Report to the Association for the Advancement of Medicine by Research on the Relation of Micro-Organisms to Tuberculosis

Handed in to the Association for the Advancement of Medicine by Research on February 1, 1883 by W. Watson Cheyne, M.B., F.R.C.S., Assistant Surgeon to King's College Hospital.

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Without entering into the history of the literature on tuberculosis, I must briefly indicate the researches which bear directly on the point at issue. In 1877 Professor Klebs published a paper[1] in which he stated that he had succeeded in cultivating an organism from tuberculosis material with which he was able to produce tuberculosis, and he looked on this organism as the cause of the disease. He gave it the name of "Monas tuberculosum." His method of cultivation was what he termed "fractionirte Cultur," i.e. if a drop of infective fluid is mixed with 50 ccm. of a cultivating solution it is diluted 1,100 times, and if a drop of this is put again into 50 ccm. the original material is now diluted 1,110,000 times, and so on. In this way he made cultivations from artificial tuberculosis in white of egg, and found that the egg became turbid in two or three days from the development of micrococci and short rods about 2mm. in length. These rods possessed active movement, but it was the micrococci and not the rods which he looked on as the cause of the disease. He also found, on examining crushed tubercles, that there were numbers of dancing bodies, the movements of which were more than molecular, and he states that they are found in advance of the tubercle and cause the tubercle. He only mentions one experiment, where a small quantity of the cultivation (third generation) obtained from a guinea-pig inoculated with human tuberculosis was injected into the abdominal cavity of a cat. The animal was killed a month later, and the peritoneum was found to contain tubercular nodules.

1 *Prager Medicin*, Wochenschrift, Nos. 42 and 43, 1877.

Similar results were obtained in 1880 by Dr. Max Schüller.[1] He pounded a piece of human lung containing tubercles till it formed a thick liquid. This was then filtered, and the filtrate used to inoculate flasks containing Bergmann's solution. His cultivations were carried to the third generation, and he employed the fractional method introduced by Klebs. In twenty-four hours, as a rule, the fluid became turbid from the development in it of micrococci. The inoculation of these cultivations was followed in some instances by development of tubercle. It is most important to note that he was only successful as a rule with the first and second generation; the third was sometimes without result. He explains this on the view that the organisms became weakened in their infective power. Schüller went further than Klebs, and describes masses of micrococci in the tubercles, in the diseased synovial membranes of joints, in lupus, &c.

In 1881 Professor Tussaint published a paper[2] in which he states that he has cultivated from the blood of tuberculous animals and from tubercles an organism which is evidently a micrococcus, and that cultivations of this organism produce tuberculosis when injected into animals. As I shall have more to say about Toussaint's investigation in another part of this report I shall omit further mention of it here.

Aufrecht, in 1881,[3] stated that the centre of a tubercle does not consist, as is generally supposed, of broken-down cells, but contains micro-organisms, of which he has made our three kinds; very small delicate micrococci, similar micrococci united in twos and threes to short chains, and also shining, short, rod-shaped structures, very narrow and about half as long again as broad. These rod-shaped bodies are, according to him, of almost constant occurrence in tubercles. In this paper he does not state how they were demonstrated, but in a later paper he says that these bodies, which he regards as the same as Koch's bacilli, could be readily shown in sputum by staining it in a half per 1000 watery solution of fuchsin.

This brings us to the now famous research of Koch published in the Berl. klin. Wochenschr. No. 15, 1882. He showed that a peculiar kind of bacillus was constantly present in tubercle, natural or artificial, and in all animals, and he was able to cultivate this bacillus, and by inoculation with the cultivated organism to produce the same disease as follows the inoculation of tuberculous material.

About ten days after Koch published, Baumgarten wrote on a bacillus which he had found in artificial tuberculosis, and which was undoubtedly the same as that described by Dr. Koch. His observation was made quite independently of Koch's, but he did not furnish any proof that the bacillus was the cause of the disease.

In July and August last I visited Toulouse and Berlin for the purpose of seeing the methods and the work of those who have been chiefly engaged in these investigations.

I arrived at Toulouse on July 21st, and was very kindly received by Professor Toussaint, who showed me all his methods and the results of his work.

[1] *Die skrophulöse und tuberculöse Gelenkleiden*, 1880.
[2] Comptes rendus, 1881
[3] *Pathologische Mittheilungen*, Magdeburg, 1881.

The materials which he employs as cultivating fluids are either an infusion of chicken or of rabbit, or blood-serum; the last is preferred. Blood-serum is obtained in the following manner: - A glass retort is perforated at the upper part for the reception of a cork through which a tube containing cotton-wool passes. The mouth of the retort is connected by means of a piece of caoutchouc tubing with a smaller glass tube. The apparatus thus arranged is purified by washing with carbolic acid lotion and afterwards by boiling off some distilled water in it, the end of the small tube being sealed. When the apparatus is cool the end of the small tube is introduced into an artery of some large animal, a horse for example, the seal end is broken and blood is allowed to flow in till the retort is half full. The end is then sealed, and when the serum has separated a purified pipette is passed through the hole at the top of the retort and a suitable quantity of the serum sucked up and put into smaller vessels. These vessels have necks of ground glass over which a cap fits closely, the upper part of the cap being drawn out in the form of a tube which is plugged with cotton-wool. They are purified by heat. When it is desired to examine the cultivation or to inoculate a fresh flask the following procedure is adopted:- The narrow end of a pipette is sealed, the upper part packed with cotton, and the whole purified by heat. The point is then broken off, the broken part heated in the flame, the cap of the flask rapidly lifted, a small quantity of the fluid sucked up into the pipette, the cap reapplied and another vessel inoculated or the material examined.

He examines the organisms in the fluid with a Vérick's No. 8 objective without a condenser and without staining them. He has not investigated the tissues with the view of finding the organisms which he describes. He showed me a specimen of the organisms with which he works, and they were undoubtedly micrococci chiefly in groups.

He generally cultivates from the blood of the heart of tuberculous animals. The skin is stripped off without any special precautions and the chest opened. Then with a heated knife a hole is made in the heart, and the heated end of one of the sealed pipettes introduced through this hole, and end broken off and some blood sucked up and put into the flask containing the cultivating liquid. The inoculated flasks are then placed in an incubator and kept at the temperature of 37ºC. In a day or two they become muddy from the development in them of micrococci. He has made about fifty cultivations from tuberculous animals and always finds the same micro-organisms.

He always, in the first instance, uses tuberculous material from cows (*perlsucht*) to infect the animals with which he experiments; but in one case he introduced tuberculosis in a rabbit with phthisical sputum, got precisely the same lesions, and on inoculation of the blood of this animal into serum he obtained precisely the same form of organism.

In his experiments on animals he always uses for purposes of injection of blood, cultivations &c. an ordinary subcutaneous syringe. After using it he washes it out with 1-20 carbolic acid lotion and sometimes with alcohol as well, but he does not allow the carbolic acid to act for any length of time.

In injecting cultivations he generally introduces ten drops. He has never used less than six or eight, but he thinks the quantity is a matter of indifference. He marks the animals by clipping their ears.

He showed me a series of specimens illustrating the production of tuberculosis by inoculation of tuberculous material from one animal to another. The disease produced was evidently the same as that obtained by all other observers by the inoculation of tuberculous material.

He finds that the injection of the micrococci cultivated in infusions very often fails to produce tuberculosis in rabbits, but the cultivations in serum are much more successful. I was shown three cases in which tuberculosis had been produced in this way. The cultivations take as long or even longer to produce tuberculosis than tuberculous material does (two or three months).

He has caused tuberculosis in animals by injection of the saliva, urine and blood of tuberculous animals, and by vaccination from vaccine pustules developed on tuberculous calves.

He has not has any case of spontaneous tuberculosis in uninoculated animals kept separate from tuberculous ones.

Professor Toussaint gave me specimens of his micrococci, and also portions of various organs of tuberculous animals for examination. The result of my investigations will be given later.

While there I saw five post-mortem examinations on rabbits (besides one to be noticed later). These were:-

1. A rabbit which had eaten bread soaked with the blood and juice of the muscle of another tuberculous rabbit. This was killed after the lapse of about two and a-half months. The submaxillary glands were enlarged and caseous, the lungs full of tubercles, also the kidneys, and a few nodules and ulcers in the small intestine. Spleen free.
2. A rabbit which had been vaccinated at the root of the ear with lymph from a tuberculous calf. The lungs, kidneys and bronchial glands were full of tubercle. There was very slight alteration at the point of infection, but the glands at the angle of the jaw were enlarged. He says that this is so in all cases. The vaccination is done in the same way as in the case of children.
3. A rabbit into which had been injected six months previously three drops of blood from a tuberculous pig. Here there was no recent tubercle, only one or two nodules which may or may not have been tuberculous; nothing to show that the experiment had been successful. However he told me that two other rabbits which had been inoculated with the same blood at the same time had died some time previously with extensive tuberculosis.
4. and 5. Two rabbits inoculated with tuberculous material. Extensive tuberculosis of all the organs.

