0389	0·0278	0·0056	0·00022
Mid level	0·0613	0·0836	0·0724	0·0446	0·0334	0·0081	0·00027
Floor	0·0836	0·0946	0·0613	0·0446	0·0389	0·0100	0·00036
Mean	0·0706	0·0687	0·0594	0·0427	0·0334	0·0079	0·00028

The apparent loss was 86 per cent. of the theoretical pro-

portion (5 per cent.) as against 95 per cent. in the last experiment.

Besides the loss by unavoidable leakage, by condensation upon exposed surfaces, by absorption, and by chemical reaction, it must be remembered that the vapour is only evolved very slowly, so that during the first two hours the loss and gain are about equal. The higher proportion of bromine in Experiment II. than in Experiment I. is accounted for by the less degree of humidity, and the consequent diminished loss by absorption and chemical reaction. The distribution of the vapour was very irregular in both vertical and horizontal directions. Upon the whole, the proportion was higher below than above; higher near the window at the upper part of the room, but higher at the centre of the room in the lower part. In some instances the variation at the same level exceeded 2 : 1.

The room was opened after twenty-four hours. The effect upon various fabrics was similar to that found in Experiment I.

As the proportion of bromine still fell far short of that required, and as there was less moisture than in the previous experiment, it is not surprising to find that the result, as regards disinfection, was very unsatisfactory, and even less favourable than in Experiment I.:—

Of	20	samples of	garden earth,	2	were destroyed.
,,	8	,,	anthrax spores,	0	,,
,,	8	,,	*Aspergillus niger*,	2	,,
,,	8	,,	*Micrococcus prodigiosus*,	0	,,

As before, the neighbourhood of the ceiling and of the upper part of the window gave rather better results than elsewhere. Objects which had been kept in moist air were again found to be more readily affected than otherwise exactly similar objects which were air-dried.

It is possible that if the proportion of bromine were raised to 0·2 per cent., the air being fully saturated with moisture, equally good results might be obtained upon the large scale as in the flask experiments. If means could be found of evolving it rapidly, its effect would no doubt be greatly increased, but liquid bromine as such cannot be entrusted to the public with safety. It is clear also that much is gained by protracted exposure of

the test objects to moist air, or even by merely wetting them, before disinfection.

The inequality of diffusion is a grave objection to the use of bromine as a disinfectant. Not only is it difficult to obtain the requisite 0·2 per cent., but the presence of this average proportion in a room is no guarantee that in every part there is even an approximation to the necessary concentration. Where there are shelves or other mechanical impediments the diffusion would be still more sluggish. After a time the diffusion tends to become more uniform, but meanwhile the proportion is becoming less, and the period of most active disinfection is past. For purposes of practical disinfection, chlorine is to be preferred to bromine, especially as the expense is less. In the preceding experiments the proportion of chlorine needed for complete disinfection was obtained at a cost of 0·15 *marks* per cubic metre,* whereas in the case of bromine the proportion which cost 0·18 *marks* per cubic metre was only one-third of that required.

* About 4d. per 1,000 cubic feet.—B. A. W.

RECENT RESEARCHES ON

ATTENUATION OF VIRUS

AND

PROTECTIVE VACCINATION.

ABSTRACTS AND TRANSLATIONS.

BY

DAWSON WILLIAMS, M.D.

I.—THE ATTENUATION OF THE VIRUS OF FOWL-CHOLERA.*

AMONG the various facts which I have had the honour of communicating to the Academy regarding the disease commonly called fowl-cholera, I take the liberty of recapitulating the following :—

1st. Fowl-cholera is, in the highest degree, an infective disease.

2nd. The virus is a microscopic parasite which can be easily multiplied by cultivation outside the body of those animals which can be affected by the disease. Hence the possibility of obtaining the virus in a condition of perfect purity, and an irrefutable demonstration that it is the sole cause of the disease and of death.

* "De l'atténuation du virus du choléra des poules," par M. L. Pasteur, *Comptes Rendus*, tome xci. p. 673. (Séance du Mardi, 26 Octobre, 1880.)

In a communication made to the Academy of Sciences on February 9th, 1880, M. Pasteur, after speaking at some length on the dependence of fowl-cholera on a micro-organism which grew readily in chicken-infusion without any alteration in its virulent properties, stated that by making a certain change in the method of cultivation it was possible to bring about a diminution in the virulence of the micro-organism. This diminution in virulence was accompanied by a slight decrease in the rapidity with which the growth of the micro-organism took place, but by no other visible change. Yet the fowls inoculated with the attenuated virus suffered from the disease but recovered, while those inoculated with the unaltered virus all died; after recovering from the effects of the attenuated virus, the birds were incapable of being infected by the disease again.

M. Pasteur pointed out that this observation might be compared with the practice of variolisation (or inoculation for small-pox) universally followed before the discovery of Jenner; variola was inoculated to preserve from variola, just as at the present time in certain countries sheep are inoculated with sheep-pox (variola ovina), and as cows are inoculated with contagious pleuro-pneumonia to preserve them from those diseases.

The inoculation of the attenuated virus did not confer immunity with mathematical certainty, but a second inoculation rendered the preservative influence stronger.—D. W.

3rd. The virus occurs in conditions of variable virulence. Sometimes the disease is followed by death; sometimes, after producing morbid symptoms of varying intensity, it is followed by recovery.

4th. The differences which can be shown to exist in the power of the virus are not merely the result of observation grounded on natural facts; the experimenter can produce them at will.

5th. As is commonly the case with all infective diseases, fowl-cholera does not recur, or rather the severity of the recurrence is in inverse proportion to the greater or less severity of the first attack of the disease, and it is always possible to push the protection so far that no effect of any kind is produced by the inoculation of the most virulent virus.

6th. Without wishing to affirm anything at the present moment with reference to the variolous virus and the vaccine virus of man, it is clear from the above facts that in fowl-cholera there are conditions of the virus which, in their relation to the most virulent virus, hold the same place as human vaccine holds in relation to the virus of small-pox. The vaccine virus, properly speaking, produces a benign disease, vaccinia, which protects from a more serious disease, variola. In the same way the virus of fowl-cholera presents certain states of attenuated virulence, which though they produce the disease do not cause death, but bring about such conditions that, after recovery, the animal can brave the inoculation of a very virulent virus. Still there is in certain respects a great difference between the two orders of facts, and it is not unprofitable to remark that in regard to knowledge of facts and principles the advantage is on the side of the observations made on fowl-cholera; whereas the relations between variola and vaccinia are still under discussion, we are certain that the attenuated virus of fowl-cholera is derived from the very virulent virus of that disease itself, that we can pass directly from the first of these kinds of virus to the second, and that, in one word, their fundamental nature is the same.

The time has come for me to offer an explanation with regard to the main assertion upon which the greater part of the preceding propositions is founded, namely, that there are variable degrees of virulence in fowl-cholera; assuredly a strange result, when we reflect that the virus of this disease is a microscopic organism which can be worked with in the perfectly pure state

in the same way as the yeast of beer, or the mycoderma of vinegar. And yet if we calmly consider this mysterious property of variable virulence, it will be quickly seen that it is probably common to the various species of the group of infective diseases. Where then is the unity in one or other of the scourges of which this group is made up? To cite only one example, do we not see epidemics of very severe small-pox side by side with others almost benign, without being able to attribute the difference to external conditions of climate or peculiarity of constitution in the persons attacked? Do we not also see virulent epidemics die out little by little, again re-appear at a later date, and again die out?

The idea that there are variable degrees of intensity of the same virus is not calculated, strictly speaking, to astonish the physician or the man of the world, although it is of immense interest that this can be established scientifically. The wonder, in the particular case which occupies us, lies especially in the fact that the virus being a microscopic parasite, the variations in its virulence are under the command of the observer. This is the point which I must rigorously prove.

Let us take for our starting-point the virus of fowl-cholera in a very virulent state, the most virulent state possible, if I may so say. I have previously made known a curious method by which it may be obtained with this property. The virus is taken from a fowl which has just died, not of the acute, but of the chronic malady. I have made the observation that fowl-cholera sometimes occurs in the latter form. Such cases are rare, although it is not very difficult to meet with instances. In such cases the fowl, after having been very ill, grows thinner and thinner, and struggles against death for weeks and months. When it perishes, an event which takes place shortly after the parasite, hitherto localized in certain organs, has entered the blood and developed there, it is found that whatever may have been the original virulence of the virus at the time of inoculation, the virus obtained from the blood of the animal which has been so long a-dying is of considerable virulence, and usually kills ten times in ten, twenty times in twenty.

This much being granted, let us make successive cultures of this virus in a pure state, in an infusion made from the muscular tissue of fowls, taking each time the seed for the one culture

from the preceding culture, and let us test the virulence of these various cultures. Experience shows that this virulence does not sensibly alter. In other words, if we agree to consider two virulences identical when, working under the same conditions, with the same number of animals of the same species, the proportion of deaths is the same in the same time, we shall find that in our successive cultures the virulence is the same.*

In what I have just said I have passed over in silence the length of the interval between one culture and the next, or if the word be preferred, the interval between one sowing and the next sowing, and the possible influence this may have on the successive degrees of virulence. However small may appear the importance of this point, let us now turn our attention to it. With an interval varying from a day to a week, the successive virulences have not changed. With an interval of a fortnight we have the same result. Nor do we observe any change in the degree of virulence with an interval of one month, of six weeks, of two months. Nevertheless, as the interval increases, certain signs of little apparent value may occasionally be noted, which appear to point to an enfeeblement of the virus inoculated. For example, the rapidity with which death occurs, if not the proportion of deaths, decreases. In the various series of inoculated fowls some birds may be seen to linger on very ill, often very lame, because the parasite in growing through the muscles has involved those of the thigh ; pericarditis runs a prolonged course; abscesses appear around the eyes ; in fine, the virus has lost, so to say, its sudden overwhelming character. Let us next go beyond even the intervals mentioned above before again taking

* NOTE BY M. PASTEUR.—Equality of virulence, while thus defined, must not be considered a precise notion ; it bears a certain relation to the number of animals inoculated. The meaning attached to the term as used in the text leads us to say, if the mortality be the same in two series of ten animals, that the virulence of the two kinds of virus inoculated is the same ; there would have been an opportunity for a difference to appear if, instead of two series of ten animals, two series of a hundred had been used. Two kinds of virus, each used separately for inoculating a hundred fowls, might furnish in the one case a mortality of sixty, and in the other of a hundred ; the test applied to two sets of ten fowls only would have pointed, even if the experiment had been repeated several times, to an equality of virulence, if the method of estimating equality stated in the text were adhered to. But we see that in reality the virulence in the two cases would have differed in the ratio of 60 to 100.

It is, however, necessary to adopt a conventional method of estimation, because in investigations of this kind we are restrained by the expediency of not increasing excessively the number of victims, and of not increasing beyond measure the expense, always great, of these experiments.

material for the renewal of the cultures. Let us prolong the interval to three months, to four, to five, to eight months and more, before we examine the virulence of the growths of the new microscopic being. The scene is now completely changed. Differences between the successive virulences which had not hitherto shown themselves, or had shown themselves in a doubtful manner, now become clearly apparent.

With such intervals between the sowings we find that on resuming the cultivations instead of identical degrees of virulence, that is to say a mortality of ten fowls in ten inoculated, we have decreasing rates of mortality of nine, eight, seven, six, five, four, three, two, or one in ten, and that sometimes there is no mortality—that is to say, the disease shows itself in all the inoculated birds, and all recover. In other words, by a simple change in the mode of cultivating the parasite, by merely putting off the period of inoculation, we have a method by which progressively decreasing degrees of virulence, and finally a true vaccinal virus, which does not kill, but produces a benign malady and protects from the mortal disease, may be obtained.

It must not be supposed that in all these attenuations the results follow with mathematical certainty and regularity. The virulence of one culture which is not renewed for five or six months may be always considerable, while the virulence of others having the same origin may have become very attenuated after three or four months. We shall soon see the explanation of these anomalies, which are only apparent. Often there is a quick jump, an interval of short duration, from a very high degree of virulence to the death of the microscopic parasite; in passing from one cultivation to the next we are surprised by the impossibility of obtaining any growth; the parasite is dead. Death of the parasite is, moreover, a habitual and constant occurrence whenever a sufficient length of time elapses before resuming the cultivations.

The Academy understands the true motive which has led me to keep silence, and why I have claimed the liberty of delay before informing it of my method of attenuation. Time was an element in my research.

What, then, happens to the microscopic organism during the course of these phenomena? Does it alter in form or aspect in changing its virulence so profoundly? I would not dare to

affirm that some morphological correspondences do not exist between the parasite and the various degrees of virulence which it presents, but I must admit that up to the present time I have been unable to perceive them, and that if such really exist the eye armed with the microscope is prevented from perceiving them by the exceeding smallness of the virus. For all degrees of virulence the cultivations are alike. If sometimes it seems possible to recognize slight changes, they are soon perceived to be merely accidental, for they disappear or peculiarities of an opposite kind occur in new cultivations.

It is worthy of remark that if each variety of virulence be taken as the point of departure for new cultivations, made in succession, at short intervals, each variety of virulence preserves its own intensity. A virus, for instance, so attenuated that it kills only one in ten, preserves this degree of virulence in its cultivations if the intervals between the sowings are not increased.* An equally interesting fact, and one which agrees with the general tendency of the observations already described, is that an interval between the sowings which suffices to cause the destruction of an attenuated virus permits the survival of a more virulent virus, which may indeed be attenuated by it but is not necessarily killed.

At this stage of our inquiry an important question arises. What is the cause of the diminution of virulence? The cultivations of the parasite are necessarily carried on in contact with the air, because our virus is an aerobic organism, and cannot develop if air is excluded. It is natural, therefore, to ask at once if contact with the oxygen of the air be not the influence which diminishes the virulence? May it not be that the little organism which constitutes the virus, being left exposed to the oxygen of the purified air in the cultivation-material in which it has been growing, undergoes certain modifications which remain permanent after the organism had been withdrawn from the modifying influence? It might further, it is true, be asked, whether some principle in the atmospheric air other than oxygen, some chemical or fluid principle, may not take part in the production of a phenomenon so strange that it justifies any supposition?

* "In the same way a cultivation which has lost all virulence gives rise to cultivations which are not virulent."—Pasteur, *Trans. Int. Med. Cong.*, Lond., 1881, vol. i. p. 87.

It is easy to understand that the solution of this problem in the way suggested by our first hypothesis, that is, some influence of the oxygen of the air, may easily be put to the test of experiment; if the oxygen of the air is in reality the agent which modifies the virulence, we shall probably have a proof of this by withdrawing it.

Let us with this object make our cultivations in the following way. A suitable quantity of fowl-broth having been inoculated with our very virulent virus, glass tubes are filled with it to two-thirds, or three-fourths,* of their volume, and then sealed with the blow-pipe. Thanks to the small quantity of air which remains in the tube, the growth of the virus commences, as is shown by an increasing turbidity of the liquid. By degrees the progress of the culture leads to the disappearance of all the oxygen in the tube. Then the material which causes the turbidity falls to the bottom, the virus is deposited on the sides of the glass, and the liquid becomes clear. This result is produced in two or three days. Henceforth the organism is withdrawn from all contact with oxygen, and will remain in this state so long as the tube is unopened.† What now happens with regard to the virulence? In order to render our investigation more trustworthy, a large number of similar tubes are prepared, and at the same time an equal number of flasks containing the same kind of cultivations, but freely exposed to the purified air. The change which occurs in the cultivations in contact with air we have already mentioned; we know that their virulence undergoes a progressive diminution. Let us speak only of cultivations in closed tubes cut off from the air. Open

* The statement in the original is not very precise, "*aux deux tiers, aux trois quarts, &c.*," *i.e.*, to two-thirds, to three-fourths, &c.—D. W.

† NOTE BY M. PASTEUR.—The aspect of the sealed tubes undergoes a great change after a time; they remain almost limpid even after shaking. The granulations into which the original elements of the initial growth become resolved have a refracting power similar to that of water, and do not cause any sensible turbidity of the liquid. Are these true spores comparable to the germ-corpuscles of the bacillus of anthrax? I do not think so. It is not probable that our parasite gives origin to true spores. If it produced spores it would be difficult to understand how, whether in contact with the air or in closed tubes, it in the end loses all vitality, all power of reproduction. Further, true spores survive a higher temperature than the elements of the organism during its growth. Nothing of the kind occurs with the microbe of fowl-cholera. Old cultivations kept in contact with the air (I have not yet proved it in the case of the others) perish at temperatures even lower than those which kill recent cultivations. This is a usual characteristic of the micrococcus-group.

one after an interval of one month, and after having, by inoculating with some of its contents, made a cultivation, test its virulence; open another after an interval of two months, and so on others after intervals of three, four, five, six, seven, eight, nine, and ten months. For the present I do not go beyond that period. It is remarkable that, as shown by the experiment, the virulence is found always to be the same as that of the virus introduced at the beginning into the closed tubes. The cultivations in contact with air are found to be dead, or to have a very slight virulence.

The question which we asked is thus answered: it is the oxygen of the air which enfeebles and destroys the virulence.*

Here, in all probability, is more than a mere isolated fact; we must be in possession of a principle. We may hope that a property inherent in the oxygen of the air, a natural force everywhere present, will be efficacious with other kinds of virus. The possible general applicability of this method of attenuation of virulence by an influence which is, to some extent, cosmic, is at any rate a circumstance worthy of attention.† May we not henceforth presume that the limitation of great epidemics must be, in the present as in the past, attributed to this influence?

The facts which I have now had the honour of communicating to the Academy suggest numerous inductions, obvious or remote.

* NOTE BY M. PASTEUR.—As attenuation does not take place when air is excluded, if the parasite be deposited in a layer of some thickness, in a cultivation in free contact with pure air, it may be assumed that the deeper layers will be in fact out of contact with air; the more superficial layers are in a totally different condition. This circumstance alone, taken along with the intensity of the virulence, whatever may be, so to say, the quantity of the virus, enables us to understand that the attenuation of a flask is not necessarily proportional to the time of exposure to the air.

† NOTE BY M. PASTEUR.—I have in this paper passed over in silence a difficult question to which I have devoted a considerable amount of time. I was persuaded (why, I cannot, in truth, say) that the facts which I had observed with regard to attenuation would be better explained in conformity with natural laws, by the hypothesis of a mixture of two kinds of virus, one very virulent, the other very attenuated, in variable determined proportions, than by that of the existence of a virus varying progressively in virulence. After having become, so to say, desperate in my search for an experimental proof of this hypothesis of the existence of two kinds of virus only, I have arrived at the conviction that it is not true.

M. Pasteur thus describes the method by which he commences a series of cultivations:—The extremity of a glass rod drawn out to a fine point is dipped, with the usual precautions, into the blood of a fowl which has just died of fowl-cholera; chicken-broth which is quite limpid, which has previously been sterilized at a temperature of about 115° C. (239° F.), and has been placed under such conditions that neither the external air nor the vessels in which it is contained can introduce into it any external organisms (any of the organisms which float in the air, or lie on the

With regard to both I maintain great reserve. I shall not consider myself justified in presenting them to the public until I am able to render them demonstrated truths.

surface of all objects), is touched with the point which has been contaminated by the blood. Some hours later, if the little vessel containing the cultivation is kept at a temperature of, for example, 25° to 35° C. (77° to 95° F.), it is seen to become turbid, and to be full of a small microbe of figure-of-8 form, but so small that often, with a high magnifying power, they appear only as points. From this vessel a drop, as small as may be, only as much as can be carried on the point of a glass rod drawn out as fine as a needle, is taken, a fresh vessel of sterilized broth is touched with the point, and the phenomenon is reproduced, the same happens with subsequent cultivations; in all, the turbidity disappears after the cultivation has been kept for two or three days at a temperature of 30° C. (86° F.), and a deposit gathers at the bottom; this is due to the cessation of the growth of the organism, all the minute points which rendered the liquid turbid having fallen to the bottom.—*Trans. Internat. Medical Congress*, London, 1881, p. 85, *et seq.*

NOTE.—In a communication made to the Académie des Sciences on July 25th, 1881 (*Comptes Rendus*, T. xciii., p. 219), M. H. Toussaint referred to a previous communication (*Comptes Rendus*, T. lxxxvii., p. 69, July 8th, 1878), which contained a description of a disease due to a micro-organism; this disease he considered to be the same as that studied by M. Davaine in 1864 and 1865. On first becoming acquainted with fowl-cholera M. Toussaint was convinced that it was identical with the disease he had previously been studying, and on August 2nd, 1880 (*Comptes Rendus*, T. xci., p. 301), he made a communication showing that in his opinion, founded on five series of experiments, fowl-cholera could be produced by inoculating the micro-organism of septicæmia (*le microbe de la septicémie*). He stated that he had produced a disease identical with "fowl-cholera" by feeding the birds on blood or other matters derived from septicæmic animals. He subsequently compared the action of a micro-organism obtained from decomposing blood (presumed to be the same micro-organism as that referred to in his previous papers) with the action of the micro-organism of fowl-cholera, and could detect no difference in the symptoms, in the lesions of the skin, the muscles, and blood, nor in the cultivations of the organism from the different sources. It then occurred to him "to inoculate chickens directly with the blood of rabbits dead of septicæmia. The results were those produced by an attenuated virus, slight lesions of the skin and of the subcutaneous tissue, sometimes a very slight alteration in the muscular fibres; but in all cases the birds recovered, and were refractory to inoculation with cholera. Cultivations of the blood of a septicæmic rabbit acted in the same way; at the most in this case the inoculation of the fowls determined in the skin a slight cicatrix, round and regular, as though made with a punch. This variety of rabbit's-septicæmia, therefore, could be used in practice as a vaccine, and would enable us to arrest the serious epizootics so often observed in the birds of the poultry-yard."

He had found that rabbits inoculated with the blood containing the micro-organism (the blood was obtained from a case of anthrax, but had travelled from the Vosges, and was not pure, but contained a micro-organism exactly like that of fowl-cholera) died in seven or eight hours of septicæmia. Pigeons inoculated with it (the micro-organism), died at first in four or five days, then in three days, finally in two days or in one day. Inoculated from the pigeon to fowls, the same results were obtained, that is to say, the first fowl died in four or five days, and the others in three, two, and one day, successively. When this septicæmia had killed the fowl after having passed through the pigeon, its very virulent properties towards these two species were preserved even after being inoculated in the rabbit.—D. W.

II.—VACCINATION FOR ANTHRAX

BY THE

INOCULATION OF THE ATTENUATED VIRUS.

[*Prevalence of the Disease.*—**The disease to which the term anthrax has been, in recent years, generally applied in pathological writings exists as a widely spread epizootic disease, very fatal to sheep in certain countries. It is commonly called *charbon* in France, from the colour (red) of the local lesion; owing to the frequency with which the spleen is conspicuously affected the name in common use in England is splenic fever, and in Germany *Milzbrand;* in France also the term *sang-de-rate* is used** by some writers. The names applied to the disease when it occurs in horned cattle seem to be commonly, black quarter, *mal noir*, glossanthrax. The rodents can easily be infected by inoculation. It occasionally occurs in man; the persons who have suffered from the disease in England have been generally wool-sorters or hide-porters, and the disease has received the trivial names of wool-sorters' disease, and malignant pustule. In Russia, where it has occasionally assumed epidemic proportions, it has been known as Siberian plague.

The mortality caused by the disease among the flocks and herds of France appears to be very considerable, but to have been declining during the last forty years. According to a report* made by M. Delafond in 1842 on a district then known as *La Beauce*, which included the country about Orleans, the average annual mortality among sheep was about 20 per cent.: in certain localities where the soil was dry and calcareous the

* Quoted by Dr. Ch. Chamberland in his handbook (*Le Charbon et la Vaccination Charbonneuse d'après les travaux récents de M. Pasteur.* Paris, Tignol, 1883), page 9, *et seq.*

mortality rose as high as a quarter, a third, and sometimes exceeded a half of the flock. The death rate has since decreased very considerably, and was estimated in 1881 at 10 per cent.; at the same time, the number of sheep kept by the farmers was much smaller. The pecuniary loss to the farmers of the district of *La Beauce* was estimated at seven million francs a year in 1842, and at three million francs in 1881.

Mode of Infection. — In a communication made to the Académie des Sciences on July 12th, 1880,* M. Pasteur, after referring to the magnitude of the losses produced by the disease, and stating that he had been entrusted by the Ministre de l'Agriculture with the means of investigating the mode in which infection commonly took place, gave a lengthy account of the experiments made. The following statements have an interest in connection with discussions which have arisen with regard to preventive vaccination :—]

The experiments commenced in the early days of August, 1878. They consisted at first in feeding certain lots of sheep with lucerne which had been watered with artificial cultivations of the bacterium of anthrax full of the parasite and its spores. . . . Notwithstanding the immense number of the spores of the bacterium swallowed by all the sheep of each lot many of them, often after having been distinctly ill, escaped death; a smaller number died with all the symptoms of spontaneous anthrax, after a period of incubation which might extend to eight or ten days, although, at the end, the disease took on the almost sudden characters frequently noted by observers, who have thus been led to believe in a very short period of incubation.†

The mortality was increased by mixing with the food sprinkled with the spores sharp-pointed objects, especially the pointed extremities of the leaves of dried thistles, and above all the beards of ears of barley cut into small fragments about ·01 millimetre long.

* *Comptes Rendus*, T. xci. p. 87.

† NOTE BY M. PASTEUR.—It is much more difficult to communicate the disease by food soiled by anthrax-spores to guinea-pigs than to sheep. In a considerable number of experiments we did not meet with one example. The spores in this case are found in the excrement. They are also found intact in the excrement of the sheep. [That is to say, the excrement could give origin to anthrax. See *Comptes Rendus*, T. xci. p. 536.]

It was of great importance to ascertain whether the autopsy of animals dying under these conditions would show similar lesions to those observed in animals dying spontaneously in stables, or in flocks penned in the open air. The lesions in the two cases are identical, and their nature authorized the conclusion that the disease begins in the mouth or pharynx.*

M. PASTEUR'S RESEARCHES WITH REGARD TO THE ATTENUATION OF THE VIRUS.†

I have made known in papers recently published the first example of the attenuation of a virus by experimental means alone. This virus, which is a distinct microbe of extremely small size, can be multiplied in artificial cultivations outside the bodies of animals. Such cultivations, when allowed to remain uncontaminated, undergo in process of time more or less profound modifications of virulence. It seems probable that the oxygen of the air is the chief cause of these attenuations, that is to say, of these diminutions in the facility with which the microbe multiplies; for it is clear that the various degrees of virulence are identified with the varying power of the parasite to develop in the economy.

There is no need to insist on the interest which attaches to these results, and to the deductions from them. To seek to diminish the virulence of virus by rational means is to found upon experiment the hope that from the active kinds of virus which easily grow in the bodies of men and animals, varieties of vaccine-virus developing in a limited way, and capable of preventing the deadly effects of the former, may be prepared. We

* NOTE BY M. PASTEUR. — One particular circumstance in our experiments deserves to be mentioned. Eight of the sheep used in our experiments were directly inoculated with cultivations of the bacteria by punctures, some even with infective blood full of bacteria, from a sheep which had died some hours earlier. All the sheep became ill, and their temperature was observed to be raised; only one, which had been pricked under the tongue, died. . . . Fowls which have been fed on food contaminated with the microbe of fowl cholera when they do not die may be vaccinated. There is reason therefore to ask if it might not be possible to vaccinate sheep against anthrax by previously gradually giving them food contaminated by the spores of the parasite.

For Dr. Koch's experiments on this point see p. 599.