I called on Dr. Schüller, in Berlin, on August 5th, and had a long conversation with him on the subject of his experiments.

He admits that Koch's tubercle bacilli can cause tuberculosis. This he considers absolutely proved by Koch. But he thinks that other things may also be the carriers of contagion, and he still holds that it may have been transmitted by the micrococci

described by him. He did not examine human tuberculous organs for micro-organisms, but in animals he found bodies which, in his opinion, were often without doubt micrococci. He did not have any specimens at hand, but he showed me several of his original drawings, which were of course similar to the figures in his book.

In reference to his views it is important to note that his cultivations did not always produce tuberculosis, that they did not always kill so rapidly as tuberculous material, and that phthisical sputum was more virulent.

I cannot sufficiently acknowledge Dr. Koch's kindness, nor the readiness with which he placed the results of his experiments and his methods at my disposal. I spent ten days in his laboratory, and was able to appreciate his extreme care and accuracy in experimenting and his entire want of bias in drawing his conclusions. I can only indicate briefly some of the principle results of my visit.

Koch's method of cultivating on solid materials instead of in liquids is so well known that a reference to its advantages in unnecessary. In the case of the tubercle bacillus it was found that the organism would only grow at the temperature of the human body, and therefore it was necessary to use some other material than gelatinised infusions. He accordingly solidifies blood-serum at such a temperature as to leave the serum as transparent as possible. The serum is put into purified test-tubes plugged with cotton-wool, and for several days in succession (usually six) these tubes are kept at the temperature of 58ºC. for an hour. At the end of that time the tubes are laid obliquely in the incubator so as to have a large surface for inoculation, and kept at the temperature of 65ºC., or a little higher, till solidification is complete. This occurs in a few hours. The tubes are then kept for some days till it is certain that the serum has been thoroughly sterilised, and then they are ready for use. Koch generally employs sheep's serum.

In inoculating from animals immediately after death the skin is dissected back, and it is well to wet the skin with bichloride of mercury solution to prevent the hairs flying about. A large number of knives, forceps and scissors and purified by heat and allowed to cool, and every fresh cut is made with a fresh instrument, which is immediately afterwards repurified by heat. The organ to be used for the experiment being exposed, tubercles are clipped out and introduced into the serum-tubes by platinum wires purified by heat, and then crushed out as much as possible. Tubes so inoculated are placed in the incubator, and if development occurs small opaque masses will be seen to appear at various points in about ten days. These masses gradually increase in size, and after a time are picked up and spread out on fresh serum, the fresh masses which form being again treated in the same way, and so on. By proceeding in this manner all trace of original tuberculous material other than the bacilli is soon lost.

In inoculating from the lung of an animal which has died some hours previously (man or cow) the procedure is somewhat different. The lung is removed from the body and the surface washed with bichloride of mercury solution. A series of cuts are then made with pure knives throughout the organ till it may be supposed that a pure portion has been got, and then tubes are inoculated in the same way as before.

During my visit Dr. Koch showed me a large number of cultivations, among which may be mentioned cultivations from phthisical lungs, from acute tuberculosis in man, from scrofulous glands, from cheesy pneumonia, from perlsucht, from spontaneous tuberculosis of a monkey, from artificial tuberculosis of animals &c. These all exhibited the same appearances of growth and varied from the third and fourth to the sixteenth generation.

[Fig. 1]

For purposes of injection of the cultivations into animals, special syringes are used. These consist of a glass tube, on each end of which the thread of a screw is cut and the metal fittings are thus screwed directly onto the glass. The piston is graduated, and the bulbous end is hollowed in the middle, and does not fill up the calibre of the tube (see Fig. 1). A sufficient amount of cotton-thread is wrapped around this end of the piston to make it fit the tube accurately. The washers are made of thin pieces of cork. After each injection the cotton-thread and the washers are removed and the syringe washed. Before use fresh thread and fresh washers are arranged, and the whole is purified by heat. In this way the syringe is thoroughly purified and there is no chance of contamination from its previous use. The process of inoculation is as follows: - A tube of serum on which bacilli are growing being taken, the surface is scraped with a heated platinum wire and the masses of bacilli ground up in a purified mortar with a little boiled distilled water so as to break up the masses. This material is then injected.

On August 2nd, Dr. Koch inoculated a series of animals before me in the following manner with a cultivation of bacilli from a case of phthisis. This was the eighth cultivation, the first cultivation having been made directly from the lungs of the patient on January 24th, 1882:-

a. About half a syringeful of the cultivation was injected into the vein of the ear of a rabbit. This animal was killed on August 10th, and numerous tubercles were seen in the lungs. I brought back the organs of this animal with me, and will refer to them later.
b. About two drops of the fluid were injected into the anterior chamber of each eye of a rabbit, less being put into the left eye than into the right. On August 4th one could perceive minute white masses on the surface of the iris, more marked in the right than in the left. On August 10th both eyes showed well-marked tuberculosis of the iris. Dr. Koch writes me that he killed this animal on September 12th, and that there were numerous tubercles in the lungs.
c. A speck of the cultivation was introduced into the anterior chamber of the eye of a rabbit by means of a pure needle. On August 10th there was no very marked change perceptible. Dr. Koch killed this animal on September 12th, and found that there were numerous tubercles in the lungs. (Tuberculosis of the iris had previously developed.)
d. A syringeful of the cultivation was injected into the abdomen of a guinea-pig, and a small bit was put under the skin of the abdomen. On August 10th there was a hard nodule at the seat of inoculation, and the nearest lymphatic glands were much enlarged. Dr. Koch writes that this animal died on September 5th, and was found to be extremely tuberculous.

While I was in Berlin I also saw post-mortem examinations of a number of animals which had been inoculated with cultivations of bacilli.

1. On August 1st a rabbit died which was inoculated on July 6th into the eyes with the sixteenth cultivation from a case of spontaneous tuberculosis of a monkey, the first cultivation having been made on October 14th, 1881. In the right eye the point of the syringe was introduced into the anterior chamber and the piston only just touched. In the left eye the point was introduced, but immediately withdrawn again, the piston not being touched. The right eye was seen to be completely converted into a cheesy mass and tubercles were present in the conjunctiva, especially at its reflection. The left eye still showed well-marked tuberculosis of the iris, with commencing caseation at the point of inoculation, and tubercles in the cornea. The glands below the jaw were enlarged and beset with greyish points. The lungs, liver and spleen were full of minute tubercles. These were the largest in the lung where there was commencing caseation at the centre of the nodules with hæmorrhages around the cheesy points.
2. Another rabbit inoculated on the same day with the same material, and in the same manner, was found dying, and was killed on August 1st. The post-mortem appearances were exactly the same as in rabbit No 1. Tubercles cut out of the lung, crushed between two cover glasses and stained by Ehrlich's method, showed numerous bacilli.
3. On August 2nd a third rabbit inoculated in the same manner, at the same time, and with the same material as Nos. 1 and 2, was found dead. The post-mortem appearances were essentially the same, but the tubercles were in the main limited to the lungs.
4. On August 5th, a fourth rabbit of the same series was killed, and the post-mortem appearances were essentially the same. Tubercles of the lung crushed and examined were found to contain bacilli.

There were also other two post-mortems, but as I brought back the organs with me for examination I shall refer to them again.

To show me his method of cultivation, Dr. Koch inoculated several tubes of blood-serum as follows:- (a) Several little flat vessels and tubes were inoculated from a cultivation from the mesentery of a guinea-pig inoculated with a cultivation from acute tuberculosis. This tube had been kept for some time, and fungi were growing from the cotton-wool plug. As a result, of seven tubes three had fungi develop in them and in four the bacilli grew well. All the small flat vessels became impure, but nevertheless it could be seen that the tubercle bacilli were growing. (b) From rabbit No. 3 a number of tubes were inoculated from one lung. In this lung there was pneumonia as well as tubercles, and in all the tubes both tubercle bacilli and another form developed. (c) Several tubes were inoculated from tubercles from the lung of rabbit No. 4, but no development occurred. Koch thought that this was probably bad serum, for there were masses of crystals in it, and he has noticed that serum containing blood-crystals is not favourable for development.

I made sections of a variety of cases of tuberculosis, and Dr. Koch gave me several tubes containing bacilli and several pieces of tuberculous tissue for further examination. The result of my investigation of these will be narrated presently.