† "De l'attennation des virus et de leur retour à la virulence," par M. L. Pasteur avec la collaboration de MM. Chamberland et Roux. *Comptes Rendus*, Tome xcii. p. 429 (February 28th, 1881).

have further used our best endeavours to ascertain whether the action of the oxygen of the air in causing the attenuation of virus may not be of general application.

The virus of anthrax, being one of the best studied, must be the first to attract our attention. Yet at the threshhold we stumbled on a difficulty. An essential difference exists between the micro-organism of fowl-cholera and the micro-organism of anthrax; the new research therefore cannot rigorously follow the older. The microbe of fowl-cholera in fact does not in cultivations appear to become resolved into true spores. In these cultivations there are only cellules, or elements, always ready to multiply by scission, but the special conditions under which they give rise to true spores are not known.*

The yeast plant strikingly exemplifies how these cellular bodies can go on multiplying indefinitely without the appearance of spores. There are many kinds of mucor with tubular mycelia, which under certain conditions of cultivation give rise to chains of more or less spherical cells, termed *gonidia*. Detached from their branches these can reproduce themselves as cells, without the appearance at any time, unless the conditions of cultivation are changed, of the spores of their respective mucors. This kind of vegetable organization may be compared with those plants which are propagated by slips, the fruits or seeds not being used to reproduce the original plant.

The bacterium of anthrax, in artificial cultivations, behaves itself very differently. Its mycelial filaments, if one may so term them, have been multiplying for scarcely twenty-four or forty-eight hours when they are seen, especially those which are in free contact with the air, to become transformed into highly refracting ovoid corpuscles which become isolated by degrees, and constitute the true spores of the micro-organism. Further observation shows that these spores, which are so quickly formed in the cultivations, do not undergo with the lapse of time any alteration due to the action of atmospheric air, either in their vitality or their virulence. I could show to the Academy a tube containing spores of the bacillus of anthrax formed four years ago, on March 21st, 1877. Each year the

* Note by M. Pasteur.—I have previously observed that the minute elements of the micro-organism become resolved into granules of very small diameter. It is difficult to believe that these granules are the true spores, because after a time the micro-organism dies. Can they be granules without inherent vitality?

ability of these small corpuscles to grow was tested, and each year the growth occurred with the same ease and the same rapidity as at the beginning; each year also the virulence of the new cultivations was tested, and no apparent enfeeblement was to be observed. This being the case, how was it possible to test the action of atmospheric air upon the virus of anthrax in the hope of causing an attenuation?

It seems probable that the difficulty is entirely due to the rapid production of spores upon which we have just dwelt. Is not this organism when in its filamentous form, and multiplying by scission, comparable, in all points, to the micro-organism of fowl-cholera? It is easy to conceive that a spore properly so-called, a seed, may not undergo any modification by the action of the air, but it is equally easy to conceive that, if there be any change at all, a fragment of mycelium would be most likely to experience the change. A cutting allowed to remain upon the ground exposed to the air soon loses all vitality, although a seed, on the other hand, would be preserved, ready to reproduce the plant. We are led to think that if these views have any foundation, in order to test the action of the oxygen of the air upon the bacterium of anthrax it would be necessary to be able to subject the mycelial growth of the micro-organism to this action, under such circumstances that not a single spore could be produced. This being the case, the problem how to subject the bacterium to the action of oxygen resolved itself into this, how to entirely prevent the formation of spores. When the problem is thus stated it is, as we shall see, capable of being solved.

The appearance of spores in artificial cultivations of the bacterium of anthrax can in fact be prevented in several ways. At the lowest temperature at which it grows—namely, about 16° C. (60·8° F.)—the bacterium does not, at least for a very long time, form spores. At this lower limit of its development the shape of the microbes is irregular; they are globular, pear-shaped, monsters in a word, but there are no spores. The same holds good, with regard to this latter point, for temperatures as high as are compatible with the growth of the parasite, temperatures which vary a little according to the medium. In neutral chicken broth the bacterium ceases to grow at 45° C. (113° F.) Its growth is, on the contrary, easy and abundant at from 42° to 43° C.

(107·6° to 109·4° F.), and yet there is no possible formation of spores. Consequently a mycelial growth of the bacterium entirely free from spores can be maintained in contact with pure air at a temperature between 42° C. and 43° C. Under these circumstances the following very remarkable results are obtained : after an interval of about one month the cultivation is found to be dead, that is to say, fresh broth inoculated with it remains completely sterile. On the day before that on which this inability to grow is noted, and on every preceding day during the month, reproduction of the growth is, on the contrary, easy. So much as to the life and nutrition of the organism. With regard to its virulence we discover this remarkable fact : after remaining for eight days at a temperature of 42° to 43° C., and ever afterwards the bacterium has lost its virulence ; at least its cultivations are innocuous to the guinea-pig, the rabbit, and the sheep, three of the animals most liable to contract splenic fever. We are therefore, by using a simple artifice in cultivating, able to produce not merely an attenuation of virulence, but a suppression which is apparently complete. More than this, we have the power of preserving and cultivating the terrible microbe in this inoffensive condition. What is it that occurs during these eight days at 43° C. which suffice to deprive the bacterium of all virulence ? We remember that the microbe of fowl-cholera also perishes in cultivations which are in contact with the air, though after, it is true, a much longer time, and that during this period it undergoes successive attenuations. Are we not authorised to expect that it will be the same with the microbe of splenic fever ? The conjecture is confirmed by experiment. Before the extinction of its virulence the microbe of anthrax passes through various stages of attenuation, and moreover, as is also the case with the microbe of fowl-cholera, each one of these states of attenuated virulence can be reproduced by cultivation. Lastly, since, as has been shown in our recent papers, splenic fever does not occur a second time, each of these cultivations of the attenuated bacterium of anthrax is a vaccine for the more virulent—that is to say, a virus capable of producing a more benign malady. What, then, can be easier than to find among these successive kinds of virus, varieties of virus capable of producing splenic fever in sheep, cows, or horses, without causing death, but serving subsequently to protect them from the disease in its

deadly form? We have performed this operation on sheep with great success. As soon as the time comes for folding the flocks and herds in *La Beauce*, we will test it on a large scale.

M. Toussaint has already announced that sheep can be protected by preventive inoculations; but when that skilful observer publishes his results, with reference to which we have made a thorough investigation, as yet unpublished, we shall be able to show the great difference between the two methods—the uncertainty of the one, the certainty of the other. The method which we make known has, further, the very great advantage of depending upon the existence of virus-vaccines which can be cultivated at pleasure, which can be multiplied to infinity in the course of a few hours, without ever having recourse to blood infected with anthrax.

The above facts raise a question of great interest: I refer to the possible return of the virulence of a virus which has been attenuated or extinguished. We have obtained, for instance, a bacterium of anthrax, which has lost all virulence for the guinea-pig, the rabbit, and the sheep. Can it be again rendered active with regard to these species? In the same way we have prepared the microbe of fowl-cholera deprived of all virulence for fowls. How can it be again rendered capable of developing in these Gallinaceæ?

At the present time the secret of their return to a virulent condition is merely their growth in the body of certain animals in series.

Our bacterium innocuous to guinea-pigs is not innocuous to these animals at all ages; but how short is the period during which it is virulent! A guinea-pig several years old, one year, six months, one month, a few weeks, eight, seven, six, or even fewer days old, does not after inoculation with the enfeebled bacterium of which we are speaking run any danger of the disease or of death; but this same virus, on the contrary, and the result is very surprising, kills a guinea-pig one day old. In our experiments there has not yet been one exception with regard to this. If now we pass from the first guinea-pig one day old to another, by the inoculation of the second with the blood of the first, and from the second to a third, and so on in series, the virulence of the bacterium, in other words, its habit of developing in the economy, is strengthened progressively. In consequence we can soon kill guinea-pigs three days, four days, one

week, one month, several years old, and in the end even sheep. The bacterium has returned to its original virulence. We can say without hesitation, although we have not yet had an opportunity of putting it to the proof, that it would kill cows and horses; moreover, it preserves this degree of virulence indefinitely, if no steps are taken to attenuate it afresh.

In the case of the microbe of fowl-cholera, when it has arrived at the point at which it has no action on fowls, its virulence can be restored by operating with it on small birds—canaries, sparrows, &c.; all these species it kills at once. By passing it, thus, through the bodies of the animals, we can little by little cause it to take on a virulence capable of once more producing an effect on adult fowls.

Need I add that, while this return to a virulent condition is in progress, virus-vaccines of all degrees of virulence can be prepared both in the case of the bacterium of anthrax and of the microbe of fowl-cholera?

This question of the return to a virulent condition is of the greatest interest in its bearing on the etiology of infective diseases.

I concluded my paper* on October 26th last with the remark that the attenuation of the different virus by the influence of the air might be one of the factors in the extinction of great epidemics. The above facts afford in their turn an explanation of the so-called *spontaneous* appearance of these plagues. An epidemic extinguished by an enfeeblement of its virus can come to life again owing to its virus being strengthened by certain influences. The accounts which I have read of the spontaneous appearance of plague appear to me to afford examples of this—witness the plague at Benghazi in 1856—58, where the outbreak could not be traced to any origin by contagion. Plague is an infective disease peculiar to certain countries. Its attenuated virus may exist, in all these countries, ready once more to take on its active condition, when certain conditions of climate, of famine, of poverty again appear. There are other infective diseases which appear *spontaneously* in all countries: such is the typhus of camps. Without any doubt the spores of micro-organisms, which are the authors of these latter diseases, are everywhere present. Man carries them about on his body, or

* "The Attenuation of the Virus of Fowl-cholera." See above, page 551.

in his intestinal canal, without any great inconvenience, yet they are ready to become dangerous when, under conditions of overcrowding, and of development successively on the surface of a series of wounds on bodies which are enfeebled or otherwise, their virulence becomes progressively strengthened.

Thus infectivity appears to us in a new light, which is in truth disquieting for humanity, unless indeed Nature in her evolution through ages past has already met all the opportunities for the production of infectious or contagious maladies, which is exceedingly improbable.

A micro-organism innocuous for man or for some particular animal, what is it? It is a being which cannot develop in our body, or in the body of that animal; but there is nothing to prove that if this microscopic being penetrates into some other of the thousands and thousands of species in creation, it may not be able to invade it and to render it diseased. Its virulence, strengthened by successive passages through individuals of that species, might pass into a state in which it could attack such or such an animal of larger bulk—man, or some of the domestic animals. In this way new virulences and contagions might be created. I am very much inclined to believe that it has been in this way that during past ages small-pox, syphilis, plague, yellow fever, &c., have appeared, and that it is owing to phenomena of this kind that from time to time certain great epidemics, such as those of typhus, which I have mentioned, appear.

The facts observed during the period when variolation (inoculation of small-pox) was in use have introduced the inverse opinion into science, that, namely, of the possible diminution of virulence by the passage of the different kinds of virus through certain subjects. Jenner was one of those who looked at the matter in this way, which has nothing improbable about it. Still up to the present time we have met with no examples of it, although we have deliberately searched for them.

These inductions will find, I hope, fresh support in later communications.

[In a paper read before the Académie des Sciences* on March 21st, 1881, M. Pasteur, after recalling the fact that he had

* *Comptes Rendus*, Tome xcii. p. 666, "Le vaccin du Charbon," par M. Pasteur, avec la collaboration de MM. Chamberland et Roux.

obtained the bacillus in a form so attenuated that it was incapable of producing any effect upon guinea-pigs more than one day old, proceeds as follows :—]

We were naturally led by the existence of this virulence, so much enfeebled, so nearly extinct, to continue our experiments in order to produce, if possible, even greater degrees of attenuation. We attained the end in view by taking as the starting point the most virulent bacterium we have yet possessed. It was in fact that growth of which I spoke in my paper of February 28th, starting from the development of spores which had existed for four years. This bacterium could be maintained at a temperature of 42°—43° C. for more than six weeks without dying. The experiment commenced on January 28th. After February 9th cultivations made from it no longer killed adult guinea-pigs. Thirty-one days after, on February 29th, a cultivation grown at 35° C.* started from the flask, which had been constantly kept at 42°—43°, still killed very young mice, but not guinea-pigs, rabbits, and sheep.† On March 12th, that is to say, forty-three days after January 28th, a fresh cultivation could kill neither mice, nor guinea-pigs, not even guinea-pigs only a few hours old. We thus obtained a bacterium which it was impossible to bring back again into a virulent state. If ever again it be obtained in a virulent condition, we may feel assured that it will be by having recourse to fresh species of animals, not at present known to be inoculable, and differing entirely from those which are at the present time known to be susceptible to anthrax. In other words, we now possess, and have a simple means of procuring, a bacterium which is the progeny of a most virulent bacterium, but is yet itself entirely inoffensive, and quite comparable to those numerous microscopic organisms with which our food, our intestinal canal, the dust which we breathe, are full, which do not produce illness or death, and amongst which we often even seek helpers in our industrial processes.

How unexpected is this result when we remember that this inoffensive bacterium grows in artificial media as easily as the most virulent, and cannot, except perhaps by certain transitory characters, be distinguished from it morphologically !‡

* 95° F.

† NOTE BY M. PASTEUR.—Mice are more sensitive than guinea-pigs to anthrax.

‡ NOTE BY M. PASTEUR.—When the bacterium is very attenuated its filaments are shorter, and more divided. The growth, less abundant, forms a uniform deposit on

The observations and facts which follow are not less worthy of attention.

In my paper of February 28th I observed that the microbe of anthrax differs from the microbe of fowl-cholera in that spores properly so-called were probably not present in cultivations of the latter. In fact all the cultivations of the micro-organism of fowl-cholera perish in the end, whether they be preserved in contact with the air, or in closed tubes in contact with inert gases such as nitrogen and carbonic acid. The micro-organism of anthrax, on the contrary, becomes resolved in cultivations into brilliant corpuscles like dust, which are true spores. It is these which we have seen grow in the earth around the bodies of animals dead of anthrax, brought back again by earth-worms to the surface, where they defile the crops, and become the agents whereby this terrible disease is propagated in the byres or pasture lands.*

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the sides of the vessel, whereas in the virulent state it is usual to see cotton-like flocculi made up of very long threads. Yet in order to see the attenuated take on again the characters of growth of the virulent bacterium, it is only necessary to wait until spores are formed, and to make a new cultivation with these.

* In a paper read before the Académie des Sciences on July 12th, 1880, M. Pasteur stated that the surface-soil of a grave in which an animal which had died of anthrax had been buried contained the virus of the disease. In one case investigated certain cows had been buried about six feet and a half deep two years earlier, and the land had been cultivated in the interval; in another case, where the animal buried was a sheep, the ground was left uncultivated, and the examination was made after fourteen months. A commission (Académie de Medecine, 17 Mai, 1881) consisting of MM. Bouley, Davaine, Alphonse Guerin, and Villemin, examined the evidence adduced. Earth was taken from the surface of two trenches used as burial-places for diseased animals; the one had been in constant use for three years before the experiment, in the other no carcase had been buried for twelve years; the earth was pounded in a sterilized mortar, shaken up with distilled water in a sterilized flask, a little chloride of lime added to hasten the precipitation of the coarser earthy matters, and the turbid supernatant fluid decanted into a sterilized flask; this operation was repeated three times, and the turbid fluid allowed to settle. The deposits were collected and introduced into a sterilized tube, which was then raised to a temperature of 90° C. (194° Fah.) in order to destroy if possible all micro-organisms except the spores of anthrax, which retain the power of germinating after exposure to that temperature. Eight guinea-pigs were then inoculated with the deposits thus obtained from the washings of the soil over the two trenches; two of the guinea-pigs inoculated with washings from the soil over the trench which had been used twelve years before, and one inoculated with washings from the soil over the trench which had been in use for the three years immediately antecedent to the experiments, died of anthrax; all the other animals died of septicæmia; guinea-pigs were also inoculated with washings obtained in the same way from soil from an adjoining field where no burials had been made; a small encysted abscess formed at the seat of inoculation but the animals were not rendered ill. M. Pasteur had expressed the belief, founded on the examination of

We are thus brought to ask ourselves the following question, well worthy of meditation when we come to consider the matter from the lofty point of view afforded by the principles of natural philosophy: Are all these attenuated degrees of the virus of anthrax also capable of giving rise to spores, and, if the answer is in the affirmative, what are the characters of these spores? Do they at once return to that degree of virulence possessed by the germs of the virulent bacterium from which they were derived by the method of attenuation previously explained? If not, are they practically the same as those of a bacterium without any virulence at all? Or, finally, have these spores various natures, and do they fix and maintain for ever the several degrees of virulence which characterized the bacteria from which they were formed, adding thus to the fund of medical knowledge and the great laws of nature this new principle, the existence of as many kinds of spores as there are degrees of virulence in certain kinds of animal virus?

It is this latter proposition which is true. Corresponding to all the bacteria of diverse degrees of virulence there are spores, each endowed with the power of reproducing the degree of virulence peculiar to the bacterium from which it emanates.

Need I add that a practical application of great importance follows from this? While reserving for future study the difficulties of detail which we must encounter in putting in practice a wide prophylactic system against anthrax, it is not the less true that we have at our disposal to serve as virus-vaccines for anthrax not only the filamentous bacteria, but virus-vaccines with all their peculiar qualities fixed in their spores and transportable without any possible alteration.*

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their casts, that the spores of the bacillus were brought to the surface by earth-worms. The commission obtained worms from the trench in which animals had recently been buried; small quantities of the excrement (casts) from certain of these worms were mixed with distilled water and injected into three guinea-pigs; two died of septicæmia, and one of anthrax; cultivations made from the blood of this animal gave pure growths of the bacillus anthracis. In another experiment pure cultivations of the bacillus capable of killing guinea-pigs were directly obtained from the excrement (casts) of earth-worms obtained from the soil over the trench which had been used for burials twelve years earlier.

M. Feltz (C. R., T. c ii. p. 132) has since stated that the virulence of the micro-organism gradually diminishes in the soil.—D. W.

* This statement was subsequently found to require modification, see page 583.—D. W.

EXPERIMENTAL APPLICATION OF THE METHOD.

[On April 28th, 1881, M. Pasteur* arranged with the President of the Agricultural Society of Melun to put preventive inoculation to a practical test. M. Pasteur proposed that 60 sheep should be used for this experiment, and consented, at the request of the President of the Society, to extend the experiment to 10 cows. He foretold that all the sheep not protected by inoculations of attenuated virus would die, and that all the cows not so protected would be, at least, made ill and that some would die when inoculated with a very virulent virus; while all the protected sheep would survive the inoculation with this very virulent virus, and that the cows would not be made ill; 10 sheep were not to be dealt with in any way, but kept for ultimate comparison with the inoculated sheep.

The experiments were commenced on May 5th upon a farm belonging to M. Rossignol, at Pouilly-le-Fort, near Melun, a small town twenty-eight miles from Paris on the road to Fontainebleau, and were attended by a large number of persons interested in agriculture or in veterinary science.

The animals used in this experiment were 58 sheep of both sexes, and of varying age, 2 goats, 8 cows, 1 ox, and 1 bull.

On May 5th, 1881, 24 sheep, 1 goat, and 6 cows were inoculated, by means of a Pravaz' syringe, with five drops of a cultivation of the attenuated virus of anthrax. On May 17th all these animals were again inoculated with a second attenuated virus more virulent than the first.

On May 31st the inoculations with very virulent virus, designed to test the efficacy of the previous preventive inoculations, were made. The very virulent virus used was derived by cultivation from the stock preserved in M. Pasteur's laboratory from March 21st, 1877.† Sixty animals were inoculated with this virus—namely, 48 sheep (24 vaccinated and 24 not vaccinated), 2 goats (1 vaccinated and 1 not vaccinated), 10 animals of the bovine species (6 vaccinated and 4 not vaccinated).

* *Comptes Rendus*, Tome xcii. p. 1378. "Compte rendu sommaire des expériences faites à Pouilly-le-Fort, près Melun; par M. Pasteur, avec la collaboration de MM. Chamberland et Roux."

† See above, page 563 and page 569.

10 sheep, neither vaccinated nor inoculated, were reserved as test animals. On June 2nd, when the spectators assembled, about forty-eight hours after the inoculations with the very virulent virus, they found 21 of the 24 sheep which had not been protected by the inoculation of the attenuated virus dead, and the remaining 3 died before evening; the unprotected goat was also dead. The 24 sheep and the goat protected by inoculation with attenuated virus appeared to be in good health. None of the bovine animals were dead, but the 4 not protected by inoculation with attenuated virus each presented extensive œdematous swelling around the point of inoculation; on the following day these swellings were very much larger; the temperature of these animals was 3° C. above the normal. The 6 animals protected by inoculation with attenuated virus did not suffer any elevation of temperature, swelling, or loss of appetite. The test therefore was, says M. Pasteur, as completely successful for cows as for sheep.*

The first complete experiment on the horse was made by M. Rossignol. On August 6th, 1881, he vaccinated a healthy colt† with the vaccine of the first degree. The contents of ten divisions of a Pravaz' syringe were injected into the subcutaneous cellular tissue. The animal's temperature was 38·5° C.‡ on that day, and on the following day varied only by a few tenths of a degree. On August 19th the colt was vaccinated with vaccine of the second degree. The temperature remained about normal on this and the following days. On September 2nd this colt

* In the night between June 3rd and June 4th one of the ewes which had been vaccinated with the attenuated virus, and subsequently inoculated with the virulent virus, died. The body was examined on June 4th by M. Rossignol, in the presence of MM. Chamberland, Roux, Gassend, and Garrouste. From the lesions found in the womb and the fœtus MM. Rossignol and Garrouste (veterinary surgeons) concluded that the ewe died from the consequences of abortion, which had not been completely effected, because the animal was at the time under the influence of the fever produced by infection with anthrax. The lesions peculiar to charbon were absent; only one or two bacilli were found in the blood from the veins and heart, and these were attributed to the inoculation which had been made three days earlier. The lesions found in the abdominal and pelvic cavities were certainly, in the opinion of MM. Rossignol and Garrouste, sufficient to bring about the death of the animal. (*Le Charbon et la Vaccination Charbonneuse*, par Ch. Chamberland.) However this may have been in this particular case, there appears to be a good deal of evidence tending to show that pregnant animals offer a diminished resistance to infective diseases communicated by inoculation. See page 587 *passim*.—D. W.

† *Le Charbon et la Vaccination Charbonneuse d'après les travaux récents de M. Pasteur*, p. 145.

‡ 101·3° F.

was inoculated with a very virulent liquid, furnished by M. Roux; at the same time a stallion, in vigorous condition but broken down in the fore-legs, was inoculated with the same virulent liquid; in both cases fifteen divisions of the syringe were injected into the subcutaneous tissue. A rabbit was also inoculated, and died subsequently with the characteristic symptoms of anthrax. Neither of the horses appeared at all incommoded on the two days following the inoculation, but on the morning of the next day, September 5th, the stallion was found dead; the examination of the body showed the ordinary lesions of anthrax; the blood contained numerous rod-shaped bacteria, either isolated or agglomerated.]

THE MORTALITY DUE TO VACCINATION.*

After the experiments at Pouilly-le-Fort a large number of farmers did not hesitate to have all or some of their animals vaccinated. During the summer of the year 1881 M. Pasteur, wishing to judge more surely of the effect produced, advised that only about half of each flock should be vaccinated, and that the other half should serve as tests. This was done, except on a few farms, where the owners insisted on having all the animals vaccinated.

During the months of June, July, and August, 32,550 sheep belonging to 138 flocks were thus vaccinated by M. Pasteur's assistants and by certain veterinary surgeons taught by them. The test animals numbered 25,160; they constantly mixed with the vaccinated, and were submitted to exactly the same management. At the time the inoculations were made some of the flocks were infected by spontaneous anthrax. Other flocks, not infected, were vaccinated as a preventive measure. Among the latter (45 flocks, comprising a total of 10,500 sheep) not a single death occurred either during the vaccination or in the subsequent months. This fact at once clearly shows how small is the danger attending vaccination. In the other flocks the mortality continued. 194 vaccinated sheep died between the first and the second inoculation. 87 vaccinated sheep died within

* Condensed from Chamberland's *Le Charbon et la Vaccination Charbonneuse*, chap. xxvii., p. 250.

ten days after the second vaccination. Thus the total loss among the vaccinated sheep from the first vaccination to ten days after the second was 281, or 1 in 116. Among the sheep not vaccinated 120 died during the period between the first and the second inoculation; within ten days after the second inoculation 50 unvaccinated sheep died. The total loss among the unvaccinated sheep from the time when the first vaccination was made to ten days after the second was 170, or 1 in 147.

If the mortality among the vaccinated sheep had been the same as among the unvaccinated, 220 sheep would have died. But 281 sheep died. Vaccination therefore had caused the death of 61 sheep, or 1 in about 533; 1 in 533 is the only mortality which can be attributed to vaccination in sheep.

During the same period 1,254 oxen and cows were vaccinated; 888 were kept as test animals. Not a single vaccinated animal died during vaccination; 3 died among the unvaccinated; 142 horses were vaccinated, and 81 kept as test animals. One vaccinated animal died during vaccination; the veterinary surgeon who performed the operation was satisfied that it died of septicæmia, and not of anthrax; no death occurred among the unvaccinated animals.

During the year 1882 there were vaccinated 34,870 sheep, 47,817 oxen and cows, and 2,325 horses. For this year it is not possible to state what part of the total mortality should be attributed to vaccination, and what part was due to spontaneous anthrax. All, or almost all the farmers, having been convinced by the results obtained the year before, had all their flocks vaccinated.

Table showing the Number of Animals Vaccinated and the Number of Deaths during Vaccination, and for ten days subsequently (1881, 1882):—

SHEEP.

Year.	Total number vaccinated.	Number of deaths after first vaccination.	Number of deaths during ten days after second vaccination.	Total number of deaths.	Proportion of deaths to total number vaccinated.	Proportion of deaths among unvaccinated animals to the total number kept as tests.
1881	32,550	194	87	281	1 in 116, or ·86 per cent.	1 in 147
1882	155,153	421	526	947	1 in 164, or ·61 per cent.	...
			HORNED CATTLE.			
1881	1,254	0	0	0	.....	1 in 296
1882	14,769	12	4	16	1 in 923, or ·1 per cent.	...
			HORSES.			
1881	142	1*	...	1	1 in 142, or ·7 per cent.	No deaths.
1882	962	0	6	6	1 in 160, or ·62 per cent.	...

THE DEGREE OF PROTECTION AFFORDED AGAINST THE "SPONTANEOUS" DISEASE.†

It was not sufficient to prove that vaccination for anthrax was almost free from danger to the animals vaccinated, it was necessary to show further that the vaccinated animals were protected from the spontaneous disease.

Experiments made by us at Chartres in 1878 showed that sheep succumbed less easily to the ingestion of the spores of anthrax than to the direct inoculation of these spores into the subcutaneous cellular tissue. In our opinion this was to be attributed to inoculation in the spontaneous disease being effected by a few spores only, while in inoculation under the skin a relatively large quantity of the virus was always introduced. It was therefore to be anticipated that animals would resist the natural causes of contagion better and more easily than artificial inoculations. It was, however, necessary to verify these preconceived views by carefully noting what happened, in practice, to the vaccinated and non-vaccinated animals,

* Due to septicæmia.

† Condensed from chap. xxviii. Chamberland's *Le Charbon*, &c., p. 255.

the two classes living together, and being in consequence submitted to the same sources of contagion.

The results of the vaccinations made during the year 1881 were quite conclusive on this point.