I received the following letter from Professor Klebs in reply to a request from me that he would state his present position with regard to the question of the organisms of tubercle. I will translate the part of his letter which refers to this subject. His letter is dated January 18th, 1883. He says: -

The question as to the organisms of tubercle turns at the present moment on the decision of the preliminary question whether the bacilli described by Koch , those rod-like bodies which hold fast crystallised acid fuchsin even when subjected to the action of mineral acids, are to be regarded as the carriers of the virus of the disease. I must in the first place state, with regard to these bodies, that developmental processes have not as yet been demonstrated in them; their multiplication on cultivations would not disprove their inorganic nature. Although I believe that they are organisms, the proof of it must yet be furnished by the demonstration of phenomena of life on their part, such as movement or division under the eye of the observer. I think it proper to leave this task to their discoverer. On the other hand, I can definitely state that even in the purest cultivations (Koch's own) there are always present finely granular masses which appear to possess the characters of micrococci. The same bodies also appear in cultivations on microscopic slides. They may, since they are present in cultivations which are active, be the carriers of the virus just as much as the rods which can be stained, and to which alone Koch attributes that property. It is possible that these forms are different stages of development of the same organism, but it is also possible that the rods are of an inorganic nature, just as one finds crystals in the form of rods in many cultivations, as for example, the characteristic albuminoid crystals in cultivations of monads on isinglass jelly.

This question cannot be regarded as settled at present, it requires further investigations. Nevertheless it seems to me certain that the development of tuberculosis by a virus capable of self-multiplication, i.e. an organism, as first asserted by me as a result of experiments in which active cultivations were obtained, has received an important confirmation by Koch's experiments. And further, the diagnostic meaning of Koch's rods seems to me to be of great value.

Professor Klebs ends by recommending the study of and the adoption of measures against tuberculosis in cattle, which is in his opinion "the most frequent source of human tuberculosis (scrofula)."[1]

[1] The following is the original of Prof. Klebs' letter: -

Zurich, January 18, 1883.

Dear Sir, - In answer to your request to express my opinion upon the particular forms of organisms associated with tuberculosis, I cannot give you but a very short abstract of an extensive publication that will be published in the *Arch. of Exp. Pathology*. But you will excuse me if I prefer to write in my language.

Die Frage nach den Tuberkelorganismen wird sich im Augenblick um die Entscheidung der Vorfrage drehen, ob die Koch'schen Bacillen, also jene Stäbchenförmigen Körper, welche crystallisirter, saures Fuchsin auch gegenüber der Einwirkung von Mineral-säuren festhalten, als die Träger des Kran[k]heitsvirus zu betrachten sind. Ich muss zunächst bezüglich dieser Körper constatiren, dass

Entwicklungsvorgänge an denselben bis jetzt nicht nachgewiesen sind. Ihre Vermehrung an den Culturen würde nicht ihre unorganische Natur ausschliessen. Obwohl ich glaube, dass es Organismen sind , müsste doch erst der Beweis geliefert werden indem man an denselben Lebens-erscheingungen nachweist, Bewegung oder Theilung unter der Augen des Beobachters. Ich halte es für zweckmässig diese Aufgabe dem Entdecker zu überlasson. Dagegen kann ich mit Bestimmtheit behaupten, dass auch in den reinsten Culturen (von Koch selbst) regelmässig feinkörnige Massen vorkommen, welche den Charakter von Micrococcen zu besitzen scheinen. Dieselben Bildungen treten auch bei Object-trägerculturen auf. Sie können, da sie in wirksamen Culturen vorhanden sind ebenso die Träger des Virus sein, wie die sich farbenden Stäbchen denen Koch ausschliesslich diese Function vindiciren will Es ist möglich, dass beide Formen zu derselben Entwicklungsreihe gehören; es ist aber auch möglich, dass die Stäbchen Beimischungen unorganisierter Art sind, wie man solche in Gastalt von Crystallen in vielen Culturen antrifft, zum Beispiel die characteristischen Eiweiss-crystalle in Culturen von Monadinen auf Hausenblasegallerte.

Diese Frage kann vorläufig nicht als entschieden betrachtet-werden, sie erfordert weitere Studien. Dagegen erscheint es mir unzweifelhaft, dass die Erzeugung der Tuberculose durch die mit Vermehrungsfähigkeit ausgestatteter Virus, also einen Organismus, von mir zuerst auf Grund wirksamer Culturen behauptet, durch Koch's [sic – should read Kochs] Versuche eine wesentliche Bestätigung und Begründung gefunden hat. Ferner aber erscheint auch die diagnostische Bedeutung der Koch-schen Stäbchen von hohem Werth.

Ich hoffe[,] dass diese beiden letzten Sätze, namentlich die Vorletzte durch die Discussion, welche Sie einleiten werden, die allgemeinste Verbreitung in England finden und die Collegen und das Publlicum veranlassen werden mit ihrer bewährten Energie alle Consequenzen zu ziehen. Ich empfehle namentlich das Studium und die Bekämpfung der Tuberculose der Rinder als die häufigsten Quelle menschlicher Tuberculose (Scrofula) Mit Gruss der Ihrige

E. Klebs

Koch's original method of demonstrating the tubercle bacilli was to stain them with an alkaline solution of several of the aniline dyes. He recommended an alkaline solution of methylen blue prepared according to the following formula:-
Distilled water, 200ccm.
Saturated alcoholic solution of methylen blue, 1ccm.
Shake, and while shaking add of a 10 p.c. solution of caustic potash, .2ccm.

The specimens to be stained are left in this solution for twenty to twenty-four hours, or if kept at the temperature of 40ºC. for half to one hour. Afterwards they are put in a saturated watery solution of vesuvin, which drives the blue out of everything but the tubercle bacilli, and as a result we have blue bacilli and brown surroundings. He states that other aniline dyes except brown, can be used in place of the methylen blue though they are not so good, that other alkalies can be used in place of potash, and that a more strongly alkaline solution, though it spoils the sections, stains bacilli which were not previously apparent. Hence he concludes that the essence of the matter is

alkalinity of the stain. Other bacteria stained in this way become brown except the bacillus of leprosy.

Baumgarten's method of demonstrating the bacillus was quite different from Koch's. He had found that the mycelia of fungi occurring in sections of tissue, though intensely stained by aniline dyes, were concealed by the stained tissues, while on the other hand if everything was treated with dilute alkali, the mycelium threads stood out as unstained bodies. In order to demonstrate the tubercle bacillus, he therefore treats the section with very weak caustic soda or potash. They are best found in sections of recent tubercle which has been hardened for twenty-four hours in absolute alcohol. If the tissue is afterwards stained with an alcoholic solution of safranin, the bacilli stand out as unstained bodies. Ziehl describes Baumgarten's method of treating sputum. He says that Baumgarten first treats it with very weak caustic potash, spreads it on a cover glass, dries it, and passes it two or three times through a gas flame. He then stains it with aniline blue, and everything becomes blue except the tubercle bacilli, which remain colourless.

On May 1st 1882, Professor Ehrlich brought forward his method of staining tubercle bacilli, which is now so well known and has been universally adopted. He also holds that an alkaline solution is necessary to stain the bacilli, but instead of potash or soda he uses a saturated watery solution of aniline, to which he adds a saturated alcoholic solution of any of the basic aniline dyes till precipitation commences. The dyes which he specially recommends are fuchsin, methyl violet, and gentian violet, but he states that he has tried all the basic aniline dyes in this way. The sections after being stained in the solution are then decolorised in nitric acid (one part to two of water), and after being washed in water are put in some contrast stain. The contrast stains generally employed are in the case of the red stain in the first instance, methylen blue, and in the case of the violet stain chrysoidin or vesuvin. By this process he obtains quicker staining, more intense staining of the bacilli, less intense staining of the tissue, and hence easier demonstration than by Koch's original method, while he thinks that possibly, also, more bacilli are stained. If other forms of bacteria are present, they are decolorised by the nitric acid and afterwards stained by the contrast dye in contradistinction to the tubercle bacillus.

Ehrlich holds that the relation of these bacilli to the basic aniline dyes is the same as that of other bacteria, but that the difference depends on the presence of a sheath which can be penetrated by colouring matters under the influence of alkalies, while it becomes impenetrable after the use of acids. Hence he draws the conclusion that disinfective substances must be alkaline.

Weigert has made out the best proportions between the saturated alcoholic solution of the aniline dye and the saturated watery solution of aniline, and thus the difficulty which some have experienced in making Ehrlich's solution has been removed. Weigert's formula is:-
Saturated watery solution of aniline, 100ccm.
Sat. alcoh. sol. Of fuchsin, methyl violet, &c., 11ccm.

The views of Koch and Ehrlich with regard to the necessity of alkalinity of the solution, and the conclusion of Ehrlich as to the presence of a sheath with peculiar properties were upset by the researches of Dr. Franz Ziehl, published on August 12th,

1882. He found that the tubercle bacilli were stained even though acetic acid were added to the solution in sufficient quantity to make it acid. He further found that aniline left blue and red litmus unaltered, but behaved to a weak reddened solution of phenolphthalein like an acid, the colour disappearing. The staining solution made with an aniline which did not act in this way, did not colour the tubercle bacilli. Hence he concluded that the material which gave this reaction was the cause of the success, but he could not tell what it was, though it probably belonged to the aromatic series. He then tried a substance with like reactions in place of the aniline, viz. carbolic acid. This succeeded, though not quite so quickly as the solution made with aniline. He also succeeded with resorcin and pyrogallic acid in place of aniline. From these facts it is evident that the alkaline reaction of the fluid is immaterial.