We have seen that 32,550 sheep distributed among 138 flocks were vaccinated, and 25,160 were kept as test animals. The reports on the mortality were transmitted to us at the commencement of the month of November, 1881. From the time when the vaccinations ceased up to the beginning of November, 44 sheep died of charbon among the vaccinated sheep; a mortality of 1 in 740. During the same period 320 died among the non-vaccinated sheep; a mortality of 1 in 78. The mortality was thus about ten times lower among the vaccinated than among the non-vaccinated.

These figures, I repeat, are deduced from the reports which the veterinary surgeons and farmers have transmitted to us.

If the mortality had been the same among the vaccinated as the non-vaccinated, 413 sheep of the former class would have died; 44 of them died. Vaccination, therefore, had preserved 369. But this vaccination during the period that it was in progress produced a slight mortality of 61 sheep according to the figures previously given. Vaccination then actually preserved 308 sheep from death.

This number is not very considerable, but the fact that it is only applicable to a period of two months after vaccination must be taken into account, as well as the further fact that the mortality from anthrax was small during that year (1881). In many flocks in fact we had a mortality of one, two, or three among the non-vaccinated, and of one or two among the vaccinated. This is equivalent to saying that these flocks had not been attacked by anthrax, and that nothing could be concluded with regard to them. In order to appreciate the effect produced by vaccination, flocks in which a distinct mortality was produced must be taken. In fifteen flocks almost all suffering from anthrax at the time when vaccination was performed, the mortality among the vaccinated was completely stopped, while it continued among the non-vaccinated.

Table showing the Number of Deaths among the Vaccinated and Non-Vaccinated in fifteen flocks, which were suffering from Anthrax at or soon after the time when Vaccination was performed:—

	Number of deaths before vaccination.	Number of animals.	Deaths during vaccination.	Deaths for two months after vaccination.	Total deaths during and after vaccination.
Vaccinated	589	3,663	58	0	58
Non-Vaccinated		2,867	60	141	201

Table showing the Total Number of Deaths among the Vaccinated and Non-Vaccinated Sheep during, and for two months after, Vaccination:—

	Total number of animals.	Deaths during vaccination.	Deaths for two months after vaccination.	Total.	Rate per mille.
Vaccinated	32,550	281	44	325	9·98
Non-vaccinated	25,160	170	320	490	19·47

The mortality during vaccination was 60 sheep among the non-vaccinated and 58 among the vaccinated; maintaining the same proportion, 77 of the vaccinated sheep would have died. It follows that the first vaccination alone preserved a certain number of sheep (about 20).*

In 1881 the number of horned cattle vaccinated was 1,254, and 888 were kept as test animals. After vaccination, and up to the beginning of November,† 1 vaccinated and 10 non-

* The area over which anthrax spreads is often very limited, though the plague is very persistent in localities which it infects. "In the department of Seine et Marne certain farms pay a heavy tribute every year to this plague; the districts of Provins, Fontainebleau, and Meaux always suffer severely; many farms in these districts go by the name of *fermes à charbon* (splenic-fever farms); the best farmers take them with fear and trembling. In the high lands of Auvergne, in Cantal for example, there are *montagnes maudites* (accursed hills), on which flocks lose 10 or 15 per cent. of their number, owing to the *mal de montagne* (anthrax); nor need it astonish us to find that in this part of the country the probability of the occurrence of anthrax is an element in the price of farms." (Chamberland's *Le Charbon et la Vaccination Charbonneuse*, pp. 10, 11.)—D. W.

† About two months.

vaccinated animals died of anthrax. If vaccination had not been performed 14 of the vaccinated cattle would have died.

In 1882, among the 155,153 vaccinated sheep, concerning which reports are now in our hands, 759 died of spontaneous anthrax, or ·5 per cent. nearly. The mortality during vaccination was 947, or ·61 per cent. The total loss was therefore 1·11 per cent. The average of the deaths in the preceding years is about 10 per cent. Vaccination would thus have reduced the mortality in the proportion of 10 to 1·11. Among the 14,769 oxen or cows vaccinated, 21 succumbed to spontaneous anthrax, or ·14 per cent. The mortality during vaccination was 16 animals, or ·10 per cent. The total loss, therefore, is ·24 per cent. The average of the losses in the preceding years was 7 or 8 per cent. Vaccination, therefore, has been very efficacious among horned cattle. Of the 962 horses vaccinated, 2 died after vaccination ; 6 succumbed during vaccination. The total mortality then was 1 in 120 (·84 per cent.). The mortality in preceding years was, on an average, 5 per cent. The comparison which I have just made between the mortality among the vaccinated animals during the year 1882, and during the preceding year, is open to a serious objection, namely, that the natural mortality from anthrax was very feeble in that year. Happily, without our knowledge the experiments which we had made in 1881 were repeated by the Veterinary Society of Eure-et-Loir ; the report was presented to that Society at its meeting on October 29th, 1882, by M. E. Boutet. This report removes all doubts.

"The number of sheep vaccinated during the year reaches 79,392 ; the average of the annual loss in these flocks during ten years was 7,237, or 9·01 per cent. Since vaccination only 518 animals, or ·65 per cent., have died of anthrax. It must be noted that this year, probably on account of the great humidity, the mortality in Eure-et-Loir only amounted to 3 per cent. The loss, therefore, would have been 2,382 instead of 518 after vaccination.

"In the flocks partly vaccinated we have 2,308 vaccinated, and 1,659 non-vaccinated ; the loss among the first has been 8, or ·4 per cent. ; among the second the mortality has amounted to 60, or 3·9 per cent. We may remark that in these flocks, taken from different cantons of the department, the vaccinated

and non-vaccinated sheep were submitted to the same conditions of soil, housing, food, and temperature, and that consequently they were subject to absolutely identical influences.

"The veterinary surgeons of Eure-et-Loir have vaccinated 4,562 animals of the bovine species; the annual loss among these was 322. Since vaccination only 11 cows have died. The annual mortality, which used to be 7·03 per cent., falls to ·24 per cent. Angina, generally slight, having occurred after vaccination in the horse, and the mortality from anthrax being not high (1·5 per cent.), veterinary surgeons have not deemed it prudent to vaccinate upon a large scale. Only 524 have been vaccinated, and of these three died between the two vaccinations.

"This result appears to us irrefutable; in view of such figures it is no longer possible to doubt the efficacy of vaccination for anthrax."

"E. BOUTET, Reporter."*

DURATION OF THE IMMUNITY.†

[The evidence as to the length of time during which sheep are protected by Pasteur's vaccination is somewhat conflicting. On June 2nd, 1881, M. Pasteur and his assistants inoculated with the *premier vaccin* 225 sheep on the State farm la Faisanderie at Joinville-le-Pont. On July 15th following, 150 of these sheep received the *deuxième vaccin*, and on July 20th 75 of this last lot were inoculated with virulent virus; all the animals

* *Comptes Rendus*, T. xcv. p. 1251.

While these pages were passing through the press a preliminary report of the results of a series of experimental vaccinations made in South Russia, in the summer of 1885, was published (*Centr. f. d. Med. Wiss.*, 1, 1886). Professor Zenkowski prepared his own vaccines premier and deuxième in the botanical laboratory of the University of Charkow, and during the summer of 1885 inoculated 1,333 sheep. The loss after the first vaccination was 1·55 to 1·6 per cent.; after the second ·039 to ·3 per cent.; no subsequent inoculations with diluted blood from a case of anthrax caused loss. An independent commission chose 30 sheep from this herd, and this number, together with ten other sheep which had not been vaccinated, were inoculated on November 4th on the inner side of the hind leg with 1 c.c. of blood from a case of anthrax. Of the vaccinated sheep three died of anthrax, with well-marked symptoms, in between 36 and 40 hours, and one on the sixth day; one survived. The ten unvaccinated sheep showed a rise of temperature to 39·9—41·6° C., but within four days the normal temperature, 38·5—40° C., was regained. One of the vaccinated sheep was killed to death by dogs on November 10th, and a second died on November 18th of pleuro-pneumonia and pericarditis. In neither of these animals could any anthrax bacilli be found either in the blood or in the tissues. The annual mortality from anthrax among the flocks in the Cherson district was stated to be from 12 to 20 per cent.—[EDD. W.]

† Condensed from Chamberland's *Le Charbon*, &c., p. 261.

survived, and were kept together until the following November. The 75 sheep which had received the *premier vaccin* were designated series A; the 75 sheep which had received also the *deuxième vaccin* were designated series B; the 75 sheep which had, in addition to complete vaccination, been inoculated also with virulent virus were designated series C. Certain animals of each series were inoculated with virulent virus after intervals of about five, seven, and nine months. The dates and results are indicated in the following table :—

Table showing the results of Inoculation with Virulent Virus at various dates after Vaccination and Inoculation :—

DATE.	November 17th, 1881.			January 16th, 1882.			March 18th, 1882.		
SERIES.	A	B	C	A	B	C	A	B	C
Inoculated	6	6	6	12	12	6	12	12	6
Died	2	0	0	1	1	0	1	1	0
Made ill	4	a few	0	11	many	0	11	11	a few

As the result of experiments made for the Société d' Agriculture de Melun by M. Rossignol, it appeared that sheep withstood inoculation with virulent material eleven months after vaccination, but at the same time one of five sheep not only vaccinated but inoculated (as in Series C above) died. At Chartres M. Boutet inoculated on May 16th, 1882, twelve sheep vaccinated on August 2nd and 16th, 1881. The material used for inoculation was serous fluid from a sheep which had died three hours earlier of spontaneous anthrax; five of these twelve sheep died. M. Pasteur objected to the very large dose of virulent material injected. The experiments were consequently repeated, only two drops of blood being used. The result of these experiments is indicated in the subjoined table :—

Table showing the Number of Deaths caused by Inoculation of Virulent Material at various intervals after Vaccination :—

Number of sheep.	Interval between vaccination and inoculation with virulent material.	Number of deaths after the inoculation.	Percentage of deaths.
12	13 months	4	33
12	8½	2	17
12	4½	3	25
10	3	2	20
8	not vaccinated	8	100

An experiment made for the Société Générale de Médecine Vétérinaire gave less satisfactory results. The inoculation with virulent material was made on July 28th, 1882. Twenty sheep vaccinated in July, 1881, all survived, while seven out of ten vaccinated in February, 1882, died; this vaccine sent out during February was, as will be shortly explained, subsequently ascertained to be too weak.]

"From a consideration of all the experiments which I have reported, we may conclude that at the end of a year sheep are still vaccinated against inoculation of virulent virus in the proportion of at least 60 per cent. If we take account of the fact that subcutaneous inoculations are more dangerous to sheep than the ingestion of bacterial spores from the fields; if we remark also that the immunity acquired by vaccination need only produce its effect upon those animals which would be spontaneously attacked; if, further, farmers will have their animals vaccinated in spring, that is to say, a short time before anthrax ordinarily appears, we may boldly conclude that vaccination will preserve the animals during at least one year, and that it will perhaps even last longer. But for the present, and until new experiments have been made, it would appear to me to be necessary to practise re-vaccination every year. Further, as a universal rule, if a flock, although vaccinated, is accidentally attacked by anthrax, there ought to be no hesitation about re-vaccinating it immediately."

VARIATIONS IN THE VIRULENCE OF THE VACCINES.*

A tube containing a cultivation of the virulent virus had been preserved by M. Pasteur from the commencement of his study of anthrax—that is to say, for more than five years, and every six months it was tested to ascertain if the spores were still living, and whether their virulence had changed. For this purpose a flask of broth was inoculated with a drop of the original tube. Growth occurred as in ordinary cases, but there was a slight delay in the development, as if the old spores needed a longer time than the young to germinate. But the cultivation once made when used for inoculating sheep caused their death as

* Extracted from Chamberland's *Le Charbon et la Vaccination Charbonneuse*, chap. xxx. p. 281.

surely and as rapidly whether the cultivation had been made at the end of four or five years as after a few months. The spores of the vaccines seemed to have the same properties as those of the virulent virus with regard to resistance to the action of heat, alcohol, compressed oxygen, &c., and it was natural to believe that they would also have analogous properties with regard to the preservation of their virulence. I may add that in numerous successive cultivations of the virulent virus made in the course of our researches we had not observed any sensible diminution in their virulence. We thought, therefore, that successive cultivations of the vaccines would also all preserve their own proper virulence.

[At the beginning of 1882 facts became known which showed that this assumption was unfounded, and on June 8th, 1882, M. Pasteur, in the course of a speech before the Société Centrale de Médecine Vétérinaire, spoke as follows :—]

"Unfortunately the facts observed in practice have shown that the vaccines were enfeebled, and that in consequence accidents of various kinds have occurred. In certain cases the 'first-vaccine' being, in relation to the 'second-vaccine,' too feeble, deaths were observed in the flocks immediately after the second vaccination, which, instead of being a vaccination, was a virulent inoculation. In other cases, the first and the second vaccines being both enfeebled, no longer afforded sufficient protection, and splenic fever was seen to cause the death of vaccinated sheep after one or two months. . . . The accidents of which I speak did not all occur at once; when they were understood it was necessary to make vaccines *de novo*. This led to a double loss of time, and it must be recognized that the vaccinations from the month of November, 1881, to the month of March, 1882, have been insufficient . . .

"The conclusion from all these facts is that the different kinds of virus, instead of being, as was formerly supposed, fixed and immovable, are on the contrary variable, becoming modified by the influence of time, of climacteric circumstances, &c. . . .

"We must guard ourselves against putting down to the account of the attenuation of the vaccines all the deaths observed after the first or the second vaccinations. Thus in the report of M. Leblanc, I find that MM. Leblanc and Cagny in 1881 vaccinated a stable of thirty animals under the following circumstances:

The mortality had ceased for ten years, and, suddenly, from the Saturday to the Tuesday six oxen and cows died of splenic fever. If the first vaccination, instead of having been made a month after, had been made just at this time, the deaths of these animals would have been attributed to the vaccine. An analogous fact has been reported to me from the neighbourhood of Meaux. It must not be forgotten, therefore, that if the animals are at the time of the operation already spontaneously inoculated, vaccination will not protect them. It is well known that in the human species vaccinia* does not protect when small-pox is already in the period of incubation.

"Racial differences must also be taken into account. Some breeds of sheep are much more sensitive than others from the point of view of splenic fever, and then the first vaccine is relatively too feeble to enable them to resist the second vaccine. It is, therefore, sometimes necessary at the first to test such and such a race, in order to learn what strength of vaccine suits it...

"The importance of the accidents which occur ought not to be exaggerated; the accidents may be alarming to the proprietor or the veterinary surgeon who witnesses them, but to him who takes all the facts into consideration they are a small matter: taking, thus, account of all the facts, I conclude that vaccination ought to be a general practice. The best way would be to vaccinate with a guarantee. By adding to the price of each vaccination a sum of ten centimes, a reserve fund would be formed sufficient to afford a guarantee against all losses."

M. Chamberland states that new vaccines were prepared and were used extensively up to the month of October, 1882.

At this time, it was ascertained from the information supplied to us by different veterinary surgeons, that the "first-vaccine" was a little too strong, and that in a few flocks a certain number of sheep had succumbed after the inoculation of this "first-vaccine." Some cows and oxen also had suffered from œdematous swellings, generally not severe. This first vaccine possessed the great advantage that it vaccinated directly and completely 95 per cent. of the animals, so that after the first

* *La vaccine.* The statement is at variance with facts generally accepted in this country. See the late Mr. Marson's article on Variola, in Reynolds' *System of Medicine*, First Edition, vol. i. p. 477; Second Edition, vol. i. p. 268; see also *A Handbook of Vaccination*, by Edward C. Seaton, M.D., p. 103, *et seq.*—D. W.

vaccination the animals were protected from spontaneous anthrax. This vaccine has been employed in the re-vaccination of flocks incompletely vaccinated, and the results have everywhere been excellent. This vaccine, therefore, might be used with great advantage in all cases where the disease is already raging in a flock, because the mortality would thus be almost instantaneously arrested. Some animals would succumb after the inoculation, but it would be better to run the chance of losing even 1 per cent. of the animals than to wait about three weeks before vaccination would be complete, as is ordinarily the case. During this time the natural mortality would certainly be greater than that resulting from the inoculation of the vaccine . . .

We have undertaken numerous experiments in order to ascertain the causes of this diminution of virulence, and the conditions under which the relative virulence is preserved. We have thus been able to reproduce, so to say mathematically, the vaccines employed during the summer of the year 1881.

These vaccines alone have been used since the month of October, 1882, and up to the present time we have not received any reports of mortality among the sheep, or œdema in the cows, oxen, or horses. The year 1882, if it gave rise to some disappointments, has thus powerfully served the general cause of vaccination for anthrax. Now, owing to the numerous new researches which have been made as to the relative virulence of vaccines, and as to the conditions of the preservation of this virulence, we can affirm that we are in full possession of these conditions, and that the few failures which have been reported will not recur in the future. The most important condition to observe in order to ensure success is to use, whenever possible, vaccine which is fresh, that is to say, recently prepared. We have for this reason recommended veterinary surgeons not to keep the vaccines, but to use them as soon as possible after they have been received. This recommendation is of especially great importance with regard to the "first-vaccine," for we have ascertained that the virulence of the "first-vaccine" diminished much more rapidly than that of the second. The latter can be preserved with all its properties unimpaired in the condition in which it is despatched for at least three weeks, so that in the future it will be easy to despatch the first and the second vaccines at the same time. The first should be employed imme-

diately, the second should be kept in the cold, in a cellar for instance, without opening the tubes, and should be used twelve days or a fortnight later.

Vaccines which are not to be used for a very long time, for instance, two or three months, after being put in the tubes must be prepared with special and minute precautions. We are now able to fulfil these conditions almost rigorously. Still it is not yet possible for us to affirm that the vaccine will have exactly the same properties as in the fresh state. The spores, in fact, as they grow older do not appear to have the same aptitude for developing in the bodies of animals as when they are recent. When they are too old they do not develop, and do not produce any effect. . . . The problem of preserving the virulence of vaccine stored in glass tubes is, therefore, not absolutely solved. I believe that I may even add that it will probably never be solved, for it will never be possible to make old spores, tending towards death, possess the same force and the same activity as recent spores, or as adult bacteria in full course of reproduction and development. I think, therefore, that it would be extremely advantageous, not to say indispensable, to establish, in distant countries, in all countries where it is fifteen or twenty days before the vaccine arrives at its destination, small manufactories to produce fresh vaccines which would be sent out through the neighbouring districts in the fresh state.

THE METHOD OF VACCINATING.*

Vaccination is effected by making two inoculations at an interval of twelve or fifteen days, the first with the "first-vaccine" which only partially preserves the animals, and the second with the "second-vaccine," which is much more active than the first and renders the animals completely refractory to anthrax. It is a good plan to make these two inoculations at two different parts of the body. If, for example, the first is made in the right thigh, the second should be made in the left. The inoculations may probably be made at any part of the body, but so far sheep have been vaccinated in the thighs, oxen or horses behind the shoulders, and in some cases in front. The

* Condensed from Chamberland's *Le Charbon et la Vaccination Charbonneuse*, p. 297. (*Vide supra.*)

last situation has been most frequently chosen in saddle horses in order to avoid the saddle bearing on the point of inoculation.

Full grown sheep of all ages, lambs even when very young, ewes more or less advanced in pregnancy, some on the point of lambing, have been vaccinated without bad consequences. Still accidents have sometimes been reported as occurring in young lambs when the same vaccine used at the same time in full-grown sheep produced only a more or less slight disease but not death. With the vaccines now in use even very young lambs can be vaccinated without inconvenience.

Vaccination of ewes on the point of lambing has also given rise to some accidents. Several times abortions have been reported to us. We thought at first that they ought to be attributed to the shaking which the animals underwent in turning them over on to their backs in order to present the internal aspect of the thigh to the operator, but it may also be possible that the fever from which the ewe suffered may have directly produced the abortion. According to researches which I have recently made in collaboration with M. J. Straus it appears that, contrary to the view hitherto held, bacteria introduced into the mother sometimes pass into the fœtus. It must be asked then whether the cases of abortion which have been reported are not due to the vaccine bacteria of the mother, bacteria which she can withstand without serious results, passing into the fœtus, which, being less resistant, has succumbed, and has consequently been expelled. However this may be, it is better to vaccinate the ewes when they are not pregnant, or at least during the earlier periods of gestation. Except in urgent cases, as for example when the disease is raging in a flock, ewes on the point of lambing ought not to be vaccinated.

It may be asked whether lambs born of vaccinated ewes are themselves vaccinated. This question is not completely cleared up, and we are making experiments with regard to it. Everything leads us to believe that a large number of the lambs, if not all, do not enjoy immunity, so that for the present it is necessary to vaccinate lambs when there is reason to fear that they may be exposed to the spontaneous disease.

Finally, spring ought, whenever it is possible, to be chosen for the operation, for the disease being rare at that time, we avoid, at least in the great majority of cases, the combined

action of the vaccine and of spontaneous anthrax. Moreover, sheep recently vaccinated are better able to resist the disease, which rages, generally, in summer and autumn . . .

In oxen slight œdema sometimes follows the puncture; these swellings disappear without treatment and should never be opened. Cows being also liable to suffer from œdema or perceptible fever after the inoculation, the quantity of milk diminishes; it is therefore better to vaccinate cows when they are giving little or no milk. Further, as it is not absolutely proved that the vaccine-bacterium never passes into the milk, there is an additional reason for choosing the time above indicated. . . . All that has been said above with regard to pregnant ewes applies also to cows in that condition.

The vaccines are sent out in tubes, closed by caoutchouc corks, which contain the quantity of liquid necessary for the vaccination of 100 sheep or goats, or 50 oxen, cows, or horses. The quantity of vaccine to be used for inoculating the larger animals is practically double that used for the smaller. The liquid is drawn up into a Pravaz' syringe. . . . The tube of vaccine ought to be well shaken before removing the cork so as to mix the contents thoroughly; unless this is done the vaccine-bacteria are deposited on the bottom or sides of the tube, and there is a risk of drawing up several syringefuls of liquid in which there are no bacteria, or very few; the negative results which have been observed in some cases after vaccination must be attributed to the neglect of this precaution. . . . The syringe being filled,* the runner which is at the top of the piston-rod is turned so as to descend to the division marked 1 on the rod. Then an assistant lays hold of the sheep to be vaccinated, and holds it for the operator, turning it by its fore legs into a sitting attitude. The operator introduces the needle under the skin, about the middle of the right thigh, and pushes the piston until the runner touches the syringe. The inoculation of the first animal is thus completed. The syringe is withdrawn, the runner is turned in the opposite direction so as to bring it to the division marked 2 on the piston-rod. Then the second sheep is inoculated. The runner is then brought to the division 3, and so on, each syringe being sufficient for the vaccination of 8 sheep.

* The syringe must be quite filled to the exclusion of all bubbles of air.

The syringe is filled again and the process repeated. With a little practice 150 or 200 sheep can be vaccinated in an hour. . . .

The vaccine liquid introduced under the skin ought to be pure in order to produce its proper effect, that is to say, it ought not to be contaminated by any foreign organism derived from the aerial dust, morsels of wool or straw, or scraps of dung sometimes found on the animals, for if such impurities get mixed with the vaccine they may give rise to other diseases, especially to septicæmia, phlegmons, &c. At other times these impurities may, as M. Pasteur has shown, produce no apparent effect, but may hinder the action of the vaccine. In order to attain the highest possible degree of this condition of purity, the syringes are boiled after each operation, and put together again in order to prevent the small quantity of liquid which has soaked into the piston or remained below from giving rise to new organisms; with regard to the tube containing the vaccine, the caoutchouc cork by which it is closed ought to be put back into the tube each time after the syringe has been filled, and only the extremity not directly in contact with the tube ought to be touched with the fingers. There is another cause of impurity which it is almost impossible to avoid. The outer surface of the needle, in fact, becomes quickly covered with scraps of wool, hair, or even of dung; in this way each time that the needle is put into the tube in order to draw up a fresh quantity of liquid some of these foreign bodies get mixed with the liquid and destroy its purity; in consequence when there remains but little liquid, that is to say, when the needle has been plunged into it ten times, the liquid is almost always impure. . . .

We have endeavoured to obviate these difficulties, and we believe that we have succeeded in the syringe constructed under our direction by M. Collin . . . With this syringe once filled fifty sheep can be vaccinated in succession. The vaccine is not in contact with aerial impurities, and cannot be soiled by foreign bodies sticking to the outer surface of the needle. . . . It can be easily cleaned. . . . Strictly speaking a single syringe of this pattern would do for all vaccinations; but it appears to be preferable to get two syringes—one for the first, and the other for the second inoculation. . . . This syringe has certain inconveniences; the liquid injected is not seen, and the quantity of liquid injected cannot be regulated with the same precision as

when a Pravaz' syringe is used. . . . [The vaccine for this syringe is sent out in tubes identical with ordinary tubes, except that both extremities are closed by small caoutchouc corks. The smaller cork is withdrawn and the extremity of the tube introduced into the reservoir of the syringe; on removing the other cork the liquid runs into the reservoir.]

In all cases, whatever kind of syringe be adopted, a tube of vaccine which has been opened must never be used on the next or following days, for in that interval the vaccine liquid may have been altered by foreign organisms. If the vaccine when sent out cannot be used for three, four, or five days the tubes ought to be put in a cool place, if possible in a cellar, in order to prevent the possible ulterior development of foreign organisms. As a rule, and whenever it is possible, fresh vaccines ought to be used, and used on the day or the day after they are received. When it is desired to keep vaccines for a long time—one, two, or three months—it is necessary to take special and very delicate precautions. This we do in the case of all vaccines sent to foreign countries; but for France we do not take these precautions, and the vaccines ought always to be used within eight or ten days of the day on which they are despatched. As to the vaccine liquid remaining in the tube when the operation is finished it ought to be destroyed by plunging the tubes into boiling water.*

DR. KOCH'S CRITICISM OF M. PASTEUR'S METHOD AND THEORY OF ATTENUATION.†

It is well known that M. Pasteur first made experiments upon the attenuation of the microbe of fowl-cholera, and that he arrived at the conviction that attenuation is caused by the oxygen of the air. M. Pasteur then applied the results of these experiments to the bacillus of splenic-fever, and he succeeded in attenuating its virulence also, to such a degree that some animals vaccinated with it resisted the vaccination, and after

* [In a private letter, dated April 23rd, 1885, M. Pasteur states that every year material for the vaccination of four or five hundred thousand animals (sheep, cxen, and horses) is sent out.—D. W.]

† Extracted from Dr. Koch's reply to M. Pasteur's discourse at Geneva. The pamphlet, which was published simultaneously (by Fischer, of Cassel and Berlin, 1883) in German and French, contains, as does also M. Pasteur's reply, published in the *Revue Scientifique*, S. III., T. v., p. 74, a good deal of personality and of the *odium pathologicum* with which the reader need not here be troubled.—D. W.

this preventive vaccination remained refractory when subsequently infected with the most powerful virus of anthrax.

Nevertheless in order to confer immunity on the animals against inoculation with non-attenuated virus, without encountering too many losses, it is necessary, as M. Pasteur perceived, to have recourse to two successive preventive vaccinations, the one with a highly attenuated material which he calls the first vaccine, the other with the second vaccine, the virulence of which is much less attenuated.

Even at the time of his first successes with fowl-cholera M. Pasteur was carried away by his great expectations, and after he had succeeded, by preventive vaccinations, in rendering a few sheep refractory to anthrax, he did not hesitate to draw a general conclusion from the results of these experiments. It seemed to him indubitable that immunity against anthrax could be conferred not only on sheep, but on all animals susceptible to the disease. Further, he definitely stated that all infective diseases would behave like anthrax, and that it was possible to attenuate the corresponding microbes and convert them into protective vaccines. With the fullest confidence did he proclaim a victory over the infective diseases in the near future. The results of an investigation of the subject of immunity conducted by Dr. Loeffler* in the Imperial Health Office was published at this time. M. Pasteur had described his method of attenuating the bacillus of anthrax in so incomplete a fashion that Loeffler had to make some very extensive researches for himself to rediscover and study this method. Dr. Loeffler's work thus involved almost of necessity the question of immunity in general, and from numerous experiments made on mice, rabbits, rats, and guinea-pigs, he was led to the following conclusions. That it is quite true that there are diseases, produced by bacteria, to which an individual who has once suffered from them is refractory; but that, on the other hand, it is also known that there are a considerable number of diseases, produced by bacteria, which can attack the same individual several times in succession at short intervals, and which, consequently, do not protect from a subsequent infection. . . .

First of all M. Pasteur's theory, which would make, what may be true for fowl-cholera and anthrax, a general characteristic

* *Mittheilungen a. d. k. Gesundheitsamte*, 1er Band, 1881.

of all infective maladies, must be rejected as contrary to the ascertained facts of medicine. Dr. Loeffler, in the investigation of which we have spoken, relied not only on the results of his own experiments with the infective diseases artificially produced in animals, but also on facts in practice which show that erysipelas, gonorrhœa, and intermittent fever, though they are, as has been proved, infective maladies caused by bacteria, do not confer immunity against recurrences. Tuberculosis has now been for some time added to the number of the infective diseases which can attack man several times in succession. No physician has ever yet thought of maintaining that a person who has suffered from tuberculosis, and who, after suffering from scrofula or fungous disease of the joints, for instance, has been cured, is afterwards protected from tuberculosis. On the contrary, practical experience shows that persons of this class have a stronger disposition than others towards tubercular affections, and that they frequently become phthisical later on. Neither with regard to leprosy, which is evidently a bacterial disease, has anything been ascertained which would favour the supposition that immunity against this disease could be acquired. Thus the rule which M. Pasteur believes to hold good generally cannot be admitted so to hold.

But we go further, and say that not even for anthrax can the law of immunity be maintained to the complete extent given to it by M. Pasteur. Dr. Loeffler had previously found that immunity cannot be conferred on guinea-pigs, rats, rabbits, or mice. This fact has been confirmed by all experimenters who have turned their attention to this question.* . . . At the Imperial Health Office numerous experiments have been made on rabbits, guinea-pigs, and mice with the virus of anthrax at different degrees of attenuation, and finally with the vaccine of Pasteur: in spite of all the efforts made, immunity against the effects of the non-attenuated virus has not been conferred on one of these animals: all without exception died of true anthrax after the control inoculations. It may therefore be considered certain that by M. Pasteur's method all species of animals are not capable of acquiring an immunity.† According to all appear-

* Experiments of Gotti, Guillebeau, and Klein (see XIIth Annual Report of the Local Government Board. Supplement containing the Report of the Medical Officer for 1882, p. 205, *et seq.*) are quoted.—D. W.

† M. Pasteur (*Réponse au Docteur Koch*, Rev. Scient., S. iii., T. v. p. 83) asks,

ances horses are equally refractory to protective vaccination, for at the meeting of the Société Centrale de Médecine Vétérinaire at Paris, on June 8th (1882),* numerous failures after the vaccination of horses for anthrax were mentioned, and it has also been reported elsewhere that horses bear the preventive inoculations very ill. With regard to man, Dr. Loeffler has shown by a series of examples that we are not refractory to anthrax after having once passed through that disease. This opinion has been recently confirmed by J. de Jarnowski, who has in his own practice observed fifty persons attacked by anthrax; of these he cited two, of whom one had taken anthrax twice in two years, and the other three times in three years.†

Distinct immunity has up to the present time only been produced in sheep and bovine animals, and provisionally it is only in these two species that advantage can be taken of preventive vaccination. According to M. Pasteur's statement the preventive vaccinations of sheep and of animals of the bovine species, performed according to his method, is so entirely free from danger, and the protection is so sure, and lasts so long,

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with reference to this criticism, "Have I ever recommended the vaccination of rabbits and guinea-pigs with the vaccines prepared for sheep and oxen?" He adds that Dr. Koch is mistaken in affirming that the smaller animals cannot be vaccinated against virulent anthrax, and refers to the experiments of Dr. Feltz. These experiments were reported to the Académie des Sciences on November 6th, 1882 (*Comptes Rendus*, T. xcv., p. 861 *et seq.*). Dr. Feltz says: "By placing fresh cultivations in warm chambers maintained rigorously at a temperature between 42° and 43° C. I have been able to assure myself, by inoculating animals with the contents of my flasks, that the anthrax-virus loses its strength progressively in direct proportion to the time that it remains in the warm chamber, until its virulence completely disappears. This kind of degeneration of anthrax is characterized by the slenderness of the filaments, and by a certain dwarfing of the germ-corpuscles in the cultivations. . . . The comparative study made with rabbits and guinea-pigs of the different degrees of attenuated virus proved that the rabbit resisted their action much better than the guinea-pig. If it is difficult to find the degree of attenuation of anthrax which makes the guinea-pig ill without killing it, it is far otherwise with the rabbit. . . . Confident of the possibility of effecting the attenuation of the virus of anthrax under the conditions indicated by M. Pasteur, I sought first to vaccinate rabbits, and then sheep, against anthrax. With this object I inoculated several series of rabbits every fifteen days with less and less attenuated kinds of virus. As early as my third series I obtained surprising results; almost all the rabbits treated as I have just stated resisted the most virulent cultivations of anthrax which I had and even the inoculation of the blood from a case of anthrax."—D. W.

* See p. 583.

† For an alleged example of acquired immunity in man see Cosson "Sur en cas de preservation contre la maladie Charbonneuse, observé chez l'homme," *Comptes Rendus*, T. xciv. p. 697.—D. W.

that agriculture is invited to reap great benefits from it. As a matter of fact, M. Pasteur's method has already been applied on a large scale, and the sole question now is to ascertain whether M. Pasteur's promises as to the harmlessness of vaccination and as to the efficacy of the protection will be realized. This is now the *crux* of the whole question, it is therefore worth discussing in detail. . . .

In the first place with regard to the preparation of the vaccines, M. Pasteur contents himself by saying that he cultivates the bacillus of anthrax in neutralized infusion at a temperature of 42° to 43°, and that at the end of about twenty days the virulence is sufficiently attenuated for the vaccination of sheep. With regard to the most favourable time for obtaining the second vaccine, and to the qualities which render the recognition of the degree of attenuation sufficiently certain, M. Pasteur does not express himself categorically, and yet this is the essential point. It would certainly be desirable to learn something more precise with regard to the preparation of the vaccine, and consequently I will report our experiments on this subject.* It is very important to make use of a thermostat which maintains the same temperature for weeks without variation. To this end we made use of an apparatus of the Arsonval pattern supplied to us by Wiesnegg of Paris. Small flasks containing about twenty grammes of neutralized chicken broth which had been inoculated with the usual precautions with the bacillus of anthrax were kept in this apparatus at a temperature of 42·5 C. Eight to ten days mice, full grown guinea-pigs, and large vigorous rabbits were inoculated with the liquid from one of the flasks, and at the same time a cultivation in nutrient jelly was made with the fluid used for the inoculation. At the beginning all the animals died of anthrax after the inoculation. After several days the number of days is not the same in all the experiments, and varies sometimes from one flask to another in the same experiment) inoculation is uncertain in its action on the large rabbits, for only some of them die, for instance, one or two in three or four, while all the mice and guinea-pigs are still killed by the inoculation. Still later the guinea-pigs in the same way resist the inoculation while the mice continue to perish. Finally, cultivations of the bacillus are obtained with which mice can be

* Performed in co laboration with Drs. Loeffler and Gaffky.—D. W.

inoculated without being killed. These bacilli of anthrax which have completely lost their pathogenic action do not differ morphologically from the virulent bacilli. They are entirely immobile, and in pure cultivations they, like the others, form long filaments. The cultivations which kill mice but spare guinea-pigs furnish the best material for the vaccination of sheep, and those which when inoculated produce anthrax in guinea-pigs but do not kill all the large rabbits with certainty furnish the material for the second vaccination. Between these degrees, as well as above and below, there are numerous gradations which can, according to circumstances, be used in the same way, when a second vaccination is not considered sufficient. I doubt whether M. Pasteur knows the characteristic signs, as I have indicated them, of the degree of attenuation, otherwise there would not be such numerous variations in the effects of his vaccines. I have had occasion to test a *premier vaccin* from M. Pasteur which did not even kill mice, and which was consequently too feeble, and a *deuxième vaccin* which infected with anthrax all the rabbits vaccinated, and which consequently was too strong. Dr. Klein inoculated four guinea-pigs and six mice with the *premier vaccin* furnished by M. Pasteur's agent, M. Boutroux; during the first forty-eight hours three guinea-pigs and the six mice died, which proves that this inoculating material was too strong for a first-vaccine. In Hungary, according to a report published in the Agricultural Journal, twenty-two sheep were inoculated on one day with the second vaccine without having previously received the first vaccine; in spite of that these animals remained well, and there is therefore reason to believe that this second vaccine was too feeble.

The temperature to which the cultivations are submitted has the greatest influence on the length of time within which attenuation is accomplished. The more nearly the temperature approaches 45° the more rapidly is attenuation produced, so that at that it may be complete within six days. At 42° a period of thirty days is sometimes required. Further, it is absolutely necessary to test the vaccine on mice, guinea-pigs, and rabbits. The vaccine, kept for a long time at the temperature of an inhabited room, gradually loses its virulence. M. Pasteur has made the same observation, which numerous experiments enable us to confirm.

We continued to inoculate nutrient jelly with our attenuated cultivations. This very simple and convenient method can be employed to obtain a given quantity of vaccine grown in a state of purity. If we insist on the purity of the cultures it is because the intrusion of foreign bacteria, among which may be some which are pathogenic, causing for instance septicæmia, uselessly increases the danger of vaccinations made with such fluids. Thus a part of the bad results following the vaccination of horses appears to have been due to contamination of the vaccines with bacteria which produce septicæmia; this appears the more probable to me, as on examining microscopically several samples of the original vaccines of M. Pasteur I have found them highly contaminated by numerous varieties of bacteria other than those which ought to have been met with.*

[Dr. Koch then states that a few experimental vaccinations were made with vaccines prepared as above stated, and with others obtained from M. Pasteur's agent in Paris. The *premier vaccin* (which killed mice but not guinea-pigs) produced hardly any reaction in sheep. After the inoculation made later on with the *deuxième vaccin*, a certain number of animals were attacked by anthrax and died. Owing to the small number of animals operated on he considered it useless to give the percentage of losses. The general result was in accordance with the experiments made at Kapuvar and Packisch, which he chooses for comparison with his own experiments as they were observed and controlled in a trustworthy way by a special commission. No deaths occurred after the inoculation of the *premier* either at Kapuvar or Packisch, but after the *deuxième* five out of fifty sheep died at Kapuvar, and three out of twenty-six at Packisch. Numerous other vaccinations in other localities supported the contention that no deaths occurred after the first but about 10 to 15 per cent. after the second. For the second experiment at Packisch M. Pasteur provided a less efficacious vaccine, and only one sheep in 251 died.

* M. Pasteur in his *Réponse au Dr. Koch* (*supra cit.*) contents himself by scornfully retorting, on this head, that for twenty years before Dr. Koch's scientific birth in 1876, it had been his sole occupation to isolate, and grow microbes in the pure state, and that therefore Dr. Koch's insinuation that he does not know how to make pure cultivations cannot be taken seriously. But Dr. Klein in his Report, cited above, to the Medical Officer of the Local Government Board (Twelfth Annual Report, p. 57—58) confirms Dr. Koch's statement: in the *deuxième vaccin* there were scum-forming bacilli, and in the *premier vaccin* micrococci also.—D. W.

Three weeks after the second vaccination Dr. Koch and his fellow-workers made a control inoculation with non-attenuated virus. Of six sheep vaccinated by M. Pasteur's method one died of anthrax, while two other sheep vaccinated with a different vaccine survived. That fewer deaths occurred after the control inoculations at Packisch and Kapuvar, Dr. Koch believes to have been due to the virus sent by M. Pasteur from Paris not being so virulent as that used by him; but Dr. Koch admits that his own numbers are too small, and quotes in their support the experience of Saake, veterinary surgeon of Wolfenbüttel, who lost two out of ten sheep inoculated eight weeks after vaccination with blood from a case of spontaneous anthrax; as well as the experiments of M. Bassi, of Turin,* who took twelve vaccinated sheep and inoculated six with virulent virus sent by M. Pasteur, and six with the blood of a heifer which had died two hours earlier of anthrax; two of the sheep inoculated with the heifer's blood died, but none of the others. Both the above series of vaccinations were made after April 1st, when M. Pasteur discovered that his vaccines were not strong enough. Dr. Koch also cites experimental vaccinations made in Hungary, at

* At *Turin*, the animals inoculated with the virulent blood from the heifer were six sheep, two oxen, and one horse, all vaccinated on April 20th and May 5th, 1882, and four sheep, two oxen, and two horses all non-vaccinated. Two of the vaccinated sheep died, the other vaccinated animals survived; all the non-vaccinated animals died except one ox.

The results of the experiments at Packisch are shown in the following table :—

	Totals.		Deaths after *deuxieme vaccin.*			No. inoculated with virulent material.		Number died after this inoculation.		Date of vaccination.		Date of inoculation.
Packisch.	Vaccinated.	Non-vaccinated.	Vaccinated.	Vaccinated.	Non-vaccinated.	Vaccinated.	Non-vaccinated.	First.	Second.			
First experiment...	25	25	3	22	25	0	24	April 5	April 19	May 6		
Second experiment	251	...	1	24	12	2	12	May 10	May 2(	May 30		

The virulent material used in the first experiment was obtained from a sheep which died about thirty-six hours after inoculation with virulent material sent by M. Pasteur. In the second experiment half of the animals were inoculated with virulent material sent by M. Pasteur, the other half with blood from a sheep which had died the previous day after inoculation with this same virus.—D. W.

Bauchery, at Montpothier, and at Packisch in support of his view that the vaccines furnished by M. Pasteur even after April 1st were sometimes too feeble, sometimes too strong, that is, even less sure than the vaccine distributed during the winter.* Dr. Koch then proceeds as follows :—]

M. Pasteur felt the difficulty of his position as early as the meeting of the Société Centrale de Médecine Véterinaire on June 8th. If he furnished a powerful vaccine, conferring immunity against anthrax by inoculation (or at least against inoculation with the material supposed to be virulent by M. Pasteur), then the animals perished from the effects of the second vaccine in too large numbers. If, on the contrary, he supplied too feeble a vaccine, as has been the case during last winter,† then evidently the protection was insufficient. In order to escape from this embarrassment M. Pasteur put forth the singular opinion that it is not necessary to inoculate sheep with so powerful a vaccine involving such great losses, because the chances of death are very much greater as a consequence of direct inoculation than of spontaneous inoculation, that is to say, of such inoculation as is caused by the food, or by sojourn in infected fields; to protect from the latter he said that a feebler vaccine sufficed. M. Pasteur did not succeed in producing a single argument to support this evidently arbitrary assertion, which would never have been put forward except to ward off the danger by which preventive inoculations were menaced. In reality the question whether animals could be preserved by preventive vaccination from natural infection ought to have been elucidated before introducing preventive vaccination into practice, and before making great sacrifices to vaccinate hundreds of thousands of animals. For if the facts are contrary to what M. Pasteur supposed, and if vaccinated animals enjoy immunity against artificial infection without possessing immunity, or only an insufficient immunity, against natural infection, it may be asked if the vaccination is of any use whatever. This being evidently the most important question to answer, in order to be able to pronounce upon the value of the artificial immunity to anthrax, we, from the beginning, gave it the first place in the experiments which we undertook at the Imperial Health Office . . . . .

* See M. Chamberland's statement on this head, page 584.

† See page 583.

M. Pasteur believes that coarse forage, which occasions slight lesions in the mouth of the animal, is the vehicle of the infection. If this were so we should have at bottom only to do with a particular species of inoculation with the virus of anthrax. Different arguments, which I will not here repeat, as I have previously stated them in detail, might be advanced against the explanation given by M. Pasteur. I will instead describe certain experiments which directly refute M. Pasteur's theory.

[Dr. Koch caused sheep to swallow infective material; some of the sheep received only the bacillus and not its spores (the spleen of a guinea-pig dead of anthrax), others spores as well as the bacillus (a cultivation on potato), the material was administered in a potato scooped out to receive it, and the animals were subsequently given soft food; in this way no lesion could be inflicted on the mucous membrane. The animals which received the material containing only bacilli were none the worse though the dose was repeated, while all the animals which received the material containing spores died in a few days. The infection in these cases took place through the intestine. Dr. Koch concludes that the bacillus is killed by the acid contents of the stomach, while the spores escape into the intestine; microscopical examination made it probable that infection takes place through the solitary and agminated glands. The results were the same whether the spores were fresh, or had been kept in the dry state for a year. The quantities given in these experiments were large, but natural infection, if it commonly takes place through the intestine, must be due to the ingestion of a very small quantity of spores mixed with forage cut on marshy or flooded land covered with mud and filth. To test this point ten sheep were each given every day for nineteen days a piece of potato containing a silk thread about one centimetre long which had been impregnated with a very small quantity of the spores of bacillus anthracis, and preserved for a year in the dry state; four of these animals died between the fifth and the nineteenth day. When a large quantity of spores is given a large number pass through the bowels unchanged; this opinion Dr. Koch founds on the fact that inoculations made with the fæces, dried and kept for a year, produced the disease again. The maxillary glands are very frequently swollen in anthrax and M. Pasteur had quoted this as confirmatory of his theory that infection com-

monly takes place through the mouth. Dr. Koch points out that this swelling may also be seen in cases where the inoculation has been made in the thigh, and makes the observation that the glandular affections depends not on the site of the inoculation but on that of the subcutaneous œdema.*]

After having determined, as I have just described, the mode of natural infection, we were able to turn our thoughts to the examination, with regard to their power of resistance to natural infection, of animals vaccinated preventively by M. Pasteur's method.

With this object eight vaccinated sheep and one non-vaccinated test sheep were inoculated with active material obtained from a case of spontaneous anthrax. Two days later the control sheep and one of the sheep which had been submitted to preventive vaccination were dead of anthrax. The fact that one of the vaccinated sheep contracted anthrax proved that the vaccine used for the control vaccination possessed a considerable degree of virulence. This control vaccination with very virulent material ought at the same time to be considered a new preventive inoculation, and we were justified in believing that these animals which had undergone two preventive vaccinations, and further, the inoculation with very virulent anthrax, had attained the maximum immunity.

Twelve days after the control inoculation the seven surviving sheep, together with a sheep which had not been vaccinated preventively, and was kept as a control animal, received with their food spores of anthrax. These spores had been grown on potatos, and were derived from the same anthrax material as had been used for the last vaccination of the sheep. The control animals and two of the triply vaccinated sheep died of anthrax

* M. Pasteur (*Réponse au Dr. Koch*) recalls the similar experiments made by him in 1878 (with M. Chamberland). (See page 561.) The mortality among animals fed with contaminated food was 33 per cent., while the mortality among animals inoculated was 100 per cent. The mortality in the feeding experiments was increased by mixing sharp-pointed objects with the food. M. Pasteur says that he does not dispute that sheep fed on soft food containing the spores in large numbers may die of anthrax contracted through the mucous membrane of the intestine, but contends that under natural circumstances where the number of spores ingested is comparatively small the ordinary site of infection is the mouth and pharynx. M. Chauveau (*Comptes Rendus*, T. xcii. 844) made some experiments which seem to show that even sheep very susceptible to anthrax, can resist a very minute dose of the virus. Compare the results of the experiments of MM. Arloing, Cornevin, and Thomas with the virus of Chabert's disease. See page 617.—D. W.

within the next two days. Thus the same anthrax material which at the time of the vaccination had killed one sheep in two, killed two in seven after being swallowed with forage, although the immunity of the sheep had been again reinforced by inoculation. I do not doubt but that by giving the spores of anthrax in their food to sheep which had only been vaccinated preventively twice, according to Pasteur's method, all or nearly all would in this way be infected and killed.

Our experiments have proved irrefutably that M. Pasteur is mistaken when he maintains that anthrax by natural infection is less dangerous than anthrax by inoculation. Contrary to his opinion sheep are much more accessible to natural infection through the intestine, than to vaccinal infection. We have seen that the preventive vaccinations performed in order to confer on sheep immunity against the virus of anthrax supplied by M. Pasteur, with a view to these preventive vaccinations, cause a loss which may be estimated at 12 per cent. To obtain immunity to the more virulent poison of spontaneous anthrax, as it is met with in this country, would entail a loss of about 20 per cent., and in order to protect sheep with any certainty from all kinds of anthrax-infection, and notably from natural infection, the preventive vaccinations would have to be made with material of such virulence that the losses would probably amount to double.

[Dr. Koch then complains that M. Pasteur has suppressed statistics unfavourable to the contention that vaccination by his method protects animals from spontaneous anthrax. At Kapuvar, within a little more than six months after vaccination, of the 254 vaccinated animals two (or 8 per mille) had died of spontaneous anthrax, while of the 220 non-vaccinated only four (or 18 per mille) had died of spontaneous anthrax; as the loss from anthrax during the period of vaccination had been at the rate of 48 per mille among the vaccinated, and only 4 per mille among the non-vaccinated, the total rate was much higher among the vaccinated.* At Packisch five vaccinated animals and eight of

* The large number of deaths after the second vaccination at Kapuvar was attributed by M. Thuillier, who performed the vaccinations, to septicæmia arising from impurities contaminating the vaccine fluids at the time of vaccination; there was apparently no veterinary evidence that any of the animals died of anthrax, the only one examined by a veterinary surgeon (Dr. Hartmann, chief veterinary surgeon of the State Stud at Babolna) was certified to have died from "pericarditis cum subsequente cachæxia hydræmica." In thirteen other animals which were ill but did not die M. Thuillier found suppuration at the point of inoculation.—D. W.

the non-vaccinated had died of spontaneous anthrax within three months.* At Bauchery 296 lambs were inoculated between April 28th and May 8th, 1882, and between June 22nd and 24th four died of anthrax; while 80 non-vaccinated lambs of the same flock afforded no deaths.† At Montpothier, Dr. Koch adds that the figures were still more unfavourable, as within a month of triple vaccination six sheep died of anthrax in a flock of 203.‡

Dr. Koch then proceeds as follows:—]

The materials which we now possess suffice for the formation of a sound judgment on preventive vaccination performed after M. Pasteur's method. This judgment may be summarized as follows:—The bacilli of anthrax can be attenuated by special treatment, and can be used as vaccine against materials endowed with more virulence than they themselves have in the attenuated state. Immunity cannot be conferred on all species of animals. Up to the present time, according to all appearance, M. Pasteur's method can only be employed for animals of the bovine species and for sheep. When it is desired to confer complete immunity, especially against natural infection, this procedure involves considerable losses. The smaller the losses resulting from preventive vaccination the slighter is the protection obtained.

Further, there are other circumstances of the highest importance with regard to practical usefulness. The first question which arises here is the duration of the protection afforded by vaccination. The experience on this head is, up to the present time, very insufficient, but M. Pasteur states that the animals are protected for about one year, and that they must be vaccinated afresh every year. If this be so, the losses caused by vaccination itself would much exceed those which follow the spontaneous disease, even in the countries most often attacked. Further, the signifi-

* The death-rate was 18·5 per mille among the vaccinated, and 34·5 per mille among the non-vaccinated.—D. W.

† M. Pasteur complains (*Réponse au Dr. Koch*) that Dr. Koch does not mention that the total number of animals vaccinated at Bauchery was 672, and that the 80 non-vaccinated animals belonged to a flock of 140 which had just previously lost 15.—D. W.

‡ The experience at this place appeared to be very unfavourable to vaccination: April 13th, *premier vaccin*, followed by nine deaths. April 28th, *premier vaccin*, again followed by seven deaths. May 17th, *deuxième vaccin*, followed by one death. Between June 11th and 13th six sheep died of spontaneous anthrax. June 17th, *deuxième vaccin* for the second time, followed by five deaths. M. Pasteur's comment on these figures is that anthrax was already raging on the farm when the vaccinations were performed, and that the farm was a particularly dangerous locality, every member of a flock of 250 sheep having died of anthrax in 1878.—D. W.

cance of preventive inoculations, from the point of view of public hygiene, must not be lost sight of. In fact we must not forget that vaccination is in part performed with the *deuxième vaccin*, that is to say, with a substance which can kill sheep, and which, in consequence, in its immediate effect upon these animals is not much weaker than the ordinary virus of anthrax. It is very probable also that this virus, which is only moderately attenuated, is not without danger to man. It ought, therefore, to be stated that there is some danger in scattering broadcast such a poison, by re-vaccinating thousands of sheep with it, thus increasing the possibility of infecting non-vaccinated animals, and finally creating, through the use of the wool or the consumption of the meat of animals freshly vaccinated, a real danger for man.

Consequently Pasteur's preventive vaccination cannot be considered suitable for practical use, owing to the insufficiency of the protection which it affords against the natural infection, owing to the short duration of that protection, and owing to the dangers to man and non-vaccinated animals which it involves. This does not mean that there is no future for preventive vaccination in general, but only that the method proposed by M. Pasteur is tainted by the defects which have just been discussed, and that it cannot for that reason be employed. It is possible that at a later date other improved methods will perhaps furnish the results which are now prematurely claimed for this imperfect method.

However problematical the practical usefulness of preventive vaccinations made with an attenuated virus of anthrax may be at the present moment, science will nevertheless derive great profit from the discovery that the microbe of anthrax can be attenuated and then used as a vaccine. . . .

From this point of view I acknowledge that the procedure to be followed in order to transform the virus of anthrax into a vaccine has been considerably improved by M. Pasteur. From the purely scientific point of view, as distinguished from the point of view of practice, it is of very little fundamental importance whether the number of animals which may die after preventive vaccination be great or small. Science concerns itself only with the question whether an artificial immunity can be obtained?

M. Toussaint's method gave results which were so uncertain

that at the first they appeared far from conclusive, while M. Pasteur's method has furnished the full and complete proof that artificial immunity can be obtained.

ON THE ATTENUATION OF THE BACTERIUM OF ANTHRAX BY THE INFLUENCE OF ANTISEPTIC SUBSTANCES.*

In the course of the numerous researches which we have made, under the direction of our master M. Pasteur, with regard to the bacterium of anthrax, and its spores, we have been led to examine the effect produced by a large number of antiseptic substances. During this study we have met with new conditions under which its virulence is attenuated.

Into flasks containing infusions suitable for the cultivation of the bacterium of anthrax, for example, veal or chicken infusion neutralized with potash, variable quantities of the antiseptic, the action of which we wished to ascertain, were introduced in such proportions as afforded a series of cultivation-fluids containing decreasing quantities of the antiseptic.