Ziehl concludes that it is a property of the tubercle bacillus to take up colouring matters only slowly, to hold them in spite of the action of acids and alkalies, and this taking up of the colouring matter is hastened by certain substances, such as aniline, &c.

I have tried a large number of substances which decolorise a reddened solution of phenolphthalein in place of the aniline, but I find that they act very variously. Ordinary aniline contains toluidine as well as aniline, and I obtained the two substances separate and tested them. I found that the absolutely pure aniline (sold by Hopkin and Williams at 5s. an ounce) gave a very brilliant result, better than I had been getting with the ordinary aniline, while toluidine acted very well but hardly so strongly as the ordinary aniline.

I also found that paraniline did very well, but not so well as aniline.

I was able to confirm Ziehl's statements as to carbolic acid which acts almost as satisfactorily as aniline, the difference being hardly perceptible. I found, however, that resorcin and pyrogallic acid did not act well at all.

Nitrobenzole and the alcohols, methylic, propylic, butylic, and amylic, were fairly good, but not nearly so good as aniline.

Phloroglucine acted very well, almost as well as aniline.

Boracic acid acted feebly, but apparently all the bacilli were stained though faintly. This was also the case, though to a less extent, with salicylic acid.

A solution made in Weigert's proportions with water and alcoholic solution of fuchsin, but without aniline, or any substitute for it, washed in nitric acid and stained afterwards in blue, stained a good many bacilli red but not nearly all. It seems to be always the case that a few bacilli hold the fuchsin after washing in nitric acid, even though no aniline or any substitute for it has been employed. Hence I suppose that some of these bacilli differ from others, and that it is probably at a particular stage of growth that they can be stained in this way by fuchsin alone.[1]

All the various substitutes for aniline which I have mentioned decolorise the reddish solution of phenolphthalein with the exception of methylic alcohol, which not only does not decolorise it but intensifies the colour. As methylic alcohol does act to some extent in the same way as aniline and quite as well as the other alcohols of the same group, it is probable that a neutral reaction and the decolorisation of reddened phenolphthalein solution are not essential attributes of a substitute for aniline.

How does the aniline act? It has been supposed that it forms a compound with fuchsin, &c., and that this new stain stains the bacilli. Unfortunately for this theory, when aniline and rosaniline do combine to form a compound the result is blue, not red. And also the fact that such a variety of substances can be substituted for aniline is opposed to this idea. Further, I have evaporated the Ehrlich-Weigert fuchsin solution both in an incubator and also in the cold and afterwards re-dissolved it, with the result that the solution acted like ordinary fuchsin solution without aniline and not like the original fluid. In evaporating the solution the aniline almost entirely disappeared, showing that it had not formed a compound. (Evaporate 20 grs. of aniline and there is not a residue of ¼ grain.)

Nor is it that the presence of aniline is necessary to enable the fuchsin to stain the bacilli, for I have satisfied myself that after staining with fuchsin alone, but before washing with nitric acid, the bacilli are stained.

The fact seems to be that fuchsin or other basic aniline dye alone can stain the bacilli, but that only a few of them, at a certain stage of growth probably, can retain the stain in the presence of strong nitric acid. If aniline or a variety of other substances be, however, added to the staining fluid, the bacilli are then enabled to hold the stain. The aniline seems to act in some way as a fixing agent. I have stained these bacilli first in fuchsin alone and then afterwards immersed them in aniline water, with the result that after washing in nitric acid large numbers of bacilli were seen to be stained, while similar specimens treated with fuchsin alone showed only a few bacilli. The result, however, was not so good as where the anilin and fuchsin acted together.

[1] In testing these points I used crushed tubercles or cheesy glands where no micro-organism but the tubercle bacillus was present.

I have not found any other organism, except the leprosy bacillus, which retains the stain on treatment with nitric acid, but a micrococcus has been met with by Lichtheim which does so. I have, however, seen that if a slide of sputum is immersed in the nitric acid for a very short time only, a torula often retains the colour as well as the tubercle bacillus. A little longer immersion removes the stain from the torula. Psorospermiæ and sometimes the outer coat of some parasites also retain the red stain after washing with nitric acid. Elastic tissue and cheesy matter, the former more especially, retain the red if the sections are only immersed for a short time in the nitric acid.

A great objection to Ehrlich's method is that many sections of delicate tissues, such as thin sections of the eye, shrivel up and become useless after immersion in the strong nitric acid. I have therefore made a number of experiments with the view of getting rid of the nitric acid and the following seems to me the best method.

After staining the section in Ehrlich-Weigert's fuchsin solution wash it in distilled water, immerse it in alcohol for a moment and then place it in the following solution:-
Distilled water, 100ccm.
Saturated alcoholic solution of methylen blue, 20ccm.
Formic acid (pure), 10 min.

The sections are left in this solution from one to two hours and are then treated I the ordinary manner with water, alcohol and oil of cloves.

Glacial acetic acid may be substituted for the formic acid, but it does not act quite so well, and a larger quantity (about 12 minims) must be used.

I do not recommend this method for sputum, because it takes longer than the nitric acid method and does not, I think, possess any advantage over it in the case of dry preparations.

The view that tuberculosis may be caused by the introduction of non-tuberculous substances beneath the skin of rabbits and guinea-pigs has been advanced by a number of observers and is widely held, more especially in this country. As this view is opposed to the recent statements that the cause of tuberculosis is a specific micro-organism, it is impossible to omit mention of this important question.

Among the first to oppose the specific view was Dr. Burdon Sanderson. He says at the end of his first report, in 1867, on the communicability of tubercle by inoculation: "From these facts it is evident that these lesions can no longer be regarded as dependent on any property or action possessed by the inoculated material in virtue of its having been taken from the diseased organs of a phthisical patient. This conclusion is rendered almost certain by the result of the following experiment" (that with the seton to be mentioned immediately).

The facts on which Dr. Burdon Sanderson bases his view are that, besides a number of experiments with tuberculous substances, when he inoculated animals with pus from pyæmic abscesses if the animals survived they became tuberculous, and that he

inserted a seton of unbleached cotton into each of two guinea-pigs, one of which afterwards became tuberculous. (He also inoculated two animals with material from a diseased suprarenal capsule in a case of Addison's disease, but this was probably tuberculous, or at least strumous, for there were traces of old tubercle in the lung. This experiment would not therefore count.)

In his second report Dr. Burdon Sanderson still holds "that in rodent animals the tuberculous process may originate not merely by the inoculation of tubercle, but by any irritation of the requisite intensity applied to the subcutaneous tissue, and that any external injury, provided that the animal survives its immediate effects, is capable of becoming the first link in a chain of pathological changes which cannot be distinguished from those produced by insertion of tuberculous material." In support of his view he does not mention any fresh experiments, but quotes Cohnheim's experiments, in which tuberculosis followed the introduction of cork, gutta-percha and other inert materials into the abdomen of animals. At the end, however, an experiment is mentioned which is different for the former one with the seton, and which seems to support the idea of accidental contamination of the material formerly employed. He says: "As regards the question of a specific contagium of tubercle, we think it is very important to note that this is not as yet disproved by the facts of traumatic tuberculosis. It still remains open to inquiry whether or not injuries which are of such a nature that air is completely excluded from contact with the injured part are capable of originating a tuberculous process." And in support of this it is said, "Setons steeped in carbolic acid were inserted in ten guinea-pigs on September 24th, 1868, each animal receiving two. At the same time extensive fractures of both scapulæ were produced on five others, care being taken not to injure the integuments. No tuberculosis or other disease of internal organs resulted in either case.

About the same time Dr. Wilson Fox published a large number of experiments on the same subject with similar results, and in which the number of cases where the inoculation of non-tuberculous substances was followed by tuberculosis was much greater. I may tabulate the certainly non-tuberculous substances, the inoculation of which was followed by the development of tuberculosis:-

	No. of Experiments	Successful Cases
Putrid muscle	5	4
Seton	4	1
Cotton thread	3	1
Vaccine fluid	4	4
Pus and lymph from rabbit	8	1
Cirrhotic kidney	1	1
Chronic pneumonia	2	2
Foul pus	5	3
Suppuration injury to knee	1	1
Sloughs	3	1
Py æmic abscess of spleen	2	2
Same spleen, unaffected part	2	1
Silver wire	24	3
	64	25

That it was true tubercle that was produced in some cases is shown by the successful reinoculation of the tubercles following inoculation of low pneumonia (two experiments), of pneumonia (rabbit, one experiment), and of putrid muscle (three experiments).