After having inoculated each one of these flasks with a drop of blood infected with very virulent anthrax they were placed in an incubator at 35° C.† In some of them, after the lapse of a longer or shorter time, the little flocculent masses which indicate that the bacterium is developing appeared. These flocculi appeared first in the liquids which contained the smallest quantities of the antiseptic; when this was above a certain proportion no sign of life appeared. We have thus as it were a measure of the sensitiveness of the bacterium to the agent in contact with which it grows. If from time to time we remove from each of the flasks in which the bacterium is growing a trace to inoculate infusions suitable for the growth of the bacterium, we shall have, as the offspring of each flask containing the antiseptic a series of successive cultivations, each one reproducing the bacterium with the properties and with the degree of virulence which it had in the original flask at the moment when the inoculation was made from that flask. It will then, in order to judge of the degree of virulence of the bacterium after it has developed during a given time in the presence of the antiseptic agent, suffice to inoculate various animals with these "daughter cultures."

Experiments conducted in this manner show that the addition of 1-400th part of carbolic acid to the veal-infusion prevents all growth of the bacterium. Further, the bacterium after remaining for forty-eight hours in such a medium is dead; if it is sown in neutral veal-infusion no development takes place. If the proportion of carbolic acid does not exceed 1-600th, 1-1000th, or 1-1200th the bacterium lives and grows, and, even after it has remained in contact with

* "Sur l'attenuation de la virulence de la bactéridie Charbonneuse sous l'influence des substances antiseptiques." Note de MM. Ch. Chamberland et E. Roux. *Comptes Rendus*, vol. xcvi. p. 1088.

† 95° Fah.

the antiseptic for a very long time, it easily reproduces itself when transported to a suitable nutrient liquid. Thus the bacteria have remained alive for more than six months in liquids containing 1-800th and 1-1200th of carbolic acid. If the proportion of the antiseptic is larger the bacterium dies more rapidly; in a flask containing 1-500th carbolic acid all life ceased at the end of five months. Is the evolution of the bacterium under these abnormal conditions, in contact with an antiseptic, complete? does it attain to the formation of spores? The power of the spores to resist a temperature above 80° C.* which kills the bacterial filament enables us to settle this point easily. Minute quantities of the cultivations to which the antiseptic has been added are sown in an appropriate medium after having been kept at a temperature of 80° C. for about ten minutes. If growth occurs the material sown contained spores. By this method we ascertained that 1-800th of carbolic acid in the culture-liquid is sufficient to prevent the formation of spores. In this medium the bacterium finally dies without having produced spores. When the proportion of carbolic acid is smaller (for instance 1-1200th) the bacterial filaments do form spores.

It appears therefore that the bacteria can be prevented from forming spores by adding carbolic acid to the culture liquid.

In a note communicated to the Academy by M. Pasteur† it was proved that the bacterium does not produce spores when cultivated at the temperature of 42° to 43° C., and that the bacterial filaments thus exposed to the prolonged action of the air and of heat, progressively lose their virulence. Does the same hold good for the bacterial filaments grown, without the production of spores in contact with carbolic acid? To ascertain this let us inoculate various animals with the daughter-cultivations from the flasks containing the antiseptic. A cultivation which is the offspring of a bacterium which has lived for twelve days in the broth containing 1-600th carbolic acid, is virulent for guinea-pigs and rabbits. A cultivation which is the offspring of this same bacterium after twenty-nine days, does not kill either guinea-pigs or rabbits. The action of the antiseptic has been to diminish the virulence of the bacterium. If flasks are inoculated with sufficient frequency from the original flask a series of cultivations of decreasing virulence are obtained which will furnish, as in the case of cultivations grown at 42° to 43° C., attenuated virus, capable of preserving animals inoculated preventively with them from the fatal form of anthrax. We have thus obtained, by a new procedure, a series of vaccines to choose from for practical purposes. Repeated cultivations of these vaccines reproduce the bacterium with its attenuated properties, and perpetuate these properties. The filaments obtained

* 176° Fah. † See page 565.

under these conditions, in place of being abundant and long, in tangled cottony flocculi, as in normal cultivations, are fewer and shorter and gather in little clots on the sides of the vessel. These bacteria thus altered in form easily give origin to numerous resistant spores.

Carbolic acid is not the only antiseptic which gives results of this kind: similar results can be obtained with bichromate of potassium. In infusions to which 1-1000th to 1-1900th of bichromate has been added the bacterium does not grow but quickly dies. A smaller dose of the bichromate, 1-2000th, 1-5000th permits the bacterium to develop; but, under these conditions, it does not give rise to spores and soon loses its virulence, to such a degree that when inoculated three days after the commencement of the experiment it gives origin to a cultivation which kills rabbits and guinea-pigs but does not cause the death of more than half the sheep inoculated with it. After ten days a cultivation made from the flask containing the bichromate still kills rabbits and guinea-pigs, but does not kill sheep: finally, after a longer time the cultivations are innocuous even for guinea-pigs. Smaller proportions of the bichromate retard the formation of spores but do not absolutely prevent them. The bacteria derived from filaments which have been submitted to the action of bichromate give origin to spores which perpetuate their properties and ensure their preservation. If, however, the action of the bichromate be prolonged, the bacterium loses the power of forming spores. Thus, cultivations derived from a flask containing 1-2000th, have never from the eighth day after the commencement of the experiment given rise to spores, and the same has been the case with all the successive cultivations derived from them. These bacteria, incapable of forming spores, cause the death of guinea-pigs inoculated with them in three or four days. A drop of the blood of these animals sown in meat-infusions gives rise to an abundant cultivation, [illegible] which do not produce spores: they remain in the filament [illegible] after from thirty to forty days die. This then is a variety of the [illegible] has lost the property of forming spores, and does not regain it even [illegible] passed through the guinea-pig.

The diminution in virulence thus produced by antiseptics is not transient; cultivation does not bring back the virulence. M. Pasteur has shown* that in M. Toussaint's procedure, where the bacteria are attenuated by being heated for ten minutes to 55° C., the attenuation of these bacteria was only transient, since their cultivations were virulent. M. Chauveau has shown by some recent experiments that the bacteria free from spores, and attenuated by the action of a temperature of 47° C. maintained for two or three hours, recovered their virulence in great part by cultivation.† The bacteria attenuated by antiseptics, whether they give origin to spores or not, preserve their

* See page 611.

† M. Chauveau's inquiry was undertaken to ascertain whether the oxygen of the air took any part in the attenuation produced by heating *b. anthracis* to 47° C. (116·6° Fah.) See page 613.—D. W.

diminished virulence through repeated cultivations. It would seem, therefore, that the new virulence of the varieties of bacteria thus produced is the better fixed the more gently the modifying influence acts on them.

Experiments now in progress enable us to say that other antiseptics exert an influence on the bacteria analogous to that of carbolic acid and bichromate of potash. Further, the dose of the antiseptic necessary to produce a determinate effect varies with the composition of the infusion in which the cultivation is made. Each variety of bacteria has a special action on various species of animals. Thus bacteria attenuated by bichromate of potassium can either kill sheep, or at the least make them very ill (they are then vaccinated), while these same bacteria do not produce any appreciable effect on guinea-pigs and rabbits (they are not even vaccinated). On the other hand bacteria attenuated by heat (cultivated at 42° to 43° C.) can kill guinea-pigs and rabbits, although they do not produce any effect on sheep, and do not vaccinate them. This shows how necessary it is to be cautious in the choice of vaccines to be used in practice.

In another series of experiments* we submitted the bacterial filaments to the action of the chemical agent in a liquid in which growth was impossible; a solution of an antiseptic in pure water, which could not supply any nutritive material, has been caused to act on the fully developed bacteria.

The bacterial filaments in a drop of virulent blood from a case of anthrax soon perish when placed in a 1-600th solution of carbolic acid; yet we have seen that the bacteria live and grow for months in nutritive broth which contains this same proportion, 1-600th, of carbolic acid. In a solution containing 1-900th carbolic acid the bacterial filaments remain alive for a very long time, as is proved by cultivations made from them even at the end of several months. During the whole time the experiment was in progress they did not give origin to spores, and their virulence grew feebler. Thus a cultivation made from bacterial filaments which had remained one month in contact with a solution containing 1-900th carbolic acid killed rabbits and guinea-pigs. A cultivation made after three months no longer killed rabbits. Under these circumstances the loss of

* *Comptes Rendus*, Tome xcvi. p. 1411.

virulence is less rapid than in the case where the bacteria grew in the presence of the antiseptic. This diminution in virulence towards rabbits can only be shown to exist a short time before the death of the filaments.

The essential condition for the attenuation of the virulence of the bacterium of anthrax, whether by the method of cultivations at 42° to 43° C., or by the use of antiseptics, is the absence of spores in the filaments submitted to the prolonged action of the air, of heat, or of the various chemical agents. The spore is the resistant form of the bacterium; it withdraws it, as it were, from the action of the surrounding medium, and preserves the properties of the filament which gave it birth. In spite of this resistance to external agents, the bacterial spore can be modified, and its virulence attenuated, just as the filament itself.

Well-formed spores of the bacterium a fortnight old were placed in contact with sulphuric acid (2 per cent.) and exposed to a temperature of 35° C.* in closed tubes, which were frequently agitated, to ensure that the acid came thoroughly into contact with the spores. Every second day a small quantity of these spores was sown in slightly alkaline veal-infusion. The cultivations thus obtained during the first days killed rabbits and guinea-pigs. The cultivation made on the eighth or tenth day killed guinea-pigs, but was innocuous to rabbits; the cultivation made on the fourteenth day killed only some of the guinea-pigs inoculated with it. Bacteria obtained in this way rapidly gave origin to numerous spores, and retained their attenuated virulence in successive cultivations.

But—and this is a fact worthy to be noted—the cultivations derived from the spores treated with sulphuric acid which have lost their virulence for guinea-pigs preserve it for sheep, and cause their death in the proportion of seven in ten. This fact, and the analogous facts reported in our first note,† show that each species of animal has a peculiar receptivity for each variety of bacterium which can be created by the artificial methods of cultivation.

The diminution of the virulence of the bacterial spores, and in the end, under the prolonged action of sulphuric acid, their death, supervenes the more rapidly the higher the tem-

* 95° Fah. † See preceding page.

perature and the more concentrated the acid, and the more slowly, the lower the temperature and the weaker the acid solution.

THE ATTENUATION OF THE VIRUS OF ANTHRAX AT HIGH TEMPERATURES.

[M. H. Toussaint, Professor in the Veterinary School at Toulouse, in July, 1880, announced* that he had been able to render certain animals refractory to anthrax (charbon or splenic fever) by vaccinating them in a manner which was not divulged until a subsequent meeting of the Academy. He had formed the opinion that though the bacillus anthracis could always develop in and kill certain animals—the French breed of sheep, for instance, and rabbits—it was yet not under absolutely normal conditions when introduced into the animal economy; it grew there with difficulty and never completed the whole cycle of its developmental changes; it never gave rise to spores, but multiplied always by fission. Further, he noted that certain animals never contracted the disease although they presented conditions of life very similar to other species which easily became affected by it; pigs, for instance, could not be infected; M. Chauveau had shown that nearly all the sheep of an Algerian breed were also refractory. M. Toussaint further pointed out that the young of the dog, horse, and ass were easily infected, while a large proportion of the adult animals could resist infection.

He took eight young hounds, the offspring of three different mothers; four he vaccinated. All were subsequently inoculated with the virus of the disease; the four not vaccinated died in from two to four days, the four vaccinated became a little feverish, and in two a slight œdema appeared at the point of inoculation, but all recovered. These four animals were inoculated with the virus three times subsequently, but not one of them died. He also vaccinated six sheep belonging to a race which easily succumbed to the disease; a single vaccination did not appear to afford sufficient protection, but after repeated vaccinations (three or four) the animals were able to resist the inoculation of the most virulent kinds of virus.

The method of vaccination discovered by M. Toussaint was

* *Comptes Rendus*, Tome xci. p. 135 (July 12th, 1880).

made known at the meeting of the Académie des Sciences on August 2nd, 1880.* On that day a sealed packet, deposited on July 12th, 1880, was opened. Its contents were as follows :—]

A Method of Vaccinating the Sheep and Puppy.—I at first resorted to filtration of the blood of the dog, sheep, or rabbit infected with anthrax. I collected the blood of an animal which had been inoculated, at the time when it was about to die, or immediately after its death. The blood was defibrinated by whisking, passed through linen, and filtered through ten or twelve folds of paper. Three puppies three months old, and the first ewe were vaccinated on this plan. But this method was dangerous and not practical; the filter often allowed the passage of bacilli not easily found with the microscope, for they were very few in number, though the animals which it was intended to protect were killed.

In view of these accidents, and in consequence of not being able to obtain a filter which would yield a sufficient quantity of filtered matter, I had recourse to heat in order to kill the organisms, and kept the defibrinated blood at a temperature of 55° C. (131° F.) for ten minutes. The result was perfect. Five sheep inoculated with three cubic centimetres† of this blood were subsequently inoculated with blood containing the virus in a very active state, and experienced no ill results.

It is, however, necessary to make a good many inoculations to insure complete protection. Thus, after the first preventive inoculation, I inserted under the skin of the ear in two sheep some infected blood from a rabbit, and some spores from a cultivation. One of these sheep died with an immense number of bacilli in its blood. I then again inoculated the four remaining sheep with blood which had been taken from the sheep which died, and heated up to 55° C.; since that time each sheep has been twice inoculated with infective blood without experiencing the least indisposition.

Not only are the animals insusceptible to anthrax, but inoculation with material very full of bacteria does not produce *any local inflammatory effect;* as the wound cicatrises like a simple wound I am led to think that the obstacle to the development of anthrax

* *Comptes Rendus*, Tome xci. p. 803.

† About fifty minims.

lies not only in the glands, but also in the blood or lymph, in the liquids of the body which have become unsuitable for the nourishment of the parasite.

Practical methods for the inoculation of all the animals of a herd will be immediately made the object of research. I hope that the difficulties will be easily surmounted, and that, in a short time, I may be able to make public the method contained in this note.

Criticism of M. Toussaint's Method.—[M. Pasteur,* assisted by MM. Chamberland and Roux, investigated this method of attenuation by heat, and came to the conclusion that it was very uncertain. One of three things might happen when the infected blood was kept at a temperature of 55° C. These three results are thus described by M. Pasteur :—]

(1) The bacterium is killed by the heat, and then the infected blood cannot serve for preventive inoculations. (2) The bacterium is not killed, but retains a degree of virulence which kills the sheep. (3) The bacterium is modified ; in this last case only is it possible that it can protect, that is to say cause an attack of anthrax which stops short and does not kill the animal. Only by direct preliminary experiments is it possible to ascertain the condition into which the bacterium has passed after the warming of the infective blood. Even if the attempt to obtain the organism in the condition in which it can afford protection be successful, still this condition cannot be perpetuated by cultivation, and even in the blood which contains it, it is often altered in a few days. A cultivation of the bacterium properly attenuated by heat gives rise again to a virulent bacterium, differing essentially in this respect from the attenuated organism of fowl-cholera. In our experiments it has even happened that a quantity of infective blood maintained for thirty minutes at 55° C., and containing a modified bacterium still capable of growing, has given origin to cultivations which were virulent and killed three sheep in three inoculated.

It follows from all this, that if flocks of sheep were inoculated by M. Toussaint's artificial method, there would be a risk of great losses, although those sheep which survived would be protected from any later attack of the disease. Further, the method assumes that the operator has at his disposal a large quantity of infective blood ; this is a serious difficulty.

* *Comptes Rendus*, Tome xcii. p. 665 (March 21st, 1881).

and it was found that the virulence was preserved for from four to eight days, and the power of reproduction for from ten to thirteen; he considers that these results further support his contention that attenuation is not produced by the oxygen of the air. He also showed that in a series of cultivations exposed in vacuo to various temperatures ranging between 50° C. and zero the virulence and the power of reproduction was lost the more rapidly the higher the temperature. If from cultivations with a virulence attenuated by heat in vacuo, other cultivations were made in the ordinary way, they formed spores, but the virulence was to some extent attenuated; this attenuation could be completed by heating to 80° C.

The following are the steps of the operation by which M. Chauveau prepares a vaccine fluid*:—

"1. A flask containing very clear chicken infusion is inoculated with a drop of blood from an animal infected with anthrax:

"2. The flask is kept for about twenty hours in a thermostat maintained constantly at the temperature of 43° C., in order to develop from the inoculation a fragmentary mycelium without spores;

"3. The cultivation is heated for three hours in a thermostat at the temperature of 47° C., the heating being designed to produce the attenuation of the virulence of the mycelium.

"The cultivation is then ready to serve for the inoculation of a large number of other cultivations of the second generation, which will be used for the preventive inoculations. This second part of the operation consists of the following steps:—

"1. New flasks are inoculated with one or two drops of the first, attenuated, cultivation;

"2. The flasks are kept for from five to seven days in a thermostat at a temperature of 35° to 37° C., in order to cause a development of the mycelium, and its transformation into attenuated spores;

"3. They are heated to 80° C., in order to complete the attenuation of the spores."

The chicken infusion used was made from one part of meat to five parts of water. For obtaining large quantities of vaccine M. Chauveau used large three-necked flasks,† holding one or two

* *Comptes Rendus*, T. xcvii. p. 1397.
† *Comptes Rendus*, T. xcviii. p. 74.

litres; the middle neck was traversed by a tube, drawn out fine at its lower extremity, which reached to the bottom of the flask, and was closed above by a plug of cotton wool; one of the other necks was traversed by a short tube, plugged with cotton wool, and connected with an aspirating apparatus; the third neck carried another tube, drawn out fine. By setting the aspirator at work the material for inoculating the flask was introduced through this third tube, which was then sealed. In such a flask kept in the incubator at the temperature of 35° to 37° C., the micro-organism developed slowly and incompletely if the liquid was left at rest; but if, by working the aspirator, air was drawn through it in a continuous stream, an abundant growth took place. The growth generally ceased in a week, and resulted in a rich formation of spores, which were attenuated to the proper degree by heating to 80° C. The best temperature for these cultivations was 40·5° C., though a temperature as low as 35° C. would suffice. The final degree of attenuation was influenced by the temperature to which the growth was raised during the last process.* If some of the contents of the large flask were introduced into a series of small flasks, which were then heated separately for an hour to various temperatures between 80° and 90° C., and flasks containing ordinary broth inoculated from them, some would be found to have lost the power of reproduction. The first vaccine liquid might then be prepared by heating the large flask to the degree centigrade of temperature next below that at which the power of reproduction was lost, and the second vaccine liquid by heating to two degrees lower than the first. These processes could not be absolutely relied on to give identical results in all cases, and it therefore became necessary to make some experimental inoculations on sheep to ascertain that the vaccine was not too virulent; this extra expense might, in M. Chauveau's opinion, reasonably be incurred, as enough material can be prepared at once to vaccinate 8,000 sheep.†

* *Comptes Rendus*, T. xcviii. p. 126.

† M. Chauveau, following up some experiments by M. Paul Bert, who had found that certain micro-organisms could be killed by compressed oxygen, succeeded in producing attenuation of the *bacillus anthracis* and the microbe of swine plague, by this means. (*Comptes Rendus*, T. xcviii. p. 1232.) He states that the virulence of cultivations attenuated in this way does not gradually decrease subsequently, as is the case with cultivations attenuated by being grown at 42° to 43° C., or by heating to a higher temperature.—D. W.

III.—CHABERT'S DISEASE.*

(CHARBON SYMPTOMATIQUE ; RAUSCHBRAND.)

It has been recently recognized that under the term anthrax (*charbon ; milzbrand*) two essentially distinct diseases have been included. Chabert at the end of the last century had described three clinical varieties of anthrax : *fièvre charbonneuse*, *charbon essentiel*, and *charbon symptomatique*. As the newly recognized disease embraced the two last named varieties the term Chabert's disease has come into use in France, and may perhaps be adopted here as presenting fewer inconveniences than any other yet proposed. Chabert's disease is a wide-spread epizootic ; it is known to occur in France, Belgium, Germany, Italy, Great Britain, Algeria, Natal, and the United States. Its victims are generally young animals—calves, heifers, young oxen, and lambs.

The onset of the disease is sudden ; the characteristic lesion is an irregular tumour without well-marked limits which extends with great rapidity in every direction if the part affected contains much connective tissue. Fever is high, the general symptoms well marked, and the termination in a large majority of cases is death.

The disease is chiefly interesting, in the present connection, on account of the numerous ways in which an animal can be protected from the disease by inoculation. MM. Arloing, Cornevin, and Thomas have made investigations, extending over several years, into this subject. The following pages contain a summary, as far as possible in their own words, of the results obtained by them.

Immunity can be produced by—1. Inoculating the unaltered virus obtained from a fresh tumour. 2. Inoculating an attenuated (vaccinal) virus.

* *Du Charbon Bactérien ; Charbon symptomatique et Charbon essentiel de Chabert ; Pathogenie et Inoculations Preventives*, par MM. Arloing, Cornevin et Thomas. Paris : Arselin, 1883.

Inoculation of Unaltered Fresh Virus.—(A) Immunity can be produced by introducing a very small quantity of the virus into the loose connective tissue of any part of the body; a slight indisposition follows and the animal can then resist the inoculation of a large dose.

(B) The injection into the scanty connective tissue of the tip of the tail of a very large quantity of fresh virus may be followed by the development of large tumours elsewhere, and consequently by death; but the injection of a moderate quantity is followed only by slight indisposition, and the animal then acquires immunity from the disease.

(C) The injection of a considerable quantity (in oxen even 6 cc.) of the liquid, newly obtained from a tumour, into a large vein (the jugular) has the same effect. If the virus is very active, if the animal is suffering from a severe contusion in any part of the body, or if in making the inoculation the virulent fluid is allowed to come into contact with the wall of the vein or the connective tissue, the disease develops in its most virulent form.

(D) The injection of the fresh virus into the respiratory passages also confers immunity. It is suggested that the microbes having reached the infundibula traverse the pulmonary and capillary epithelia, and so obtain entrance to the blood without coming in contact with the connective tissue.

Inoculation of Attenuated virus.—The virus may be attenuated in three ways :—

(A) By the action of certain antiseptics, such as glycerine of carbolic acid, corrosive sublimate (1 in 5,000), eucalyptol, and thymol.

(B) In an atmosphere of carbonic acid cultivations were made in broth containing sulphate of iron and glycerine. The flasks were kept at a temperature of 38° to 40° C. The virulence of the microbe was gradually diminished; the third, fourth, and fifth "generations" (so-called) produced an abortive disease, and were therefore vaccines.

(C) The virus could be attenuated by the action of heat, in the same way as the virus of anthrax had been attenuated by M. Toussaint. The results, however, were very uncertain. On the other hand it was found that if the material expressed from a tumour was thoroughly dried at a temperature between

32° and 35° C., and then after being slightly moistened exposed to a temperature above 80°, its virulence underwent a diminution proportional to the temperature. This virus heated to 100° C. produced a partial immunity in the sheep and ox. The subsequent inoculation of a virus which had been exposed to a temperature of 85° C. rendered the immunity more complete. In the case of guinea-pigs this second vaccination was not necessary. One part of the dried material was mixed with two parts of ordinary water and thoroughly ground up in a mortar; the homogeneous liquid thus obtained was spread out in thin layers, and kept at the desired temperature for seven hours. The dried lamellæ left after this operation could be kept for a year at least in a dry atmosphere without alteration. The animal is first inoculated with virus dried at 100° C., then after an interval of from five to eight days with virus dried at 80° C.; in the ox the situation chosen for the inoculation is the under surface of the tail, about two hands' breadth from the tip. The inoculation is sometimes followed by a slight rise of temperature; if the inoculation is made in the leg a very slight hardening of the subcutaneous tissue ensues.

Eighteen months after these vaccinations animals have been observed to be still protected against the effects of inoculation with a virus which had not been attenuated, and there is some evidence to show that the immunity from the disease is transmitted to the offspring.*

* A good account of recent researchee into the etiology of this disease, especially of those of the authors quoted in the text, will be found in a paper by Mr. G. F. Dowdeswell, M.A., published in the Thirteenth Annual Report of the Local Government Board, 1883–84 (Supplement containing the Report of the Medical Officer), p. 186.—D. W.

IV.—VACCINATION FOR SWINE-PLAGUE BY MEANS OF THE ATTENUATED VIRUS OF THE DISEASE.*

[In March, 1882, M. Pasteur requested M. Louis Thuillier, one of the assistants in his laboratory, to proceed to Peux, in the Department of Vienne, with the object of studying swine-plague, a disease which was at that time committing great ravages in the district. On March 15th M. Thuillier informed M. Pasteur that he had found a micro-organism in the blood and fluids of pigs dead of the disease. This micro-organism was not the same as that observed by Dr. Klein in 1877 "during the course," to quote the words of M. Pasteur, "of a long and remarkable series of autopsies and experiments."†

On November 26th, 1883, M. Pasteur made a communication to the Academy of Sciences in which, after paying a tribute to the zeal, ability, and devotion of M. Thuillier, who had died of cholera in Egypt in September, 1883, he proceeded to give an account of the results of their joint investigations in the following terms:—]

Having once entertained the idea of the existence of a micro-organism in pigs suffering from the disease, we made the necessary experiments to ascertain whether this organism was in

* *Comptes Rendus*, tome xcvii., p. 1163, "La Vaccination du rouget des porcs à l'aide du virus mortel atténué de cette maladie," par MM. Pasteur et Thuillier. (November 26, 1883.)

† On Dec. 2nd, 1882, M. Pasteur addressed a letter to M. Dumas (*Comptes Rendus*, T. xcv., p. 1120). He stated that the number of animals dying of swine-plague in the Departments of the Rhone Valley was estimated at twenty thousand for that year. Swine-plague was due to a special microbe, very small, most nearly resembling the microbe of fowl-cholera, having the figure-of-8 form, but differing from it in physiological properties. Inoculations of minute quantities of the pure cultivation produced the disease. Animals had been rendered refractory by the inoculation of the attenuated microbe. The organism described by Dr. Klein as occurring in the pneumo-enteritis of the pig, was not in any way connected with the causation of the disease observed by M. Pasteur, though he considered the two diseases to be identical.—D.W.

reality the true cause of the disease. The Academy is acquainted with the sovereign method of arriving at a proof of such a connection. In the first place it is necessary to find a medium suitable for the cultivation of the microscopic organism; this organism can grow in sterilized veal-infusion. A number of cultivations are then made in this medium, always taking for the purpose of inoculating one cultivation a drop of the preceding cultivation. The last cultivation having, when used for inoculating a certain breed of pigs, often produced the disease in a well characterized type, it has been proved, beyond dispute, that the micro-organism with which the experiment was made is the micro-organism of swine-plague.

Our first care was then to seek to attenuate the virulence of this micro-organism, and in the month of November, 1882, Thuillier and I, accompanied by a young assistant, M. Loir, went to Bollène, a district in the Department of Vaucluse, every year ravaged by swine-plague. M. Maucuer, a distinguished veterinary surgeon, had asked me, ever since the year 1877, to visit it, in order to study the plague on the spot.

We were soon convinced that the swine-plague in Vaucluse was identical with the plague in Vienne; the same symptoms, and the same micro-organism. Subsequently during the course of that year a study of the disease in the Departments of the Côtes-du-Nord, Charente, Dordogne, and Gironde, proved to us that the disease is everywhere the same, and caused by a micro-organism of the same nature.*

Certain difficulties stood in the way of vaccination with the micro-organism of swine-plague; these were due chiefly to the existence in France of numerous breeds of pigs, and to the fact that the ease with which these species became infected was very variable. Investigations are in progress in several Departments with the object of ascertaining the vaccines suitable to these various breeds. The owners, and the Agricultural and Veterinary Societies in the breeding districts have shown the greatest zeal in seconding our efforts. In addition to M. Maucuer, of Vaucluse, I have the pleasure to mention MM. Bauvillet and

* Concerning these Departments, it may be noted that Côtes-du-Nord is the north-eastern part of Brittany, that Vaucluse is in the south of France, not far from the Mediterranean coast, and that Vienne, Charente, Dordogne, and Gironde are conterminous with each other, and have a coast line in the Bay of Biscay.—D. W.

Pickeney in Charente, M. Le Berre in Côtes-du-Nord, and M. Roquebert, a large breeder in Vienne, who has placed all the animals in his piggeries, to the number of over four hundred, at our disposal.

The possibility of vaccination by inoculation with attenuated virus, and of the cultivation of this virus in considerable quantities, has been already rigorously demonstrated.

Last year we left vaccinated pigs at Bollène and the surrounding villages in Vaucluse under the charge of M. Maucuer, with the understanding that the owners should preserve them for at least one year—that is to say, beyond the period of the annual so-called spontaneous reappearance of the disease, which ceases during the cold season, but re-appears in the summer months. Down to August last, though swine-plague had already appeared and claimed many victims, M. Maucuer's letters did not give us any very significant news. But under date of September 4th, M. Maucuer wrote to me in these terms:—

"The happy effects of the vaccinations becomes day by day more and more evident. Deaths are occurring at the present time at Bollène, Saint-Restitut, Mondragon, and in the whole arrondisement of Orange, but not one vaccinated animal has died. At Saint Blaise, the animals you vaccinated are the only pigs that remain alive. There is no news yet at M. de la Gardette's, but there is a great mortality on the farms of all his neighbours . . . . .; the mortality is so great that its like has never been before. Soon there will be left at Bollène, Saint-Restitut, and Mondragon, only the vaccinated pigs. It is a complete success."

A few days later, on September 9th, M. Maucuer wrote to me again:—

"At M. de la Gardette's the non-vaccinated pigs, to the number of seven, have been without an exception attacked. Four are already dead, the three others are dying. The vaccinated pigs are all flourishing."

The above facts leave no doubt of the truth of the following conclusions: 1st. Epizootic swine-plague, even of the most violent type, may be prevented by inoculation of the attenuated virus. 2nd. It is proved that the duration of the immunity exceeds one year. This duration is amply sufficient to meet the practical needs of pig-breeding, since the process of fattening scarcely ever takes longer than one year. In spite of these happy results, however, I must repeat that the question of the various vaccines suitable to the various breeds still requires further investigation, before the vaccination of pigs can become

general. While awaiting the attainment of these definitive results, I will now make known the method which we have used for attenuating the virus of swine-plague; that is in fact the principal object of this communication.

Researches conducted in my laboratory have shown that the virus peculiar to each disease is not a fixed morbid entity, but that it may occur under a variety of forms, above all, with varying physiological properties, depending upon the media in which the virus lives and grows. Consequently, although virulence is a property of certain species of micro-organisms, it is essentially modifiable. It can be enfeebled or increased by experiment, and can be fixed in any one of these states by cultivation. A micro-organism is virulent for an animal when it has the power of growing like a parasite in its body, and when this growth causes such disturbances in the host as can bring about disease, or death. If this micro-organism has lived in a certain species, if, that is to say, it has in a series of instances escaped from one individual of this species only to enter another individual of the same species, without being subjected to any appreciable external influence during the time of passage, then the virulence of this parasite may be considered to be, to a certain extent, in a fixed state, the maximum of virulence for individuals of that race. For instance, the parasite of splenic fever in sheep varies little in the same country in its passage from one animal to another, or from year to year; this is doubtless to be attributed to the fact that the parasite in passing from sheep to sheep has attained a condition, which is, with regard to its power of thriving in the sheep, fixed and definite. This is the case too with the vaccine-virus of Jenner. The virulence, however, of a virus which is not at the maximum can be profoundly modified by passing through a series of individuals of the same race. It will be remembered that, when we wished to increase progressively the virulence of the virus-vaccines of fowl-cholera, anthrax, and other diseases, in order finally to raise it in each case to the maximum degree, we inoculated first young animals, and then a succession of older animals.

I may observe, incidentally, that these results bring the virulent micro-organisms within the general laws of life, and of its manifestations in the higher vegetable and animal species. The plasticity, if the word may be used, of these higher species shows

itself when the species are under the influence of variations in the media in which the successive generations live. The rapidity with which variations occur in the different kinds of virus, and the slowness with which they occur in the larger organisms, is the sole difference between the micro-organisms and the higher species. Each cultivation of a virus, if it last only for twenty-four hours, represents an immense number of successive generations, while in higher organisms thousands, even millions, of years would be required for the completion of a similar number of generations.

However that may be, if changes in the virulence of the various kinds of attenuated virus, or virus-vaccines, can be produced by this attenuated virus passing through a series of members of the same species, may it not be that a virus in a fixed condition of virulence for a species can be modified in its virulence by passing from one species to another? This view finds support in experiment.

The virulent micro-organism discovered by us in the saliva of animals suffering from hydrophobia will doubtless be within the recollection of the Academy.* As was shown in the communication made to the Academy on January 24th, 1881, this micro-organism was very virulent for rabbits, but harmless for adult guinea-pigs; yet it rapidly killed guinea-pigs only a few hours or a few days old. On continuing to make inoculations from one young guinea-pig to another, we observed that the virulence became more intense, so as to kill easily older guinea-pigs. In the end the lesions produced notably differed. Here we have facts of the kind just referred to, an instance of increase in virulence by passing in succession through series of individuals of one species.

The new and unexpected observation, however, which I wish to communicate to the Academy is this: the micro-organism, after its virulence has been increased by passing through a series of guinea-pigs in succession, has become less virulent for rabbits than at first.

Under these new conditions it produces in rabbits a disease which is spontaneously cured; and further, having once suffered from this malady, the animal is refractory when inoculated with

* This micro-organism however did not stand in any casual relation to hydrophobia. See page 629.

the micro-organism which is fatal to rabbits. This leads to the important conclusion, that the habit of living in one species (the guinea-pig) with a certain determinate degree of virulence, can change the relation in which the virulence stands to another species (the rabbit), can diminish it, and cause the virus to act as a vaccine for the latter species.

The importance of this result will be universally recognized, for in it is contained the secret of a new method of attenuation, which can be applied to certain most virulent kinds of virus. We will give an example, and an application.

Shortly after our arrival in Vaucluse in November, 1882, we were struck by the fact that the breeding of rabbits and pigeons was very much neglected in the Department, because these two species were liable to frequent and most deadly epizootics. Though nobody in that district had connected the two facts, the existence of these epizootics, and of epizootic swine-plague, it occurred to us to ascertain whether there were a relation of cause and effect between them. Experiments made to solve this problem soon showed us that the rabbits and pigeons died of swine-plague. It also occurred to us to ascertain if these species could be made use of in order to modify the virulence of swine-plague, under such conditions as I have just remarked we applied to the micro-organism of the saliva.

The inoculation of pigeons and rabbits with swine-plague yielded the following very curious result:—

If an inoculation with the micro-organism of swine-plague was made into the pectoral muscle of a pigeon, the bird, after showing the ordinary outward symptoms of fowl-cholera, died in six or eight days.

When the blood of this first pigeon was used for inoculating a second, the blood of the second for inoculating a third, and so on in series, the micro-organism became acclimatized in the pigeon.

The drowsiness and the tendency to roll itself up into a ball, which are the customary symptoms of the disease, appeared after a much shorter interval than in the first pigeons of the series. Death also occurred more quickly, and the blood of the later pigeons in the series was much more virulent to the pig than even the most infective products from a pig which had died of the so-called spontaneous swine-plague.

If the micro-organism of swine-plague is passed through a series of rabbits, the result is altogether different. Rabbits inoculated with the infective products of a pig which has died of swine-plague, or with cultivations made from them, are always made ill, and in the majority of instances die.

If the disease is inoculated from rabbit to rabbit, the micro-organism becomes acclimatized in the rabbit. All the animals die, death supervening in a few days. Cultivations from the blood of these rabbits in sterilized media become progressively more easy to make, and more abundant. There is a slight change in the appearance of the micro-organism; it becomes a little larger than in the pig, and has the form of a figure-of-8, without the filiform lengthening of some of its cultivations.

If the blood of the last rabbits of the series, in order to compare it with the blood of the first rabbits of the series, be used for inoculating pigs, it is found that the virulence has been progressively diminishing from the first to the later rabbits of the series. Very soon, pigs inoculated with the blood of the rabbits are not killed, although they are made ill by it. After recovery they are found to be vaccinated for the fatal form of swine-plague.

Such is the method by which certain, even very virulent, kinds of virus may be attenuated: it appears to me to be worthy to attract the attention of the Academy.

NOTE.—M. Pasteur's description of the micro-organism with which he experimented lacks precision; he stated that it resembled the microbe of fowl-cholera. "The form is still that of a figure-of-8, but more delicate and less visible." "It is so thin that it may escape even careful observation." Dr. Loeffler, in a report on an investigation conducted from July, 1882, to December, 1883, in Berlin (*Arbeiten a.d. Kaiserlichen Gesundheitsamte*: erster band [1885], p. 46), with reference to the nature of an epizootic locally known as *Schweine-Rothlauf*, states that he found the vessels in the affected part of the skin full of thin rod-shaped organisms, astonishingly like the bacilli of the septicæmia of the mouse (Koch), but somewhat shorter and a little thicker. Cultivations of this small bacillus made in gelatine discovered slight differences. Mice and rabbits inoculated with cultivations of this organism died with local symptoms resembling those of erysipelas. He found also that if a rabbit survived the inoculation a second inoculation produced no reaction of any kind. Prof. Schütz (*Ibid.*, p. 58) found the same bacillus in pigs suffering from the disease (in a different part of Germany), and ascertained that its pure cultivations (grown at 30° C.) when inoculated subcutaneously were fatal to young pigs in from three to four days. Mice were killed in two to four days, pigeons in three to four, and rabbits in six days; guinea-pigs were insusceptible. Schütz also examined vaccines sent out

by M. Pasteur; he found that they contained the small bacillus, but that they were contaminated with other organisms; they produced, however, characteristic appearances in mice, and pure cultivations could be readily obtained from them; mice and pigeons died after inoculation, but rabbits survived. The general conclusions he arrived at were—(1) That the swine-plague observed in Baden was due to a small bacillus, which in its form and properties bore a close resemblance to the bacillus of mouse septicæmia (of Koch); (2) That the disease was identical with the *rouget* or *mal rouge des porcs* seen in France; (3) That the virulence of the bacilli contained in M. Pasteur's vaccines was diminished; (4) That by inoculation with M. Pasteur's vaccines pigs could be rendered refractory to virulent cultivations. (5) That the bacilli did not possess the power of spontaneous movement, and that they measured from ·0006 to ·0018 m.m. in length. Prof. Schottelius (*Der Rothlauf der Schweine* . . . . Von Dr. A. Lydtin und Dr. M. Schottelius, 1885) has also examined M. Pasteur's vaccines, and his observations agree with those made by Schütz.

The accuracy of M. Pasteur's observations is thus on the whole confirmed by Schütz and Schottelius, who however both adduce evidence tending to show that his cultivations were not always pure. The discrepancy in the descriptions of the microscopical appearance may probably be attributed to the imperfect microscopical methods still exclusively used by M. Pasteur, who does not appear to have availed himself of the methods of staining and illumination, especially the latter, which are now generally recognized to be essential for the study of the morphology of micro-organisms.

Dr. Klein has re-asserted at length the correctness of his own views, and forcibly criticised the experiments and theories of M. Pasteur in a paper entitled "Remarks on the Etiology and Pathology of Swine Plague," published in the Thirteenth Annual Report of the Local Government Board (Supplement containing the Report of the Medical Officer for 1883), p. 166. Dr. Klein describes an organism occurring in the deeper parts of the intestine, in the lymphatic glands, in the lungs, in peritoneal exudation, and in the blood of pigs which have died of the disease; in mice and rabbits inoculated with fresh organs from an infected pig, or with an artificial cultivation, the organism is also found in the liver and spleen. The organism is a rod-shaped bacterium, about 0·001 to 0·004 m.m. long, and about a third or a fourth of this in width. The bacillus appeared to be but rarely present in the blood, as cultivations started by inoculating nutrient material with blood often failed. Cultivations made from the blood or organs in fluid media at 35° to 40° C. rendered the fluids turbid, and after three or four days incubation a slight pellicle began to form. It consisted of "moving and non-moving rods, some twice, thrice, and four times as long as others. Some being 0·005 m.m. long. . . . The longer rods include either at one end or both, or sometimes also in the middle, a bright granule, having all appearances of a spore, while the rods themselves are composed of a very pale substance." In a fresh specimen the rods varied in length between 0·0036 and 0·0054 m.m., in a specimen dried and stained they varied between 0·002 and 0·0026 m.m. In two pigs inoculated with artificial cultivations of this bacillus, enlargement of the lymphatic glands in the neighbourhood of the point of inoculation, pyrexia, and some general indisposition, were the symptoms noticed. These symptoms passed away in the second week, leaving, however, some enlargement of the glands still persisting. In mice, redness about the point of inoculation was noted within twenty-four hours, but the animals did not become ill until the fifth or sixth day, and died about the seventh; in rabbits death occurred at about the same date; the earliest deaths in mice and rabbits were on the fifth; the latest on the ninth day. In pigeons, Dr. Klein says that he has not in a single instance seen any local or general effect follow inoculation. In conclusion, he states that pigs may be protected from fatal swine-plague by two methods. (1) A cultivation, started with the bronchial glands of a pig recently dead of swine-

plague, is kept for one, two, or more days at a temperature of 30° to 40° C.; pigs inoculated with a few drops of this cultivation are affected by the disease in so mild a form that it can only be diagnosed on careful inspection, but they are thus protected from a fatal attack in the future. (2) A rabbit is inoculated with the fluid squeezed out from a diseased (inflamed) portion of the lung of a pig recently dead of swine-plague. The rabbit dies between four and seven days later; its spleen, liver, or lungs are mixed up in saline solution (0.6 per cent.), and the pigs are inoculated with a few drops. A mild attack of swine-plague is induced, and the animals acquire immunity.

The observations of Dr. Salmon in America (see *Department of Agriculture*, Annual Reports 1881, 1882, and 1885) seem to show that the organism described by Dr. Klein was not the determining cause of the disease in America at least, though Dr. Salmon is clearly mistaken in identifying the large micrococcus observed by him with M. Pasteur's "*microbe en chiffre-de-8*," which, as stated above, has been shown by Schütz and Schottelius to be a bacillus. It is important to notice that Dr. Loeffler (*loc. cit.*, p. 52) found in one pig which had died with the ordinary symptoms of "Rothlauf," not the small, thin bacillus, but an "extraordinarily small ovoid bacterium," which differed also from the bacillus in its pathogenic action towards various animals. It is clearly impossible to reconcile all these observations, and it seems highly probable, that, as Dr. Loeffler suggests, several diseases, etiologically distinct though perhaps clinically identical, have been confused together. —D. W.

V.—M. PASTEUR'S RESEARCHES ON VACCINATION FOR RABIES BY MEANS OF THE ATTENUATED VIRUS OF THE DISEASE.

[M. PASTEUR, in a communication made to the Academie des Sciences, on February 25th, 1884,* gave a short summary of the researches into the contagion of rabies which he had been carrying on for some years with the assistance of MM. Chamberland and Roux, and the late M. Thuillier.

The virus had been inoculated on two different plans; in the one case the virus was inoculated at the surface of the brain by trephining, in the other it was injected into the blood. The operation of trephining had been found to be so safe and easy that it could be performed by a laboratory assistant, and could be completed so rapidly that the whole process, including the administration of chloroform and the recovery of the animal from the anæsthetic, only occupied twenty minutes.

When the animal was inoculated by trephining, the symptoms which ensued were generally those of "furious madness," but if the virus was introduced into the vascular system or into the subcutaneous tissue, the symptoms which followed were those of paralytic rabies, the so-called "dumb madness."† It was thought probable that when the virus was injected into the blood current it at first became established in the spinal cord; dogs were killed when the first symptoms of paralysis appeared, and on comparing the virulence of the spinal cord, the lumbar enlargement being generally the part used, with that of the bulb, it was found that the cord might be capable of communicating

* *Comptes Rendus*, T. xcviii. p. 457.

† These are the two clinical types generally recognized by veterinary surgeons as occurring in dogs.

the disease at a time when the bulb was innocuous. It was further found that the virus was present, not only in the encephalon and spinal cord, but in all the peripheric nerves; that is to say, the disease could be communicated by any part of the nervous system. The virus was not constantly present in the cerebro-spinal fluid. When decomposition was prevented by a temperature between 0° C. and 12° C. (32° F. and 53·6° F.), the virus was preserved in the brain and spinal cord without diminution of virulence for several weeks. If the virus was kept free from decomposition in sealed tubes, it preserved its virulence for three weeks or a month, even at summer temperature.

The saliva was found to be virulent, whether the dog was inoculated by the intracranial or intravenous method, or had been infected by the so-called spontaneous rabies.

All attempts to cultivate the virus had failed. Among the materials used had been the cerebro-spinal fluid and the spinal cord, removed from healthy animals and preserved free from decomposition. Yet M. Pasteur stated that he found it possible to say, from the microscopical examination of the medulla oblongata, whether the animal was healthy or rabid; the molecular granulations in the infected organ were smaller and more numerous. If pure virus, taken from the medulla oblongata of an animal which had died of rabies, were injected into the veins of a rabid animal at the time when it was beginning to be asphyxiated, the blood after a few hours was found to contain only the very small granulations, all others having disappeared; in this state they could be easily stained with aniline dyes.

If the quantity of material inoculated were small, the duration of the period of inoculation was increased; if the dilution were carried beyond a certain not very extreme limit, inoculation did not produce the disease, and did not confer immunity. Occasionally, but very rarely, the earlier symptoms of rabies in the dog disappear, the disease reappearing after a time; the same thing may be observed in the rabbit, and with much greater frequency in the fowl. Sometimes the fowl finally recovered from the relapse, and one dog also recovered from a relapse.

M. Pasteur concluded his paper by making the following statement with regard to the attenuation of the virus of rabies. The following pages are a translation:—

"Rabies is pre-eminently an infective disease. The effects and

the nature of its virus are enveloped in so much mystery that it is natural to seek to ascertain if the virus itself is capable of manifesting varying degrees of virulence. Experiment has shown us that the answer to this question must be in the affirmative. In default of other methods, which are still being studied, we have ascertained that the passage of the virus of rabies through various species of animals leads to a modification, more or less profound, of the virulence of the virus. Rabbits, guinea-pigs, fowls, and monkeys take rabies. When, after passing through successive animals, the virus has attained to a fixed state proper to each race, the virulence of these kinds of virus is far from being the same, and sensibly differs from the virulence of canine rabies, a virulence which has been itself fixed by passing, from time immemorial, in a number of instances, from dog to dog by bites. In my opinion, spontaneous rabies does not occur.

"We now possess a virus which gives rabies to the rabbit in seven or eight days, with such great constancy that the duration of incubation, measured by a change in the temperature, or by the appearance of the first outward symptoms of rabies, can be foretold within, so to say, a few hours. We also possess a virus which gives rabies to guinea-pigs in five or six days, the duration of the period of incubation being definite.

"Before arriving at the fixed state for the several species of animals of which I speak, the virulence continually varies. We consider that, for the same species, the virulence is in inverse ratio to the number of days of incubation, when, that is, all other things are equal, and when, in particular, the proportion of virus inoculated is as nearly as possible equal for a given mode of inoculation. The duration of the period of incubation is generally a little shorter in young animals than in adults.

"As absolutely nothing is known as to the condition in which the virus of canine rabies, communicated to man, would be after passing in succession from man to man, we have been led to test rabies communicated from monkey to monkey."

[The thread of the story was here broken off for a time, to be resumed on May 19th, 1884,* as follows:—]

"If the virus of rabies is made to pass from the dog to the

* *Comptes Rendus*, T. xcviii. p. 1229. *Sur la rage*, par M. Pasteur, avec la collaboration de MM. Chamberland et Roux.

monkey, and afterwards from monkey to monkey, its virulence becomes feebler at each passage. If, when the virulence has been diminished by thus passing from monkey to monkey, the virus is brought back to the dog, the rabbit, or the guinea-pig, it remains attenuated. In other words, the virulence does not, by a sudden jump, return to the virulence of the dog with ordinary rabies (*rage des rues*). By passing in a small number of instances from monkey to monkey, attenuation can be easily brought to the point at which rabies is never given to the dog by subcutaneous inoculations. Even inoculation by trephining, an infallible method of communicating rabies, may not produce any result, though the animals are nevertheless rendered refractory to rabies.

"The virulence of the virus of rabies is increased by being passed from rabbit to rabbit, or from guinea-pig to guinea-pig. When the virulence thus increased is fixed at the maximum for the rabbit, it passes in this increased state to the dog, and is found to be much more intense in that animal than the virulence of the virus of the rabies of the dog with ordinary rabies (*rage des rues*); under these circumstances, this virulence is such that the virus, when inoculated into the vascular system of the dog, invariably causes fatal rabies.

"Although the virulence of rabies is increased by passing from rabbit to rabbit, or from guinea-pig to guinea-pig, it must pass through the body of these animals a good many times in order to regain its condition of maximum virulence, when that has been diminished in the monkey.

"In the same way the virulence of the dog with ordinary rabies (*rage des rues*), which, as I have just said, is not nearly the maximum virulence, when it is inoculated into the rabbit, must be passed through a good many individuals of that species before it attains its maximum.

"A rational application, such as I am about to make known, of these facts easily enables us to render dogs refractory to rabies. It will at once be understood that the experimenter can have at his disposal the attenuated virus of rabies of various strengths; some, not mortal, protecting the economy from the effects of the more active kinds of virus, and other kinds of virus of mortal intensity.

"Let us take an example. The virus of rabies from a dead

rabbit is taken, by trephining, at the end of a period of incubation longer by several days than the shortest period of incubation in the rabbit. This is invariably between seven and eight days after the inoculation by trephining of the most virulent kind of virus. This virus of longer incubation from the rabbit is inoculated, in every case by trephining, into a second rabbit, the virus of the second into a third.

"On each occasion the virus, which grows more and more powerful, is inoculated into a dog. In the end this animal is found capable of resisting a virus of mortal intensity. It becomes entirely refractory to rabies whether by intravenous inoculation or by trephining with the virus of a dog with ordinary rabies (*rage des rues*).

"I have been able, by inoculating with the blood of rabid animals under certain determinate conditions, to simplify very much the operation of vaccination, and to produce in the dog a refractory condition of the most decided kind. I will soon make known to the Academy the whole of my experiments on this point.

"At the present time and until at some distant epoch rabies is extinguished by vaccination, it would be a point of considerable interest to be able to prevent the development of the disease as the consequence of bites by rabid dogs. The first experiments which I have made give me the greatest hopes of success on this score. I have every reason to believe, thanks to the duration of the incubation of rabies following bites, that it would be possible to bring about with certainty a refractory state in the subjects bitten before the fatal malady breaks out.

"The first experiments are very favourable to this point of view; but the tests must be infinitely multiplied in various species of animals before human therapeutics can have the hardihood to try this prophylaxis upon man.

"The Academy will understand that in spite of the confidence with which my numerous experiments, pursued for the last four years, inspire me, it is not without some apprehension that I now make public facts which tend towards nothing less than a possible prophylaxis of rabies. Had I had at my disposal sufficient material means I would have been happy not to have made this communication until after having asked some of my

colleagues in this Academy, and in the Academy of Medicine, to have the kindness to control the conclusions which I have here made known. In obedience to these scruples, and with these motives, I have taken the liberty of writing within the last few days to M. Fallières, Ministre de l'Instruction Publique, asking him to be good enough to name a Commission to which I could submit my dogs which are refractory to rabies.

"The crucial experiment which I would perform in the first place would be to take from my kennels twenty dogs refractory to rabies for purposes of comparison with twenty dogs which would serve as test animals. Rabid dogs would be caused to bite these forty dogs in succession. If the facts I advance are correct the twenty dogs which I considered refractory will all resist the infection, while the twenty test animals will take rabies.*

"In a second not less decisive experiment forty dogs would be used; twenty of them would be vaccinated before the Commission and twenty would not be vaccinated. The forty dogs would subsequently all be trephined with the virus of a dog with ordinary rabies (*rage des rues*). The twenty vaccinated dogs will resist. The twenty others will all die of rabies, either in the paralytic or the furious form."

[The following is an extract from the report of this Commission, dated Paris, August 6th, 1884:—†]

The result of these experiments is, in an abridged form, as follows:—

1. On June 1st and 6th ten dogs, five vaccinated against rabies and five test animals taken from the pound, were inoculated by trephining with a virus from a dog with ordinary rabies (*rage des rues*).

2. On June 3rd, 4th, 10th, 17th, and 28th, twelve dogs, of whom six were vaccinated against rabies, and six were test animals, were caused to be bitten by mad dogs suffering from the, so-called, "spontaneous" rabies.

3. On June 19th six dogs were inoculated by the intravenous injection of the virus of ordinary rabies; on June 20th twelve

* These twenty dogs, who serve as test animals, will take rabies in an indeterminate proportion, because rabies does not always come on as a consequence of bites. Those test animals which did not become rabid after being bitten could subsequently be submitted to trephining.

† *Journal Officiel de la République Française*, No. 216 (8 Août, 1884).

dogs with a very virulent virus, obtained from the bulb of a rabbit, at the forty-sixth remove, which had been, that is to say, passed in succession through a series of forty-six rabbits. M. Pasteur showed by experiment before the Commission that this virus gave rabies to rabbits in seven or eight days, and to dogs in eight or ten, when introduced by the method of trephining. Finally, on June 26th two dogs were again inoculated with the virus from a test animal which had died after inoculation.

Up to the present time, therefore, the Commission has had under observation, during experiments of various natures, forty-two dogs; of these twenty-three were presented by M. Pasteur as refractory to rabies, and nineteen were test animals which had not undergone any preventive or vaccinal inoculation.

The results obtained by the Commission up to the present time may be classified as follows:—

The nineteen test animals have, after being bitten by mad dogs, presented three cases of rabies in six; six cases of rabies in eight after intravenous inoculations; and five cases of rabies in five after inoculation by trephining.

The twenty-three vaccinated animals, on the contrary, have not afforded a single case of rabies. During the course of the experiments, however, one refractory animal inoculated by trephining on June 6th died on July 13th after diarrhœa with black stools, which appeared in M. Bourrel's Infirmary during the early days of July. In order to see if this dog might have died of rabies three rabbits and one guinea-pig were inoculated with its bulb on July 13th. On this day, August 4th, all these animals appear to be very well, and yet they have passed beyond the customary period within which rabies appears in animals of their species after intracranial inoculation. They are retained for further observation.

The labours of the Commission are far from being concluded. By multiplying its meetings, by diversifying the proofs required of M. Pasteur, it has sought to satisfy your confidence and the impatience of public opinion.

Numerous facts still remain to be verified by continuing the study of various experiments which are not yet concluded.

The most important of all the series of experiments which remain to be undertaken will be that of the vaccination by it,

or before it, of a large number of new dogs, and the comparison which will ultimately be drawn between the dogs after their vaccination and an equal number of test dogs which will have undergone no treatment.

In other words, the series of experiments made upon dogs vaccinated by M. Pasteur has yielded decisive results. It now remains for the Commission to submit numerous animals, vaccinated by it, to multiplied and varied proofs.