Dr. Wilson Fox states distinctly that none of the animals not operated on became tuberculous. The bronchial glands only escaped eight times out of sixty-four. (Dr. Koch says that enlargement of the bronchial glands implies spontaneous infection.)

The inoculations were done by means of a trocar fitted with a piston, but no mention is made of disinfection of the trocar after each injection.

These views seemed at first to receive great support from the experiments of Cohnheim and Fraenckel,[1] who found in the pathological institution at Berlin that all guinea-pigs into whose abdominal cavities they introduced pieces of cork, paper, cotton threads, &c., became tuberculous. Cohnheim has, however, since completely retracted the conclusions derived from these experiments, because he found that he could not produce tuberculosis in the same way in the pathological laboratories of Kiel and Breslau, and because Fraenckel failed also to reproduce the results when the experiments were done at his own house in Berlin. Cohnheim is now one of the most strenuous supporters of the specific nature of tubercle.

Lebert and Waldenburg also opposed the specific nature of the disease, the latter putting forward the view that it was due to special physical properties of the material introduced, and that tubercle is due to particles taken up by the blood and deposited in the tissue. For the criticism of these works I would refer to Klebs' paper.[2]

In contrast to Tappeiner's results from inhalation of tuberculous matter, a paper was published by Schottelius,[3] who stated that miliary nodules in the lung could be produced not only by inhalation of tuberculous sputum, but also by inhalation of organic material which could undergo fermentation in the bronchi and alveoli, such as non-tuberculous sputum, cheese, &c. It is important to note that in no case did he get general tuberculosis, and that he always speaks of the effects as inflammatory. His results do not in reality contradict Tappeiner, for the latter got in some cases tubercles, not only in the lungs, but in other organs.

Numerous researches have been published which contradict the supposed traumatic origin of tuberculosis, indeed most of the recent writers on tuberculosis mention control experiments with non-tuberculous materials which have yielded entirely negative results. I may give as an example of this sort of work the results obtained by Dr. Karl Salomonsen.[4]

[1] Virchow's *Archiv*. vol. xlv
[2] Virchow's *Archiv*. vol. xliv., 1866
[3] Ibid. vol. lxxiii
[4] Aftryk fra Nord. Med. Arkiv. Bd. 11, 1879.

Salomonsen introduced the substances to be tested into the anterior chamber of the eyes of rabbits, and watched whether or not tuberculosis of the iris developed. By this method there could be no confusion from the occurrence of spontaneous tuberculosis, while different substances could be introduced into each eye and the result watched.

His results are divided into four groups, but only the second interest us where supposed non-tuberculous substances were introduced into the eye.

	No. of experiments	Length of time during which the animal was observed
Periosteum of rabbit	1	6 Weeks
Skin of rabbits	1	6 Weeks
Carcinoma of axillary glands	2	2 Months
Inflammatory induration round ulcer of prepuce	4	2 Months
Myotic tumours from tongue of cow	2	3 Months
Carcinoma of mamma (fatty degeneration)	2	4 Months
Gumma of liver	2	4 Months
Caseous gland from scrofulous child	2	6 Weeks
Chronic panaphthalmitis from rabbit (lasted 6 months)	5	2 Months
Muscle of tuberculous guinea-pig	1	4 Weeks
Testicle of tuberculous guinea-pig	1	4 Weeks
Kidney of tuberculous guinea-pig	1	4 Months
Caseous nodule from lung of a tuberculous guinea-pig, dried at 100º C, for 1½ hours	3	3-4 Months
Caseous nodule from lung of tuberculous guinea-pig, boiled for 6 minutes	3	4½ Months
Caseous nodule from lung of tuberculous guinea-pig, in absolute alcohol for 1¾ hours	2	4-5 Months

In not a single case did tuberculosis iritis or general tuberculosis develop.

In contrast to these experiments he publishes a large number with tuberculous material, all of which were followed by tuberculous iritis and, if kept longer, by general tuberculosis at least of the lungs.

As Baumgarten has also investigated this matter, I may enumerate the materials which he has inoculated without in any case obtaining tuberculosis: - All sorts of organic and inorganic foreign bodies, also chemical materials of various kinds, a great variety of pathological new formations, such as carcinoma, sarcoma, myeloid sarcoma, malignant lymphoma, leucæmic tumours, hard and soft chancres, lupus, typhus, glands, actinomycosis, &c., the products of acute and chronic inflammations, healthy and unhealthy pus, croupus and diphtheritic masses, granulation tissue and scars of different kinds from different situations, cheesy material from various sources, cheesy pus from man and animals, cheesy infarcts, caseous tumours, putrid material in different stages of decomposition, lower organisms of various kinds, gregarinæ, infusoria, cocci and bacteria from various septic fluids, and all kinds of fungi. He states that in some cases injection of bacteric fluid into the eye led to the formation of small tubercle-like masses in internal organs, but these turned out to be small abscesses. In none of the other cases was a single tubercle produced.

As this matter was so important, and as the non-specific view of tuberculosis is still held in England, I have thought it necessary to perform some similar experiments with special precautions. I have taken the greatest care to prevent contamination of the materials used, of the instruments, and of my own hands with tuberculous material. The animals have also been kept under the best possible hygienic conditions.

Experiment I., August 28th, 1882 – Experiments with setons of various kinds:-

1. A seton of cotton thread was put into the back of a black rabbit. This animal remained apparently unaffected. The seton came out at the end of September. The animal was killed November 11th, 1882. Lived seventy-eight days. On post-mortem examination it was found to be well nourished and no disease of any of the organs could be detected. On microscopical examination the lungs, liver, spleen and kidneys were found to be quite healthy.
2. A little bit of the same cotton thread was put into the anterior chamber of the eye of a red and white rabbit. This animal died September 12th, 1882. Lived fifteen days. All the organs were healthy with the exception of the liver, which was seen to be full of the large cheesy masses so common in rabbits. On microscopical examination, November 1st, 1882, all the organs were healthy except the liver. The liver was full of cheesy masses and psorospermiæ. The appearance of the masses was a cheesy indefinite mass surrounded by a definite wall of dense fibrous tissue. These were recognised as due to psorospermeæ by the fact that when sections were stained with the fuchsin aniline solution in the manner described for tubercle bacilli, the psorospermeæ remained red while everything else became blue. (See Fig. 6, Plate I.) There was, therefore, no difficulty in recognising them. I consider this fact with regard to the staining of these bodies very important as a means of diagnosis.
3. A seton of thick worsted thread put into the back of a brown rabbit. My last note with regard to this animal was on October 7th (after forty days), when the

seton was still in, but there was no inflammation or sore about it, no enlarged glands, and the animal was apparently in perfect health. This rabbit was stolen on October 8th.

4. and 5. Setons of the cotton thread used in Nos. 1 and 2 were put into the backs of two guinea-pigs. These setons had come out at the end of September, and the skin-wound was perfectly healed and there were no enlarged glands. The same condition is noted on October 7th. These animals were stolen during October.

6. A seton of worsted thread was put into the back of a white guinea-pig. No sore or enlarged glands followed, and when the animal was killed on November 2nd, 1882 (after sixty-six days, the seton being still in), all the organs were perfectly healthy. On microscopical examination this result was confirmed.

In this experiment all the animals except No. 2 were under observation quite long enough to enable me to determine whether they would or would not become tuberculous as the result of the insertion of the seton. The result was entirely negative, indeed I expected it, for I have in other experiments often sown up wounds in rabbits with cotton thread (I should think about fifty times), and left it in for weeks without in any case obtaining tuberculosis.

Experiment II., August 28th, 1882 – Experiment with vaccine lymph taken from the calf by Dr. Renner on August 26th, 1882.

1 and 2. Small pieces of the thick lymph were put into the anterior chamber of the right eyes of two rabbits. On September 9th, 1882, small opacities were found at the upper part of the corneæ of both rabbits., but the iris was perfectly healthy and the animals were well. On October 7th (forty days after inoculation), the opacities had almost disappeared, there was no iris tuberculosis and the animals were quite well. They were both stolen on October 8th.

3. A seton of cotton thread was soaked in the lymph and put into the back of a black rabbit. This seton came out at the end of September, and the animal was then perfectly well. It was killed on November 14th, 1882. Lived seventy-eight days. There was no sore, no enlarged glands, the animal was fat, and the organs perfectly healthy. On microscopical examination, the healthy state of the organs was confirmed.

4 and 5. Setons of worsted thread were soaked in the vaccine lymph and put into the backs of two guinea-pigs. These animals remained perfectly well, no sore formed at the point where the seton was, and the neighbouring glands did not enlarge. One guinea-pig disappeared during October, and the other was killed on November 2nd, 1882, and the organs were perfectly healthy. This was confirmed on microscopical examination of the lungs, liver, spleen, and kidneys.

Experiment III., August 28th, 1882 – Experiment with human vaccine lymph obtained from Dr. Sumner on August 28th, 1882.