Later on it will turn its attention to the prophylaxis of rabies in bitten dogs, by producing in them, during the period of incubation, an immunity capable of preventing the virus of the bite from producing rabies.

(Signed) BOULEY,
BÉCLARD,
E. TISSERAND,
VILLEMIN,
PAUL BERT.

A METHOD BY WHICH THE DEVELOPMENT OF RABIES AFTER A BITE MAY BE PREVENTED.*

A real progress in the study of rabies was marked, without any doubt, by the papers in which I announced, in my own name and in the name of my fellow-workers, a prophylactic method; but the progress was scientific rather than practical. Accidents were liable to occur in its application. Of twenty dogs treated, I could not undertake to render more than fifteen or sixteen refractory to rabies.

Further, it was desirable, at the end of the treatment, to inoculate with a very virulent virus—a control virus—in order to confirm and reinforce the refractory condition. More than this, prudence demanded that the dogs should be kept under observation during a period longer than the period of incubation of the disease produced by the direct inoculation of this last virus.

* "Méthode pour prevenir la rage après morsure," par M. L. Pasteur. *Comptes Rendus*, tome ci. p. 766. (Séance du Lundi, 26 Octobre, 1885.)

Therefore, in order to be quite sure that the refractory state had been produced, it was sometimes necessary to wait three or four months. The application of the method would have been very much limited by these troublesome conditions.

Finally, the method did not lend itself easily to the immediate treatment rendered necessary by the accidental and unforeseen way in which bites are inflicted by rabid animals.

It was necessary, therefore, to discover, if possible, a more rapid method, and yet one, I would venture to say, capable of affording perfect security to dogs. Otherwise who would have the temerity, before this progress had been achieved, to make any experiment on man?

After making almost innumerable experiments, I have discovered a prophylactic method which is practical and prompt, and which has already in dogs afforded me results sufficiently numerous, certain, and successful, to warrant my having confidence in its general applicability to all animals, and even to man himself.

This method depends essentially on the following facts:—

The inoculation under the dura mater, after trephining, of the infective spinal cord of a dog suffering from ordinary rabies (*rage des rues*), always produces rabies in rabbits after a period of incubation having a mean duration of about fifteen days.

If, by the above method of inoculation, the virus of the first rabbit is passed into a second, and that of the second into a third, and so on, in series, a more and more striking tendency is soon manifested towards a diminution of the duration of the incubation period of rabies in the rabbits successively inoculated.

After passing twenty or twenty-five times from rabbit to rabbit, incubation periods of eight days are met with, and continue for another interval, during which the virus is passed twenty or twenty-five times from rabbit to rabbit. Then an incubation period of seven days is reached, which is encountered with striking regularity throughout a new series extending as far as the ninetieth animal. This at least is the number which I have reached at the present time, and the most that can be said is that a slight tendency is manifested towards an incubation period of a little less than seven days.

Experiments of this class, begun in November, 1882, have now lasted for three years without any break in the continuity of

the series, and without our ever being obliged to have recourse to any other virus than that of the rabbits successively dead of rabies. Consequently, nothing is easier than to have constantly at our disposal, over considerable intervals of time, a virus of rabies, quite pure, and always quite or very nearly identical. This is the central fact in the practical application of the method.

The virus of rabies at a constant degree of virulence is contained in the spinal cords of these rabbits throughout their whole extent.

If portions, a few centimetres long, are removed from these spinal cords with every possible precaution to preserve their purity, and are then suspended in dry air, the virulence slowly disappears, until at last it entirely vanishes. The time within which this extinction of virulence is brought about varies a little with the thickness of the morsels of spinal cord, but chiefly with the external temperature. The lower the temperature the longer is the virulence preserved. These results form the central scientific point in the method.*

These facts being established, a dog may be rendered refractory to rabies in a relatively short time in the following way:—

Every day morsels of fresh infective spinal cord from a rabbit which has died of rabies developed after an incubation period of seven days, are suspended in a series of flasks, the air in which is kept dry by placing fragments of potash at the bottom of the flask. Every day also a dog is inoculated under the skin with a Pravaz' syringe full of sterilized broth, in which a small fragment of one of the spinal cords has been broken up, commencing with a spinal cord far enough removed in order of time from the day of the operation to render it certain that that cord was not at all virulent. (This date had been ascertained by previous experiments.) On the following days the same operation is performed with more recent cords, separated from each other by an interval of two days, until at last a very virulent cord, which has only been in the flask for two days, is used.

The dog has now been rendered refractory to rabies. It may be inoculated with the virus of rabies under the skin, or even

* If the spinal cord from a rabid animal be kept out of contact with air, in moist carbonic acid gas, the virulence is preserved (for several months at least) without variation in its infective intensity, provided that no alteration is brought about by foreign organisms.—(NOTE BY M. PASTEUR.)

after trephining, on the surface of the brain, without any subsequent development of rabies.

Never having once failed when using this method, I had in my possession fifty dogs, of all ages and of every race, refractory to rabies, when three individuals from Alsace unexpectedly presented themselves at my laboratory, on Monday the 6th of last July.

Théodore Vone, grocer, of Meissengott, near Schlestadt, bitten in the arm, July 4th, by his own dog, which had gone mad.

Joseph Meister, aged 9 years, also bitten on July 4th, at eight o'clock in the morning, by the same dog. This child had been knocked over by the dog and presented numerous bites, on the hands, legs, and thighs, some of them so deep as to render walking difficult. The principal bites had been cauterized at eight o'clock in the evening of July 4th, only twelve hours after the accident, with phenic acid, by Dr. Weber, of Villé.

The third person, who had not been bitten, was the mother of little Joseph Meister.

At the examination of the dog, after its death by the hand of its master, the stomach was found full of hay, straw, and scraps of wood. The dog was certainly rabid. Joseph Meister had been pulled out from under him covered with foam and blood.

M. Vone had some severe contusions on the arm, but he assured me that his shirt had not been pierced by the dog's fangs. As he had nothing to fear, I told him that he could return to Alsace the same day, which he did. But I kept young Meister and his mother with me.

The weekly meeting of the Académie des Sciences took place on July 6th. At it I met our colleague Dr. Vulpian, to whom I related what had just happened. M. Vulpian, and Dr. Grancher, Professor in the Faculté de Medicine, had the goodness to come and see little Joseph Meister at once, and to take note of the condition and the number of his wounds. There were no less than fourteen.

The opinion of our learned colleague, and of Dr. Grancher, was that, owing to the severity and the number of the bites, Joseph Meister was almost certain to take rabies. I then communicated to M. Vulpian and to M. Grancher the new results which I had obtained from the study of rabies since the address which I had given at Copenhagen a year earlier.

The death of this child appearing to be inevitable, I decided, not without lively and sore anxiety, as may well be believed, to try upon Joseph Meister the method with which I had found constantly successful with dogs.

My fifty dogs, it is true, had not been bitten before I brought them into the condition of being refractory to rabies; but I knew that that circumstance might be left out of my calculations, because I had previously rendered a large number of dogs refractory to rabies after they had been bitten. I have this year given the members of the Commission de la Rage evidence of this new and important advance.

Consequently, on July 6th, at 8 o'clock in the evening, sixty hours after the bites on July 4th, and in the presence of Drs. Vulpian and Grancher, young Meister was inoculated under a fold of skin raised in the right hypochondrium, with half a Pravaz' syringeful of the spinal cord of a rabbit, which had died of rabies on June 21st. It had been preserved since then, that is to say, fifteen days, in a flask of dry air.

On the following days fresh inoculations were made, always in the hypochondria, under the circumstances which I give in this table:—

		Half a Pravaz' syringeful of	
July 7th	9 A.M.	Spinal cord of June 23rd	Spinal cord of 14 days
„ 7th	6 P.M.	„ „ 25th	„ 12 „
„ 8th	9 A.M.	„ „ 27th	„ 11 „
„ 8th	6 P.M.	„ „ 29th	„ 9 „
„ 9th	11 A.M.	„ July 1st	„ 8 „
„ 10th	11 A.M.	„ „ 3rd	„ 7 „
„ 11th	11 A.M.	„ „ 5th	„ 6 „
„ 12th	11 A.M.	„ „ 7th	„ 5 „
„ 13th	11 A.M.	„ „ 9th	„ 4 „
„ 14th	11 A.M.	„ „ 11th	„ 3 „
„ 15th	11 A.M.	„ „ 13th	„ 2 „
„ 16th	11 A.M.	„ „ 15th	„ 1 „

I thus made thirteen inoculations, and prolonged the treatment to ten days. I shall say later on that a smaller number of inoculations would have been sufficient. But it will be understood how, in the first attempt, I would act with a very special circumspection.

In order to follow the condition as to virulence of the spinal cords, two fresh rabbits were inoculated, by trephining, with the various spinal cords employed.

Observation of these rabbits enabled us to ascertain that the spinal cords of July 6th, 7th, 8th, 9th, 10th, were not virulent, for they did not render the rabbits rabid; the spinal cords of July 11th, 12th, 14th, 15th, 16th, were all virulent, and the virulent material was present in larger and larger proportion. Rabies appeared after an incubation of seven days in the rabbits of July 15th and 16th; after eight days in those of the 12th and 14th; after fifteen days in those of July 11th.*

On the last days, therefore, I had inoculated Joseph Meister with the most virulent virus of rabies, that, namely, of the dog, reinforced by passing a great number of times from rabbit to rabbit, a virus which produced rabies after seven days incubation in these animals, after eight or ten days in dogs.

When the condition of immunity has been attained, the most virulent virus can be inoculated, in considerable quantity, without ill effects. It has always seemed to me that the only possible effect of this must be to make immunity more assured.

Joseph Meister, therefore, has escaped, not only the rabies which would have been caused by the bites he received, but also the rabies with which I have inoculated him in order to test the immunity produced by the treatment, a rabies more virulent than ordinary canine rabies.

The final inoculation with very virulent virus has this further advantage, that it puts a period to the apprehensions which arise as to the consequences of the bites. If rabies could occur it would declare itself more quickly after a more virulent virus than after the virus of the bites. Since the middle of August I have looked forward with confidence to the future good health of Joseph Meister. At the present time, three months and three weeks having elapsed since the accident, his state of health leaves nothing to be desired.

What interpretation is to be given of this new method which I have just made known, of preventing rabies after bites? I have not at the present moment any intention of treating this question in a complete manner. I wish to confine myself to certain preliminary details essential to the comprehension of

* The boy, therefore, was not inoculated with an active virus until seven days after he was bitten. The period of incubation in man is said to be rarely less than a month, the average being six or seven weeks (Gowers); probably about forty-two days.—D. W.

the significance of the experiments, which I am continuing, in order to adopt eventually the best of the various possible interpretations.

Bearing in mind the methods of progressively attenuating various lethal virus, and the prophylaxis in that way attained, and admitting also the influence of the air in bringing about this attenuation the first explanation to accounting for the effects of this method which suggests itself is, that while the morsels of spinal cord are left in contact with the dry air, the intensity of their virulence is progressively diminished, until it is entirely abolished.

This reflection would lead us to believe that the prophylactic method now described depended upon the employment at first of a virus without any appreciable activity, then of feeble intensity, and then of more and more virulence.

I will show that facts do not lend support to this view. I will prove that the increase in the length of the period of incubation of the rabies, each day, communicated to the rabbits, as I have just described, in order to test the virulence of the spinal-cords dried in contact with air, is an effect of a diminution of the quantity of the virus of rabies contained in the spinal cords, and not an effect of a diminution of its virulence.

Can it be admitted that the inoculation of a virus, always of identical virulence, could be capable of producing a refractory state, when it is used in very small but daily increasing quantities? I am studying experimentally this interpretation of the facts.

Another interpretation may be given of the new method, an interpretation certainly at first sight very strange, but which deserves every consideration, because it is in harmony with certain facts already known with regard to the vital phenomena of certain low organisms, and notably of certain pathogenic microbes.

Many microbes appear to give origin in their cultivations to matters which are injurious to their own development.

Since the year 1880, I have carried on researches in order to ascertain whether the microbe of fowl-cholera produced a kind of poison for itsel (see *Comptes Rendus*, T. xc., 1880). I have not been able to establish the presence of such a material; but I think that this study ought now to be resumed—and so

far as concerns myself I shall not be wanting—by working in an atmosphere of pure carbonic acid gas.*

The microbe of swine-plague (*rouget*) can be grown in various broths, but the weight of it which is grown is so small and so soon arrested at that proportion, that sometimes the cultivation can only be detected as slight silky waves in the nutritive material. It would be said that a product which arrests the development of this microbe comes into existence whether the cultivations be made in contact with air or in vacuo.

M. Raulin, a former assistant of mine, now Professor in the Faculty at Lyons, has proved in the remarkable thesis which he sustained at Paris on March 22, 1870, that the vegetation of *Aspergillus niger* develops a substance which in some measure checks the growth of that mould, when the nutritive material does not contain iron salts.

Can it be that that which constitutes the virus of rabies is formed of two distinct substances, and that side by side with that one which is living and capable of growing in the nervous system there is another, not living, which, when it is in suitable proportion, has the power of arresting the development of the first? In a later communication I will experimentally examine, with all the care which it deserves, this third interpretation of the method of prophylaxis of rabies.

In conclusion I need not say that perhaps the most important of the problems to be solved at the present time is that of the interval which may be allowed between the occurrence of the bites and the commencement of the treatment. In the case of Joseph Meister this interval was two days and a half. But it must be expected to be often much longer.

On Tuesday last, October 20th, with the kind assistance of MM. Vulpian and Grancher, I commenced to treat a youth† of 15 years, bitten six full days before, on both hands, under exceptionally grave circumstances. I will promptly make known to the Academy the result of this new trial.

* An excellent review of existing knowledge with regard to the chemical products of the activity of septic organisms, from the pen of Dr. Burdon Sanderson, F.R.S., will be found in the 12th Report of the Medical Officer of the Local Government Board, and the two subsequent reports contain further information, as well as the results of researches by Dr. Klein, F.R.S., and Mr. Laws, F.C.S., on the influence of the phenyl derivatives. See also in this connection the paper by MM. Chamberland and Roux, translated at p. 604.—D. W.

† J. B. Jupille.

STATISTICS.

In a communication made to the Académie des Sciences on March 1st, 1886 (*Comptes Rendus*, T. cii. p. 459), M. Pasteur stated that the three hundred and fiftieth patient was inoculated (for the first time) on February 25th. He had not treated any cases, whatever the nature of the wounds or bruises, unless the clothes had been distinctly pierced or torn by the teeth of the dog, or exposed parts of the body had been bitten. Before commencing the inoculations, he had demanded a certificate from a veterinary surgeon or a medical man stating that the dog by which the bite had been inflicted was really rabid. In some doubtful cases the existence of the disease in the dog was established by the inoculation of rabbits and guinea-pigs with portions of its nervous system. He had also treated a few persons who had been bitten by dogs which had disappeared, and could not be traced. He stated that statistics showed that rabies generally came on between forty and sixty days after the bite; in the first hundred cases treated more than two months and a half, in the second hundred more than six weeks or two months had elapsed since the patients had been bitten. The first patient (Joseph Meister), bitten nearly eight months, and the second (J. B. Jupille), bitten four months and a half before the date of M. Pasteur's paper, were both in good health. No unpleasant consequences had been noted after the inoculations, except slight redness and œdema following the later operations. Details, in most cases somewhat meagre, are given of a series of cases treated in his laboratory between November 1st and December 15th. M. Pasteur expressed the opinion that all these persons had passed through the really dangerous period.

Number.	Name.	Age.	Condition of Dog recognised by	Date of Bite.	Part Bitten.	Local Treatment.
1	Etienne Roumier	48	Veterinary Surgeon	November 4th	Both hands	None for 24 hours.
2	Chapot ♂	43	The Veterinary School of Lyons	November 6th	Left hand / Left hand; severe	Bathed with volatile alkali.
3	Chapot's daughter	14				
4	Francois St. Martin	10	Chef du Service Sanitaire des Epizooties	November 7th	Right thumb	Bathed with ammonia.
5	Marguerite Luzier	13	Bitten by a cat.	November 11th	Leg; severe	Cauterised with carbolic acid.
6	Corbillon ♂	27	Veterinary Surgeon	November 12th	Not stated	Actual cautery 8 hours after accident.
7	Bouchet ♂	5½	Veterinary Surgeon	November 12th	Left hand and thigh (clothes torn)	Actual cautery ¾ hour after accident.
8	Mme. Delcroix	—	Veterinary Surgeon	November 6th	Right foot	Actual cautery 9 hours after bite.
9	Plantin ♂	—	Veterinary Surgeon	November (beginning)	Right hand	Cauterised 48 hours after bite.
10	Jeanne Pazat	7	Medical attendant	November 12th	Not stated	None.
11	Mme. Achard	—	Veterinary Surgeon	November 9th / November 12th	Right foot / Right hand	No cauterisation.
12	Mme Legrand	—	Veterinary Surgeon	November 6th	Chin	No cauterisation.
13	A. Cattier ♂	43	Dog's master (dog had characteristic bark, refused food, tore and swallowed wood and other objects)	November 16th	Hand	Actual cautery 20 hours after bite.
14	Ternat	—	(Bitten by same dog, believed to be mad during life by Veterinary Surgeon; confirmed by examination after death)	November 15th	Not stated	Cauterisation slight or tardy.
15	Mme. Ternat	—				
16	Mme. Delzors	—				
17	Mme. Dalibard	—				
18	Dr. John Hughes (Oswestry)	—	Patient	November 13th	Lower lip, two severe wounds	No cauterisation.
19	Veuve Faure (Algeria)	—	Bitten by same dog. A fourth child bitten by this dog died of rabies two months after	September 1st	Leg; clothes torn	Not stated.
20	Algerian child	—				

Number.	Name.	Age.	Condition of Dog recognised by	Date of Bite.	Part Bitten.	Local Treatment.
23	Mme. Gréteau	—	Medical attendant	November 14th	Ring finger, two bites	Bathed with ammonia; slight cauterisation.
24	Voisenet ♂	50	Veterinary Surgeon	November 16th	Both legs	Actual cautery 4 hours after bite.
25	Guichon ♂	67	Same dog as 23	November 15th	Left hand	—
26	Walter Halfacre (London)	28	Patient sent by Sir James Paget	November 15th	Hand	Not much cauterisation.
27	Colmeau ♂	—	Veterinary Surgeon. Same dog as 24.	November 15th —16th (night)	Belly, thigh, and knee: clothes in shreds	No cauterisation.
28	Jean Lorda	36	(Seven pigs, bitten at the same time, died in a fortnight or three weeks; and two cows also bitten died in 34 and 52 days respectively)	October 25th	—	—

The cases are arranged according to the chronological order in which they applied at M. Pasteur's laboratory.

The inoculations were commenced in the middle of November in cases 19, 20, 21, 22, and on November 21st (the twenty-seventh day after the bite) in case 28; the two cows were deeply cauterised with the actual cautery.

Of the other cases M. Pasteur only describes two: (1) A boy aged 8, bitten by a rabid dog (diagnosed by a veterinary surgeon) on November 30th. The child was crying, and the animal thrust its lower jaw into his mouth; in this way the upper lip was cut, and the palate deeply pierced, at the same time the teeth in the dog's upper jaw made a wound between the right eye and the nose. Cauterisation was not practised. The boy was treated by M. Pasteur, and remained in good health. (2) A girl (Louise Pelletier), aged 10 years, severely bitten on October 3rd, 1885, in the axilla and head. She was brought to M. Pasteur on November 9th, on the thirty-seventh day after the injury; there was then a huge wound on the hairy scalp which was suppurating freely, and bleeding; the treatment of the case was undertaken with reluctance. On November 27th, eleven days after the treatment had been concluded (fifty-six days after the injury), the early symptoms of hydrophobia appeared, and on December 3rd the patient died, after suffering from well-marked symptoms. In order to ascertain whether the death of the child was to be attributed to the bites, or to the inoculations, two rabbits were inoculated by trephining with a small quantity of her brain-substance. Both the rabbits were **seized at the same time, on the eighteenth day, with symptoms** of paralytic rabies, and both died. Other rabbits were then inoculated with portions of the spinal cord of these two. In the second series rabies appeared after an incubation of fifteen days. These results show, says M. Pasteur, "that the virus which killed the girl Pelletier was the virus of the dog that bit her. If her death had been due to the effects of the virus with which the preventive inoculations were made, the duration of the incubation of the rabies which occurred in the rabbits as a consequence of these second inoculations would have been seven days at most." (See pages 636, 641.) This was the only death which had occurred among the three hundred and fifty patients.

M. Leblanc, veterinary surgeon, Director of the Sanitary Department of the Préfecture de Police de la Seine, supplied M. Pasteur with the statistics embodied in the subjoined table. The table shows the number of persons bitten, and the number who died of rabies in the Department of the Seine:—

Date.	Number of Persons Bitten.	Number who Died of Rabies.	Percentage of Deaths.	Average Percentage of Deaths.
1878	103	24	23·3	15·73
1879	76	12	15·8	
1880	68	5	7·35	
1881	156	23	14·74	
1882	67	11	16·42	
1883	45	6	13·3	
Totals ...	515	81		

A committee was appointed to take steps to found an international hospital in Paris, for the treatment of persons bitten by rabid animals in Europe or North America. On March 8th this committee presented a report, recommending the erection of a hospital in Paris, to be called L'Institut Pasteur, by public subscription, and nominated a very influential committee of management.

DESCRIPTION OF THE PLATES.

PLATE I.

FIG. 1.—Miliary tuberculosis. A portion of the wall and surroundings of a small artery of the pia mater. *a*, inner; *b*, middle; *c*, outer coat. Around these are layers of epithelioid cells, between which lie tubercle bacilli. × 700.

FIG. 2.—Giant cells with tubercle bacilli stained blue, and black pigment granules, from a miliary tubercle of the lung already caseous centrally. × 700.

FIG. 3.—A small artery from a bronchial gland in a case of acute miliary tuberculosis. It is surrounded by a blue areola consisting of stained bacilli. × 100.

PLATE II.

FIG. 4.—Part of the wall of a large phthisical cavity in the lung: on the right, the free surface of the same; on the left, compressed, airless lung tissue. × 100.

FIG. 5.—Giant cells containing rather a large number of bacilli. From the lung, in a case of bovine tuberculosis. × 700.

FIG. 6.—Radially arranged bacilli, which have retained their original arrangement, after the disappearance of the containing giant cell. From the same case as fig. 5. × 700.

FIG. 7.—Tubercular ulceration of the intestine: cover-glass preparation of the fæces, × 700. The tubercle bacilli are blue, other bacteria brown. On the right, below (*a*) is a group of more or less intensely blue-stained ovoid bacillus-spores.

FIG. 8.—A pure cultivation of tubercle bacilli on blood serum in a shallow glass cell. × 80.

PLATE III.

FIG. 9.—A pure cultivation of tubercle bacilli upon an oblique surface of coagulated blood serum, seen from the front. The growth of bacilli (*a*) is limited to the surface of the serum, and has not reached the fluid which has collected below. Natural size.

FIG. 10.—Colonies of tubercle bacilli from a necrosed area in the human kidney (section by Dr. Benda). × 700.

PLATE IV.

FIG. 11.—Erysipelas. Section through the skin at the margin of the redness, showing lymph vessels (*a a*) dilated and blocked. *b*, epidermis. × 100. (From a photograph by Dr. Koch.)

FIG. 12.—Erysipelas. Section through the skin at the margin of the redness, more highly magnified, showing a lymph vessel filled with lymph corpuscles (*b*) and micrococci. The micrococci (*a*) have also spread into the spaces of the connective tissue. × 700. (From a photograph by Dr. Koch.)

FIG. 13.—Pneumonic exudation from man, showing the pneumococci with their capsules. A cell (*a*) is also shown, containing a pneumococcus in its interior.

FIG. 14.—Test tube cultivation of pneumococcus in nutrient jelly. The nail shape is well seen.

PLATE V.

FIG. 15.—Typhoid fever. Section of the kidney, showing a mass of bacteria (*a*) in the blood-vessels. × 100. (From a photograph by Dr. Koch.)

FIG. 16.—Typhoid fever. Section of the liver, showing the border of a mass of bacteria (*a*) which at *b* is broken up, and shows the individual bacilli. × 700. (From a photograph by Dr. Koch.)

PLATE VI.

FIG. 17.—Glanders. Material squeezed out of a glanders nodule, spread out on a cover-glass and stained with fuchsin, showing large numbers of bacilli. × 600.

FIG. 18.—Glanders. Section of the lung showing a glanders nodule projecting into an alveolus and containing a few bacilli (*a*). × 600.

FIG. 19.—Section of a glanders nodule showing large numbers of bacilli. × 600.

FIG. 20.—The streptococci found by Loeffler in diphtheria. × 1250.

FIG. 21.—Section through the mucous membrane from a case of scarlatinal diphtheria. At *b* are seen masses of streptococci penetrating into the tissue. At *a* is the inflammatory layer, and between *a* and *b* is the necrotic area where no nuclei take on the stain. × 200.

FIG. 22.—Section through the membrane from a case of typical diphtheria. At *a* is the surface of the membrane containing numerous micrococci; at *b* are the clumps of the diphtheritic bacilli. × 600.

FIG. 23.—Diphtheritic bacilli from a pure cultivation. × 1250.

PLATE VII.

FIG. 24.—Section through the projecting part of a leprous tubercle (magnified 100 diameters).

a, horny layer of the epidermis; *b*, granular layer; *c*, rete mucosum; *d*, limit of the cutis; *e*, round cell infiltration between the fibres of the cutis; *v*, vessel.

FIG. 25.—Section of the cutis (magnified 300 diameters).

c, *d*, *e*, *f*, round or irregular formed cells infiltrated between the connective tissue fibres. They all contain rods coloured blue.

a, *b*, cells belonging to the wall of a vessel, *v*. They also contain rods.

FIG. 26.—Elements of a leprous tubercle, magnified 600 diameters (objective A immersion, No. 7 de Nachet).

B, D, cells filled with rods; F, a bundle of rods which were adherent to each other.

FIG. 27.—Longitudinal section of a vessel, magnified 200 diameters.

o o, lumen of the vessel; *m*, its wall; *a*, cells which are interposed between the fibres of the cutis.

FIG. 28.—Transverse section of a vessel, magnified 200 diameters.

p, wall of the vessel; *m*, peripheral tissue.

FIG. 29.—Section from the liver. Magnifying power, 750 diameters.

a, hepatic cell without bacteria; *b*, lymphatic cell containing rods; *c c*, hepatic cell containing rods.

A, a lymphatic cell from the liver containing granules and rods.

PLATE VIII.

FIGS. 30 and 31.—Staphylococcus (aureus and albus, which cannot be distinguished microscopically from each other). Fig. 30 represents them twenty-four hours old. Fig. 31 represents them several months old.

FIG. 32.—Streptococcus pyogenes.

FIG. 33.—Streptococcus erysipelas (Fehl.) from a cultivation in gelatine.

FIG. 34.—Micrococcus pyogenes tenuis from a case of empyema.

FIG. 35.—A bacillus which rapidly destroys the soil on which it grows with the production of slight putrefactive odour.

FIG. 36.—Bacillus saprogenes, No. 1.

FIG. 37.—Bacillus saprogenes, No. 2.

FIG. 38.—Small cocci from a cultivation from carious teeth.

FIG. 39.—Bacillus saprogenes, No. 3.

FIG. 40.—Bacillus from a putrid abscess of bone with general sepsis (p. 425, Binnewis).

FIG. 41.—Bacilli from gangrenous progressive emphysema (first case) from the tissue.