1 and 2. Lymph was introduced into the anterior chamber of the left eyes of two rabbits. On September 22nd, the upper part of the corneæ in both animals was opaque, but there was no appearance of iris tuberculosis, and the animals were apparently well. This was also the condition on October 7th, but the opacities were evidently rapidly disappearing. Both animals were stolen on October 8th. Observed for forty days.

3. A seton of worsted thread was soaked in the lymph and inserted into the back of a rabbit. This animal was also quite well on October 7th, the seton was still present, but was causing no irritation, and the neighbouring glands were not enlarged. Animal stolen on October 8th.
4. Seton of worsted thread soaked in the lymph and introduced into the back of a guinea-pig. This seton did not seem to cause any irritation, and the glands in the neighbourhood did not enlarge. Animal killed on November 2nd, 1882 (lived sixty-six days), and organs found healthy. On microscopical examination of the lungs, liver, spleen and kidneys, the healthy state of the organs was confirmed.
5. Seton of worsted thread soaked in the lymph was inserted into the back of a guinea-pig. This seton came out at the end of September, leaving a sore which soon healed. The neighbouring glands did not enlarge. Animal killed November 12th, 1882. Lived seventy-six days. Nothing was found either macroscopically or microscopically in any of the organs

The result of these two experiments on ten animals is also perfectly definite and negative. It will be remembered that the four cases in which Dr. Wilson Fox inoculated vaccine-lymph were all followed by tuberculosis. Here vaccine lymph was inoculated in ten cases without the production of tuberculosis in a single instance, although all the animals were under observation for a sufficient length of time.

Experiment IV., August 28th, 1882 – Experiment on the introduction of various materials into the abdominal cavity of animals.

1. A piece of cork introduced into the abdominal cavity of a brown rabbit. On September 21st this animal seemed ill, and was found dead on the morning of September 22nd. Lived twenty-five days. There were no definite post-mortem appearances, certainly no tubercles in any of the organs. On microscopical examination, the lungs were in parts engorged with blood and the capillaries ruptured. There were also plugs in the vessels which stained very strongly with gentian violet, but I was unable to resolve them into micro-organisms. In the liver were one or two old cicatrices, but no recent changes, no further appearances to account for death. (The animal had diarrhœa for a day or two before death.)
2. A piece of tubercle that had been in alcohol since June 1st, 1882 (see Experiment XII.), was introduced into the abdomen and left eye of a black rabbit. This animal seemed well in the end of September, but was found dead on the morning of October 2nd. Lived thirty-four days. Heart full of cheesy masses and much enlarged. In the lower lobes of the lungs were a number of nodules, the liver contained one or two small cheesy masses, the spleen was much enlarged, but did not contain any nodules, and the kidneys were apparently healthy. Eye healthy. On microscopical examination, the masses in the heart were seen to consist solely of inflammatory tissue which had in part undergone caseous degeneration: the masses of granulation tissue could be followed among the muscular bundles in a manner radiating from the centre of the inflammation. No epithelioid cells, and no appearance in the least like tubercle. On staining with gentian violet one could see numerous excessively minute granules, which I have no doubt were micrococci from their appearance and arrangement. There were inflammatory masses (indeed regular abscesses) in the lower part of the lungs, with the same micrococci. The

cheesy spots in the liver were evidently remnants of old inflammatory processes. There were no tubercle bacilli.

3. A piece of cork introduced into the abdominal cavity of a guinea-pig. No effect followed. The animal was observed till the end of October, and remained perfectly well. It was killed by a dog on November 1st, 1882.
4. Piece of worsted inserted into the abdominal cavity of a guinea-pig. This animal remained apparently quite well till October 7th. It was stolen on October 8th.
5. and 6. Pieces of hardened tubercle (see No. 2) introduced into the abdominal cavities of two guinea-pigs. One of these was killed on November 2nd, 1882 (lived sixty-six days), but the various organs were found healthy; no trace of the original piece of tubercle. On microscopical examination the lungs, liver, spleen, and kidneys were seen to be perfectly healthy. The other guinea-pig died on December 31st, 1882. Lived 125 days. It had diarrhœa. No disease of any organs could be found. On microscopical examination all the organs were healthy, and there was no appearance of tubercle.

The result of this experiment can only be definitely known from four of the animals. Nos. 3 and 4, though apparently well, might have been ill, and as the material was introduced into the abdominal cavity there were no enlarged glands, sores, or iris tuberculosis to guide us. In the four known cases, however, there was no development of tubercle.

Experiment V., November 7th, 1882 – Experiment with pus from the wound of a patient suffering from pyæmia. The pus was thick and foul smelling.

1. One minim was injected into the left eye of a rabbit. Panophthalmos resulted, and the animal was ill for some time. It, however, gradually recovered, and in December was apparently well. It died on January 10th, 1883. Lived sixty-four days. On post-mortem examination no disease was found anywhere but in the large intestines and cæcum. From the ileocæcal valve, involving the cæcum and extending for about nine inches along the large intestine, were numerous circular whitish patches, about the size of a pea, with greyish points in the centre and projecting somewhat above the level of the peritoneal coat. On laying open the intestine there was over each of these patches a circular ulcer, or rather a hole in the mucous membrane. On microscopical examination, part of the mass was seen to be cheesy and the rest inflammatory. Interspersed throughout were large numbers of psorosperimæ; some of these remained red on staining for tubercle bacilli, others were colourless. There were no tubercle bacilli or other bacteria.
2. Five minims of the pus were injected subcutaneously into the back of a guinea-pig. January 23rd, 1883: This animal was found dead to-day. It had diarrhœa. Lived seventy-seven days. On post-mortem examination, all the organs were seen to be healthy, and there were no tubercles.
3. Four minims of the pus were injected into the abdominal cavity of a guinea-pig. This animal did not seem to suffer anything as the result of the injection, and when examined on January 28th, 1883 (after eighty-two days), it was quite well and strong.

I may state before proceeding to the description of the results obtained in the experiments with micro-organisms that all the organs of the animals that have died or been killed have been carefully examined microscopically for the presence of tubercle bacilli, for the presence of other bacteria, and for the histological appearances. For tubercle bacilli I have used Ehrlich's fuchsin solution made according to Weigert's formula, staining afterwards with methylen blue; for other micro-organisms I have used in addition gentian violet; and for histological structure I have as a rule employed, as well as the foregoing stains, Ehrlich's triple staining, with hæmatoxylin, rubin S., and orange. In speaking of the number of bacilli present in a specimen I have drawn up a scale, reference to which will save much repetition. I say they are in *enormous masses* when there are great masses containing innumerable bacilli; *very numerous* when they are too numerous to count; *numerous*, about forty or fifty in a field; in *moderate numbers*, about twenty or thirty in a field; *few*, two or three in a field; *very few*, only one in two or three fields. These numbers refer to extremely thin sections made with Williams's microtome. Of course, if a section is thick, a comparatively small number of bacilli in each plane wound seem many on focusing.

The tubercle bacilli vary considerably in length, the longest being about 1/7000 of an inch. They are narrow (about 1/5 or 1/6 of their length), more or less rounded at the ends, and they generally present a sort of beaded appearance, clear spots with intermediate stained parts, the rod outside the clear spots being also stained (Plate II., Fig. 10). The number of beads in a single rod varies from four to eight, and is on an average six. The rods are generally straight, but they are not uncommonly more or less curved. In tissue they are generally founds singly, or sometimes in pairs, united at their ends or stuck together side by side. At other times there are two or three lying across each other, the axis of all being more or less in the same direction.

In cultivations they are as a rule shorter, and stuck together in dense masses. Perhaps their shortness is due to their being broken when spread out on the glass, but I think they are really shorter when growing rapidly than when growing slowly. (*Vide* examination of Koch's case of injection into the veins). According to Koch they are motionless.

I may also add that in all these experiments I have used Koch's syringes and observed all the precautions described by him in their purification, &c. The animals constituting each experiment have been kept in the same hutch, which was separated from the others by an interval, and there was no possibility of communication between the animals.

As I have already stated, Prof. Toussaint gave me portions of the organs of various animals illustrating the results of his experiments, and also tubes containing the cultivations themselves. I will now describe the results of the observations which I have made with these materials.

(a) Examination of the organs received:-

1. *July 22nd*, 1882 – A rabbit which was paralysed in its hind legs, and which he was sure would have died to-morrow was killed to-day. The following is the history of the experiment. In December, 1881, the

juice squeezed out from a muscle of a cow affected with tuberculosis was kept for ten minutes at a temperature of 55ºC, and then injected into a pig. This pig died 147 days later with extensive tuberculosis, and a bit of the tuberculous material was inoculated subcutaneously into two rabbits on April 7th. One of these rabbits died two days ago, and this one would have died to-morrow.

At the seat of inoculation there was an ulcer, beneath which were numerous tubercles, the neighbouring glands were enlarged and caseous, and there was very extensive tuberculosis of various organs (lungs, kidneys, spleen, mediastinum, diaphragm, costal pleura), but very little macroscopically in the liver. Portions of several of the organs were at once put into a bottle containing alcohol.

On October 3rd, 1882, I made sections of these organs with the following result:-
Lung – Almost entirely converted into a tuberculous mass, presenting the same appearances as other cases of artificial tuberculosis to be afterwards described, a good deal of caseation, tubercle bacilli present in moderate and considerable numbers, having all the characters of those described by Koch. No other micro-organisms.
Kidney – Large caseating tuberculous masses in the cortical part. Tubercle bacilli in considerable numbers in some parts and numerous in others. No other micro-organisms.
Spleen – A considerable number of caseating tuberculous masses. In some of these the tubercle bacilli are very numerous. No other micro-organisms.
Lymphatic gland – Almost entirely caseous, and containing numerous tubercle bacilli. No other micro-organisms.

2. Portion of kidney from a rabbit inoculated on December 30th, 1881, and died on March 31st, 1882. In this case some of the tenth cultivation of micrococci from the blood of a rabbit which had been inoculated with tuberculous material from a cow, was injected at the base of the right ear. The kidney and other organs were extensively tuberculous. January 20th, 1883 – The kidney was badly preserved, and stained badly, so that the histological structure could hardly be made out. There were large tuberculous masses in the cortex and the tubercle bacilli, which were as a rule numerous in these masses, stained very well. No other mocro-organisms.
3. Piece of lung from a rabbit inoculated on November 29th, 1881, with the fifteenth and sixteenth cultivations from a tuberculous rabbit, and later on, on February 11th, 1882, with the twenty-first and twenty-second cultivations of the same series. This animal died on April 19th, 1882, and extensive tuberculosis of the various organs was found.

January 20th, 1883 – The lung was extensively infiltrated with tubercles, presenting the usual appearances to be afterwards described. Tubercle bacilli were found in some places in enormous numbers. Epithelioid cells in alveoli and bacilli in the cells (see after). In one or

two places there were very interesting appearances from the point of view of the spread of the disease. In one large blood-vessel there was a mass of cells lining the wall and projecting in one part into the calibre of the vessel though not obliterating it entirely. In this mass there were numbers of tubercle bacilli, there were also some in the wall of the vessel)Plate I., Fig. 1). In two other places there were numbers of bacilli in the wall of vessels (apparently arteries) penetrating quite to the inner coat. No other organisms.

4. Organs of a cat with the following history. A pig ate part of the organs of a cow affected with tuberculosis, and became tuberculous. A drop of the blood of this pig was put into a vessel containing rabbit infusion, and micrococci developed. Ten drops of the seventh cultivation of these micrococci were injected into the peritoneal cavity of this cat, which died 109 days later with enlarged abdominal glands, and tubercles in liver, spleen, lungs. The tubercles were not large, because, according to Toussaint, the enlarged glands caused the death of the animal by arresting the flow of lymph before there was time for the tubercles to grow to a large size.
   October 3rd, 1882 – Examination of organs received:-
   Lung – Typical tuberculosis generally in considerable masses, with here and there slight caseation, a good many giant cells. Tubercle bacilli present in moderate numbers, and several seen also in the giant cells. No other micro-organisms.
   Liver – Several tubercles in the lobules, but not very numerous, commencing caseation. Tubercle bacilli present in moderate numbers. No other micro-organisms.
   Spleen – Only a few tubercles. Tubercle bacilli present, but very few. The spleen did not stain very well. No other micro-organisms.
   Lymphatic gland – For the most part caseous. The tubercle bacilli are in many places very numerous. No other micro-organisms.
5. Lung of a pig with following history. A portion of the tuberculous material from the cat just mentioned was inoculated into a rabbit, and caused tuberculosis. From the tubercles in the rabbit this pig was inoculated, and it died sixty-seven days afterwards.
   October 3rd, 1882 – The tubercles in the lung are still for the most part very small, but their structure does not seem to differ in any way from that of tubercle in the lung of rabbits. Tubercle bacilli are present in moderate numbers. In a number of very thin sections of small tubercles I found an average of nine bacilli in each. No other micro-organisms.

(b) I also received from Toussaint two tubes containing the micrococci with which he had been working, and with these the following experiments were done. Before describing these I may state that when I opened each tube I dried a small quantity of the fluid on cover-glasses, and stained it both by the ordinary methods for staining bacteria, and also by the methods for demonstrating tubercle bacilli. I did not find tubercle bacilli in either of the tubes, and they seemed to contain nothing but micrococci, very minute, but staining well with gentian violet, vesuvin, and methylen blue, like other forms of micrococci. They were chiefly in large groups, but there were also a few pairs, threes and fours, grouped in the manner characteristic of micrococci (see

Fig. 5, Plate I.). There were a few bodies more ovalish, and somewhat larger, which might be small bacteria.

No. I – A tube of serum containing micrococci. This was the fourteenth cultivation from the blood of a tuberculous rabbit, and the flask was inoculated in January, 1882. Toussaint said that the micrococci would be still alive and active, and he cited in proof thereof the fact that the cultivation from which the cat, whose organs I have referred to above, was inoculated, was six months old. The fluid was clear, and there was a thick, white deposit at the bottom which, when shaken up, made the fluid muddy, and was composed of the micrococci.

August 29th, 1882 – Further cultivation from this tube. The tube was opened, as great care as possible being taken to prevent contamination, and six tubes containing solidified sheep's serum, and three flasks containing meat infusion, were inoculated and placed in an incubator kept at 37ºC.

Development occurred in all the serum tubes very rapidly. They stood in the incubator till September 21st, when I removed them, and allowed them to stand at the ordinary temperature. They were carefully examined on September 27th, with the following result. They were numbered 1 to 6. In tubes 1 to 5 inclusive the surface of the serum was covered by a thin growth consisting of numerous little gelatinous masses, which had more or less run together, but did not penetrate the surface of the serum nor affect its solidity.
No. 1 – Contained micrococci and a few ovalish bodies.
No. 2 – Micrococci apparently quite pure.
No. 3 – Micrococci, and also very short oval bacteria, and a few longer rods.
No. 4 – Micrococci alone.
No. 5 – Micrococci alone.
No. 6 – Long delicate bacilli with spores. The serum had become fluid on the surface. This was probably accidental contamination during the re-inoculation.

Nothing developed in the flasks of meat infusion.

Experiment VI., August 29th, 1882 – Injection of the contents of this tube into animals. About a drop was injected into the anterior chamber of the left eyes of each of three rabbits, and about three drops into the abdominal cavity of each of two guinea-pigs.

September 22nd, 1882 – The eyes of the three rabbits are all right. No iris tuberculosis, and only small opacities of the cornea where the needle was inserted. Both the guinea-pigs are well.

October 8th, 1882 – The opacities in the eyes of the rabbits were smaller and hardly perceptible. The eyes were carefully examined and were seen to be perfectly normal. The guinea-pigs were well and fat.

On October 9th the three rabbits and one guinea-pig were stolen.

November 2nd, 1882 – The remaining guinea-pig was killed to-day (lived sixty-five days). It was quite fat, and macroscopically there was no evidence of disease in any of the organs.

A later microscopical examination of the lungs, liver, spleen, and kidneys, showed them to be perfectly healthy. No trace of tubercle, either recent or old.

The result of this experiment is quite definite. In none of the animals was tuberculosis produced, though all were under observation for a sufficient length of time (the three rabbits and one guinea-pig for forty days, and one guinea-pig for sixty-five days).

Experiment VII., September 30th, 1882 – Inoculation of the organisms grown on the solidified blood serum.

The growth covering the surface of the serum was in the case of each tube scraped off by means of platinum wires purified by heat every time they were used. This material was then mixed up thoroughly with a small quantity of boiled distilled water, and injected into the animals by means of a purified syringe – a fresh syringe being used for each injection.

No. 1 – A rabbit was inoculated from tube 1 into the right eye, and nine minims were injected into its back. On October 8th the cornea was seen to be somewhat opaque, but the animal seemed well.

October 15th, 1882 – Animal died to-day (lived fifteen days). Cornea opaque, no tuberculosis of the iris. No trace of the point of injection in the back. A few cheesy masses in liver. Other organs healthy.

October 27th, 1882 – Organs examined. No tubercles or tubercular bacilli in any organ. A few old cheesy masses in the liver. No bacteria of any kind.

No. 2 – A rabbit was inoculated from tube 2 into the left eye, and eight minims were injected into the back. This animal died on October 2nd (lived three days). No special post-mortem appearances, and nothing morbid was found on microscopical examination of the organs.

No. 3 – A rabbit was inoculated from tube 3 into the right eye, and six minims were injected into its back. On October 8th the animal seemed well, and there was only a little opacity of the cornea at the point of inoculation. It was stolen on October 9th.

No. 4 – A rabbit was inoculated from tube 4 into the left eye, and five minims were injected into its back. It died on October 2nd (lived three days). No special post-mortem appearances and nothing was found on microscopical examination.