FIG. 42.—Bacilli from gangrenous progressive emphysema from the tissue (second case).

FIG. 43.—The cocci which cause finger erysipeloid.

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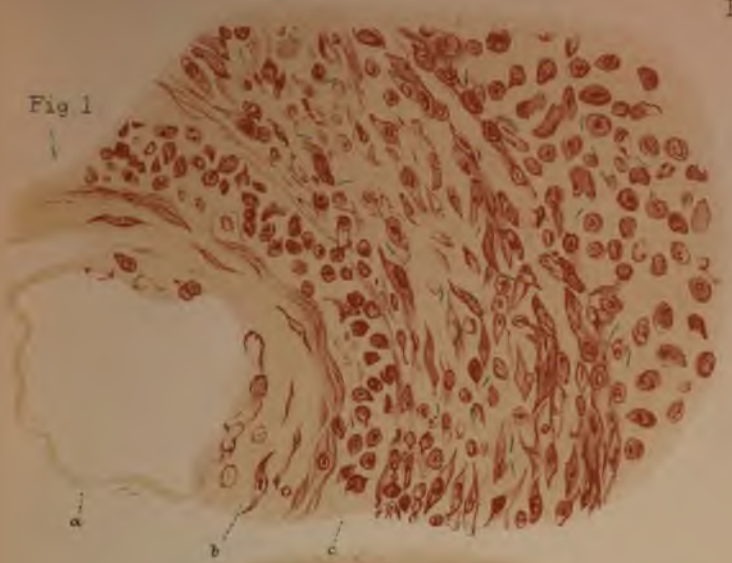
Pl I.

Fig 1



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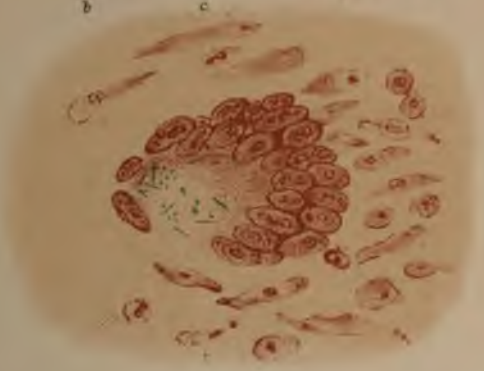

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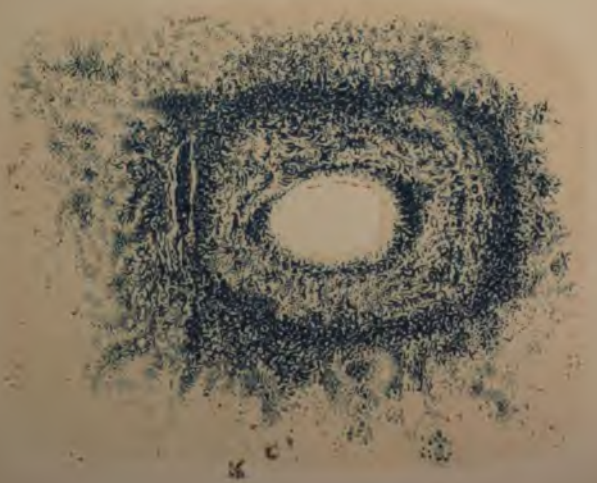

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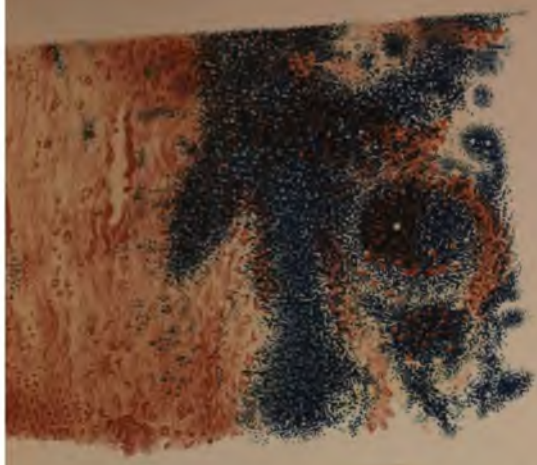

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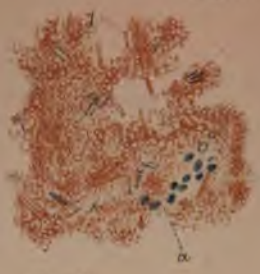

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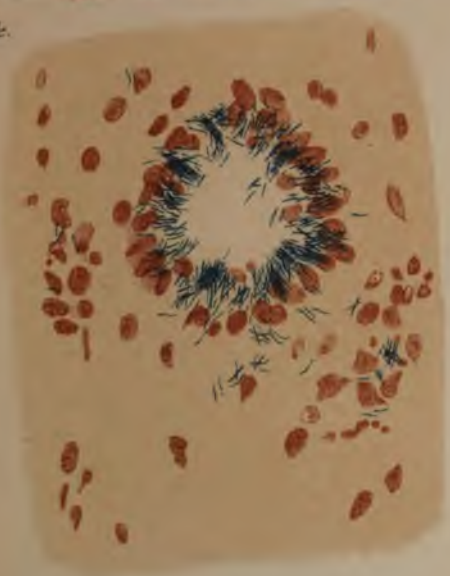

Fig. 5.

Fig. 8.

Fig. 6.

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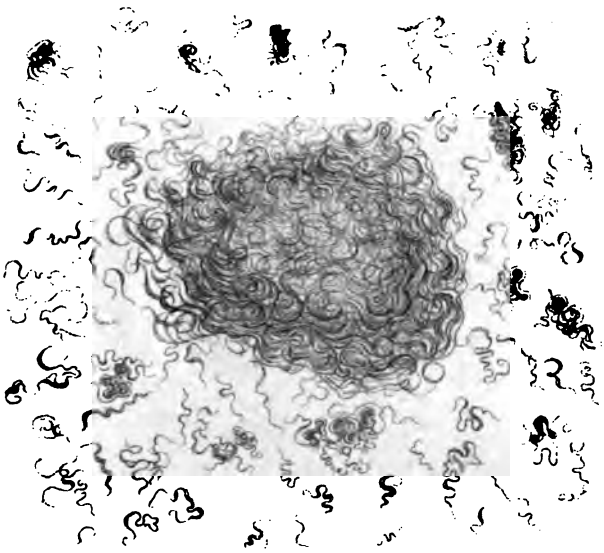

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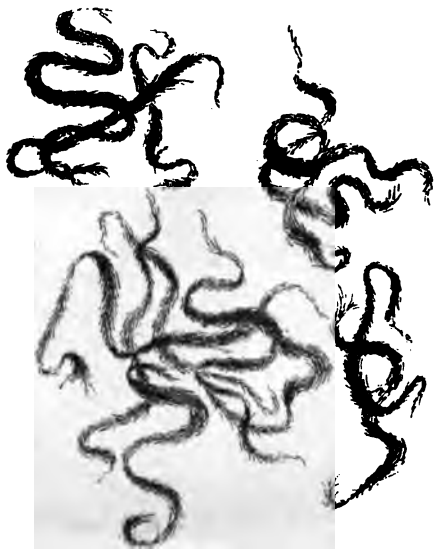

Fig. 10.

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Fig 11.

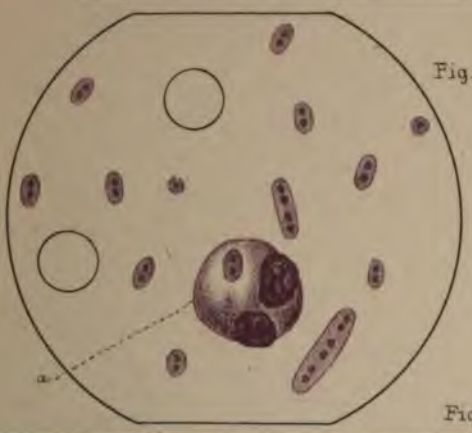

Fig. 13.

Fig. 14.

Fig. 12.

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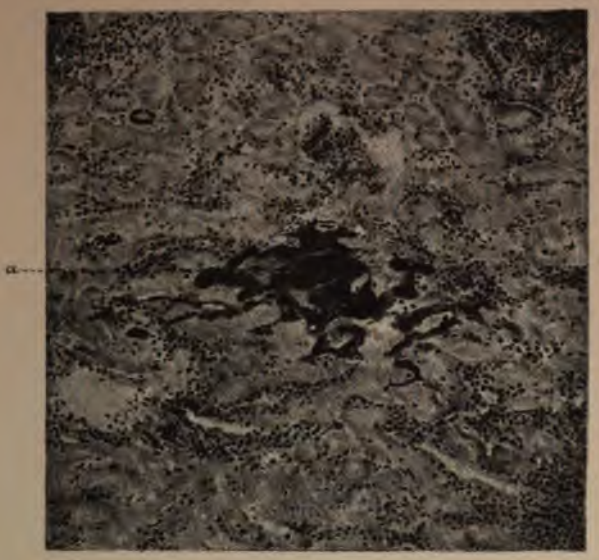

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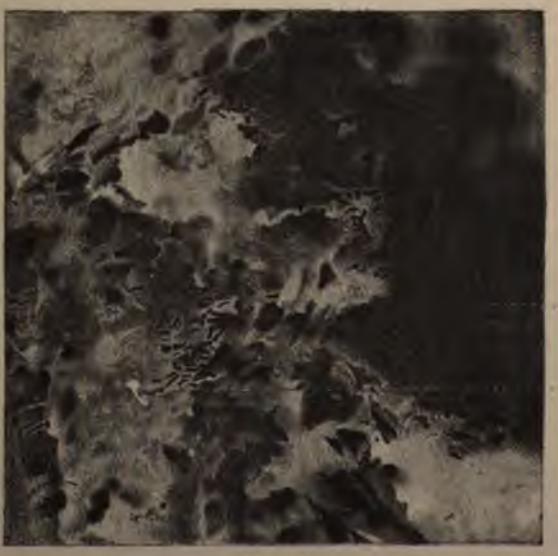

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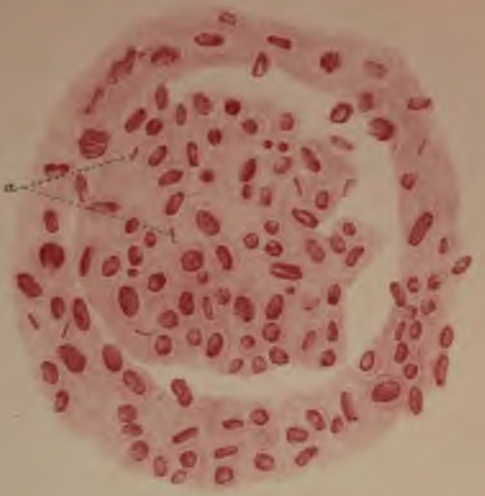
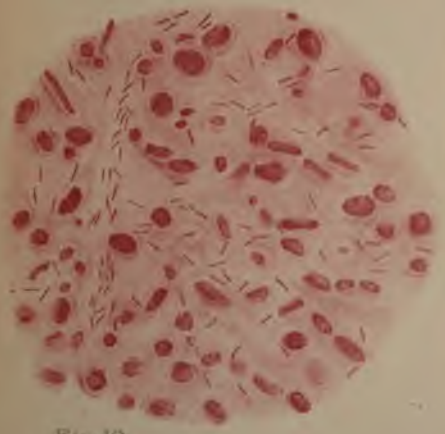


Fig.18.

Fig.20.



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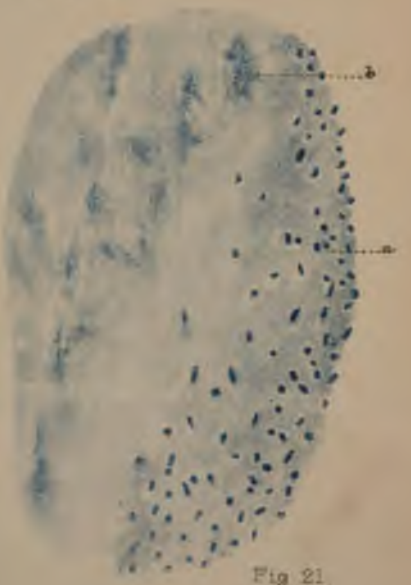
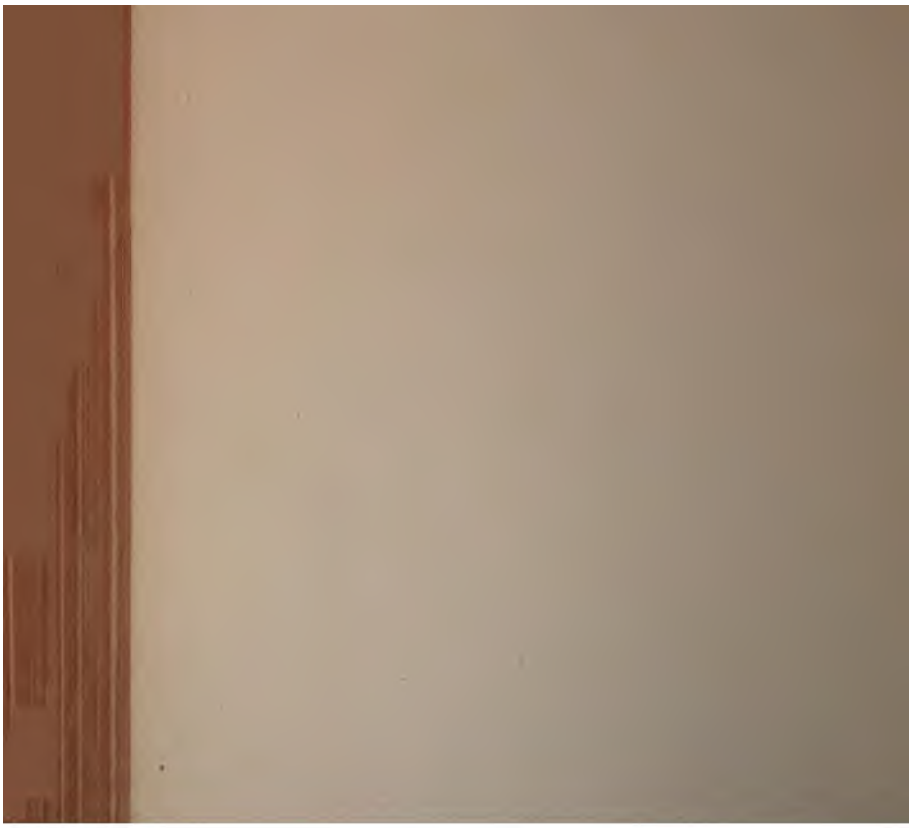
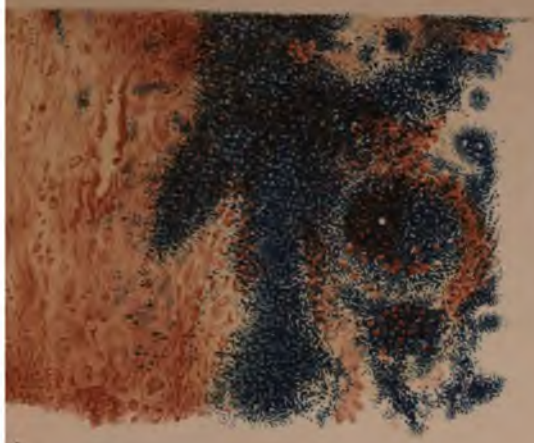


Fig.21.

Fig.22.

Fig.23.





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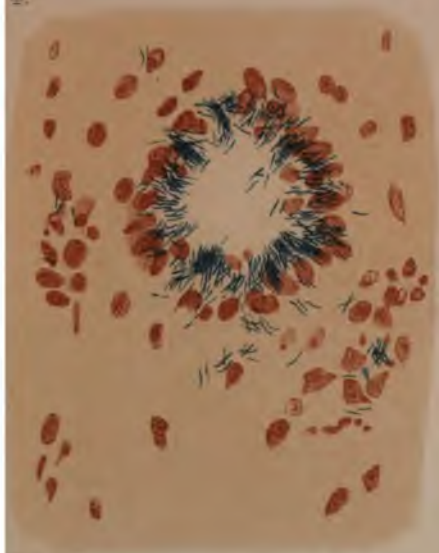

Fig. 5.

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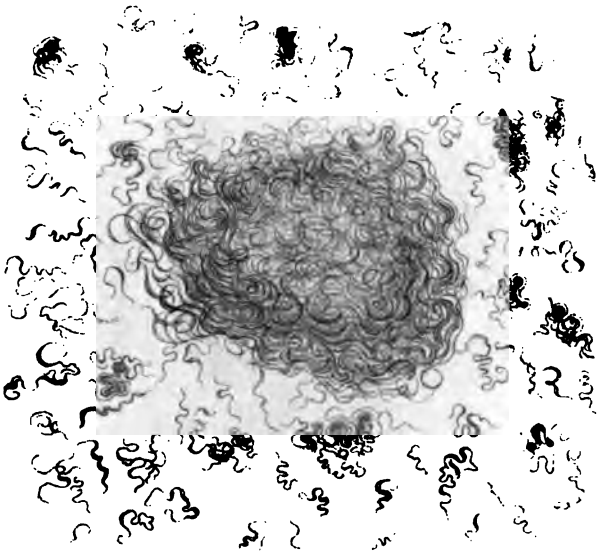

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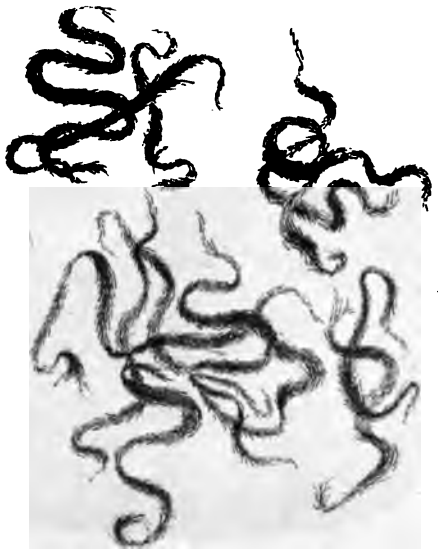

Fig. 10.

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Pl. IV

Fig. 11.

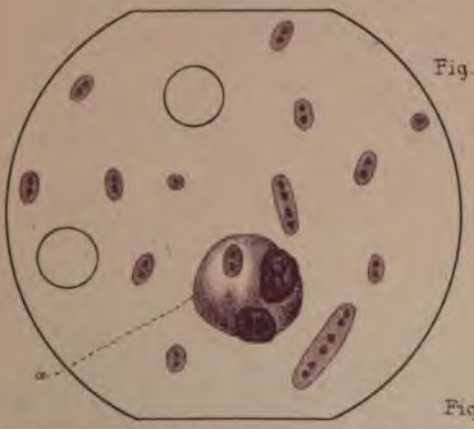
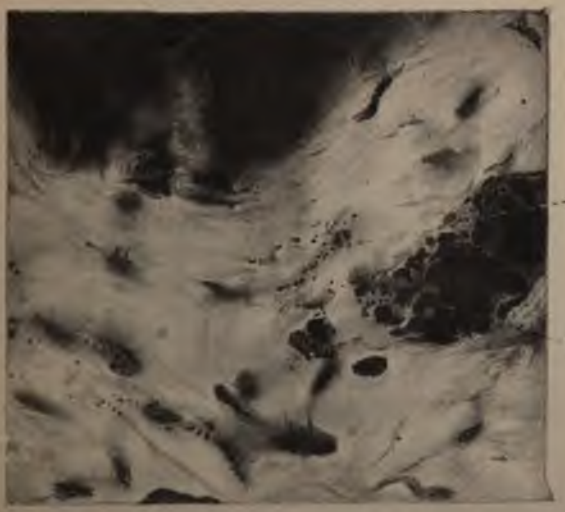


Fig. 13.

Fig. 14.

Fig. 12.



West, Newman & C° del. Lith.

Fig. 15.

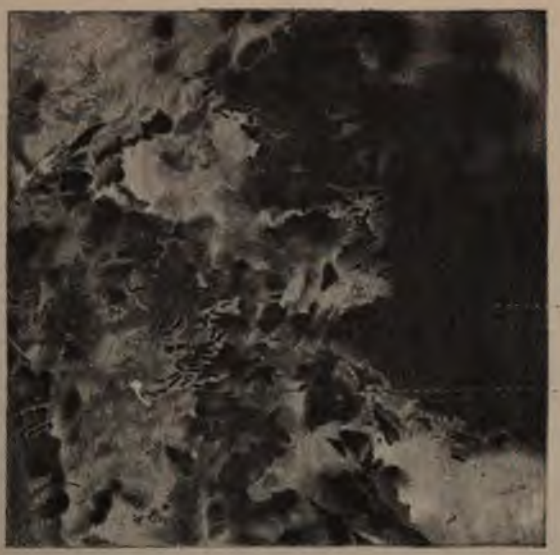


Fig. 16.

West, Newman & Co. lith.

Fig. 17.

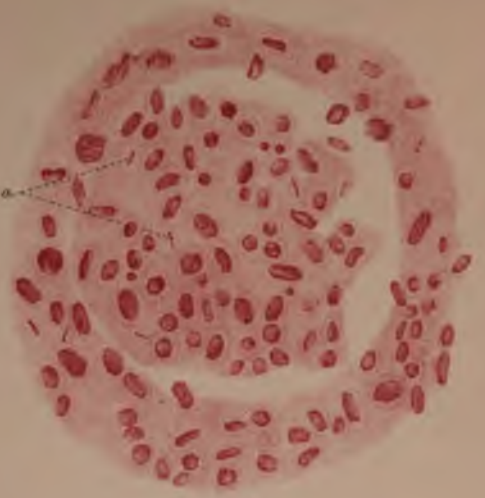


Fig. 18.

Fig. 20.

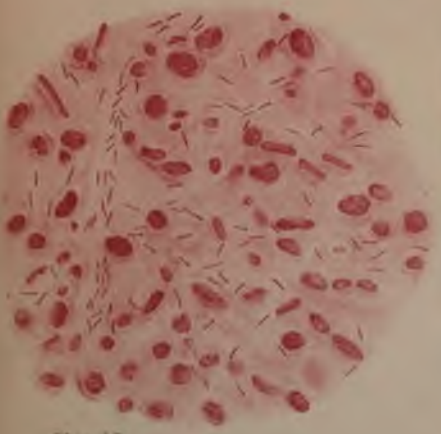


Fig. 19.

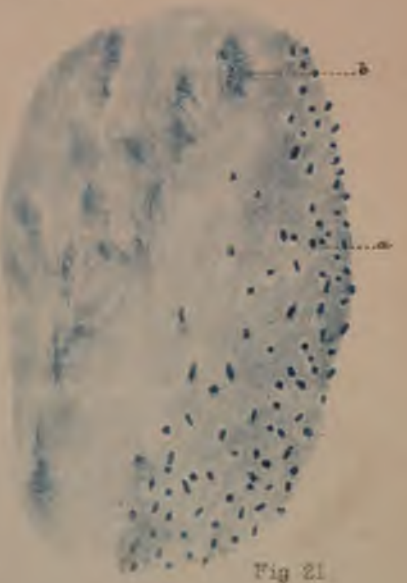


Fig. 21.

Fig. 22.

Fig. 23.

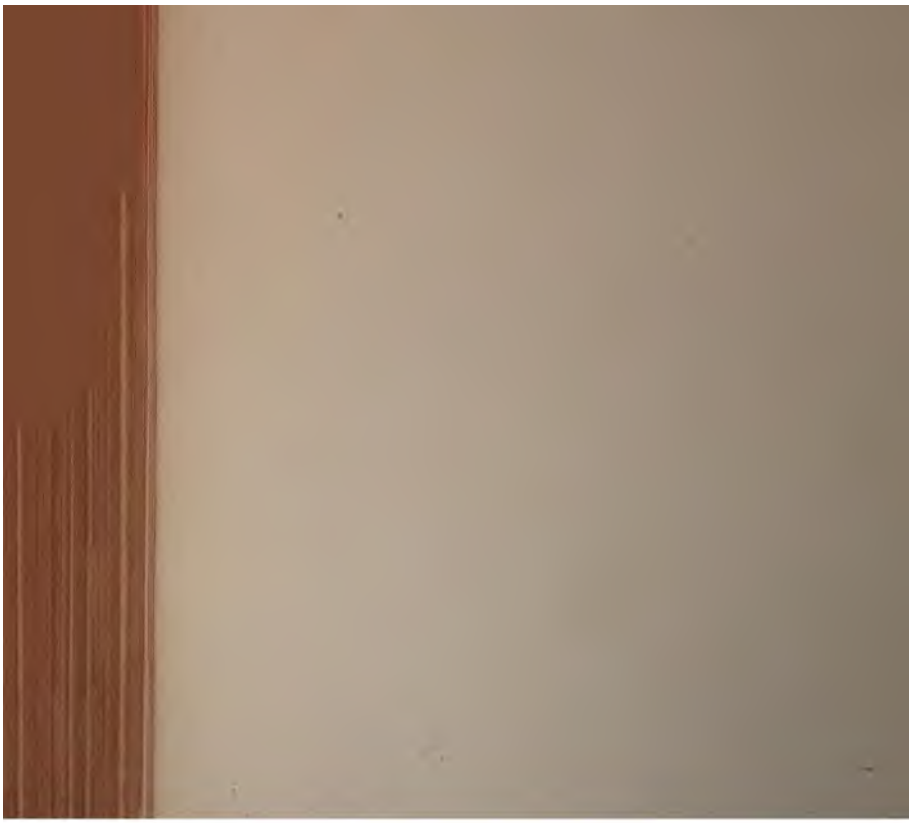


Fig 27.

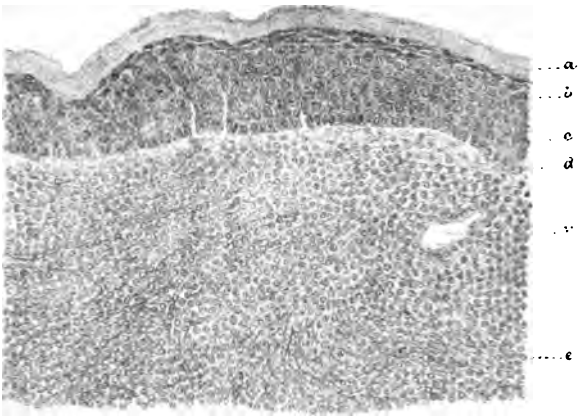


Fig 28.

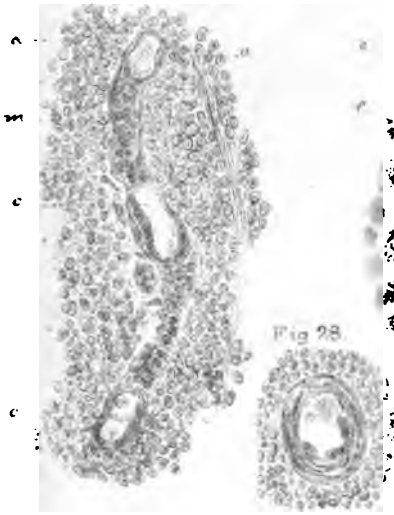


Fig 28.

Fig 29.

Fig 30.

Fig 31.

Fig 30

Fig 31

Fig 32

Fig 34

Fig 33

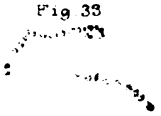


Fig 35

Fig 37

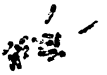


Fig 36

Fig 38

Fig 40

Fig 39

Fig 41

Fig 43

Fig 42

West Newman & Co. lith.