No. 5 – A rabbit was inoculated from tube 5 into the right eye and six minims were injected into its back. Died October 8th (lived nine days). Nothing found on post-mortem or microscopical examination.

No. 6 – A rabbit was inoculated from tube 6 into the left eye, and seven minims were injected into its back. Died on October 2nd (lived three days). Nothing found on post-mortem or microscopical examination.

No. 7 – Ten minims of a mixture of the organisms from all six flasks were injected into the abdominal cavity of a guinea-pig. This animal was examined at various dates and seemed always to be in good health. It was found dead on the morning of December 6th (lived sixty-seven days). On post-mortem examination the organs were found to be perfectly healthy, and no trace of tubercles, and this was confirmed on careful microscopical examination.

The result of this experiment can only be told from No. 7, in which a mixture of the growth from all the tubes failed to produce tuberculosis. The rabbits were a very young brood – too young for purposes of experiment. I did not ascertain this, however, till everything was prepared, and it was too late to delay the experiment. I do not think that the organisms injected had anything to do with the death of the animals.

No. II – A tube of serum containing micrococci. This was the twenty-third cultivation from the blood of a pig, and this flask was inoculated in February, 1882.

Experiment VIII, October 29th, 1882 – The contents of the tube were well shaken up, and the whole as far as possible (about four minims) was injected into the abdominal cavity of a young cat. This animal has been under observation ever since, and is, and has been, perfectly well.

At the same time about a drop of the same fluid was injected into the back of a mouse. This mouse died on November 16th (lived eighteen days), but no tubercles were found on post-mortem or microscopical examination.

The injection of the cat was done at the special request of Prof. Toussaint, who said that he had found the result of the injection of these micrococci more successful in cats than in rabbits. The result was, however, negative.

October 29th, 1882 – Cultivation of these micrococci.
Four solidified serum tubes and two tubes containing gelatinised meat infusion were inoculated from this tube. The serum tubes were put in the incubator. They were numbered 1 to 4.
Examined on November 16th, 1882.
No. 1 – Contains micrococci and long spore-bearing bacilli.
No. 2 – Contains mixrococci.
No. 3 – Contains short bacilli.
No. 4 – Contains micrococci.
In one gelatine tube micrococci alone developed; in the other micrococci and long bacilli.

Experiment IX., November 7th, 1882 – Inoculation of the growth in these tubes.

(a) Growth scraped off the surface of the serum in tube 1, and rubbed up with boiled distilled water. About two drops injected into the left eye of a rabbit, and eight minims into the abdomen of a guinea-pig.
(b) A piece of the growth from tube 2 put into the left eye of a rabbit, by means of a purified wire passed through an incision in the cornea.
(c) Growth from all four tubes mixed together, rubbed up with boiled distilled water, and two drops injected into the right eye of a rabbit, and eight minims into the abdomen of a guinea-pig.

November 12th, 1882 – All the animals well. In all the rabbits there was a little lymph in the pupil, and partial opacity of the cornea.

November 23rd, 1882 – All the animals well. No inflammation about the eyes of the rabbits, and the opacities of the cornea have almost gone. Traumatic cataract in *b*. No tubercular iritis.

December 10th, 1882 – All well.

December 28th, 1882 – All well; but one guinea-pig was missing this morning. It was apparently quite well (under observation fifty-one days).

January 28th, 1883 – The three rabbits and one guinea-pig are apparently quite well. There are now no opacities in the corneæ of the rabbits.

February 8th, 1883 – One rabbit killed. All the organs perfectly healthy. Other animals well.

The result of this experiment is that five animals inoculated with the organisms cultivated from the second of Toussaint's tubes remained well without the development of tubercle.

Dr. Koch gave me portions of the organs of several animals for further examination, and also cultivations of bacilli for experimental purposes. While in Berlin I made sections of a considerable amount of the material in his laboratory; the result of this examination will be referred to later.

(a) Examination of the organs obtained from Dr. Koch. I may state that I will refer in detail at the end of the histology of tubercle as elucidated by these researches, and shall therefore not describe the points in giving the results of the examination of each animal.
    1. Organs of rabbit, No. 2, p. 7 [p.250 in original], inoculated into eye with cultivation from a spontaneous monkey tuberculosis. Lived twenty-six days.

    Eye – Iris converted into a diffuse tuberculous mass, and containing a considerable number of tubercles. There were numerous tubercles in the lungs, liver, and spleen, those in the liver being still quite young and exhibiting very well the structure of young tubercle to be afterwards

described. In the lung the tubercle bacilli were numerous and very numerous. In the liver they were also numerous. In the spleen they were few.

2. Lung of rabbit, No. 4, p. 7 [p. 250 in original]. Inoculated in the same way as the former. Lived thirty days.

Numerous tubercles throughout the lung. Not large. No caseation. Only a few bacilli. (Plate II. Fig. 13). No other micro-organisms.

3. Guinea-pig which had a cultivation of bacilli injected into its thigh six months previously; was killed on August 1st. It looked fat and well, but had tubercles in the lungs and spleen, not in other organs. It was apparently recovering. Tubercles from the lungs were crushed and examined at the time, but no bacilli were seen.

January 4th, 1883 – *Spleen* examined. Contains a few tubercles but no caseation. Only very few bacilli.

4. About Christmas 1881, a syringeful of a cultivation of bacilli was injected into the abdominal cavities of two dogs. One of these became very ill and was killed four weeks later, when tuberculosis of various organs, more especially the abdominal organs, was found. The other dog was ill for a time but got better. At the beginning of June, 1882 two syringefuls of cultivation of bacilli (seventh cultivation from a man, the last cultivation having been done six months previously) was injected into its abdominal cavity. The animal became ill, got steadily thinner, and when killed (August 7th, 1882) it was very emaciated and hardly able to move about. Killed August 7th. On post-mortem examination there was found ascites, well marked tuberculosis of the abdominal wall somewhat resembling perlsucht, matting together of the mesentery, thickening of the omentum with tubercles in it, tuberculosis of liver, kidneys, peritoneum, and lungs, but less advanced in the lungs than in the liver. There was also an ulcer on the skin at the seat of injection affecting the skin only and not communicating with the abdominal cavity. A nodule from the liver, squeezed between two cover-glasses, showed tubercle bacilli. Animal lived eight weeks.

January 4th, 1883 – *Liver* examined. The tissue stains badly, but the bacilli stand out beautifully. The capsule is slightly thickened with little nodules in parts. Everywhere throughout the capsule the bacilli are numerous, and are often growing in little clusters. The liver is full of tubercle, the masses being as a rule continuous with each other and penetrating in all directions among the liver cells, but definitely marked off from them. There are enormous numbers of bacilli which are generally in twos and threes, side by side, or in larger masses or in circlets. They seem to have spread in from the capsule along the tracts of connective tissue. In one part the tuberculous tract was traced right across the liver with dilations at intervals. The bile-ducts were inclosed in the centre of the masses. The bacilli were mainly in epithelioid cells, and a few were seen

in liver cells at a little distance from the masses. Some of these might however have been in cells in the capillaries.

Lung – Contained a large number of tubercles and numerous bacilli. The bacilli were contained almost entirely in epithelioid cells sometimes 6-10 in one cell. These epithelioid cells seemed to be derived from the alveolar epithelium, and in one bronchus where the epithelium was in the main intact, I found numerous bacilli in the mucous lining it. No cavities in the lungs, and no caseation.

5. The rabbit which received the injection into the vein of the ear on August 2nd, killed August 10th. Lived eight days (See *a* p. 6 [p. 249 in original].

January 23rd, 1883 – *Lungs* examined. A large number of small tubercles in the lungs. Bacilli as a rule numerous in the nodules. They are shorter than usual and are often united together in dense masses. They present the appearances seen on examination of cultivations rather than those seen in the tissues. In parts, however, where they are growing in the epithelioid cells, and not in the blood-vessels, they present the normal appearance.

The spleen and kidneys were apparently perfectly normal. In the liver, however, I saw one or two minute plugs in the capillaries of the liver consisting of five or six white blood corpuscles with a few bacilli. I also saw in one section one or two bacilli at the margin of and probably inside the liver cells.

6. Perlsucht nodule from a cow. Well developed fibrous tissue and numerous giant cells, also a considerable tract of caseous material. The bacilli were very numerous in all the giant cells and also in the caseous material where radiating groups of them could often be seen, looking as if they had previously been in the interior of a giant cell which had disappeared leaving the bacilli. A few bacilli were also scattered through the fibrous tissue.

7. Tuberculous nodule from a horse. The histological appearances and the number of bacilli were identical to No. 6, except that giant cells were more numerous.

(b) I also received from Dr. Koch several tubes containing bacilli growing on blood-serum. These have been carefully examined microscopically, and nothing but tubercle bacilli have been found; no other micro-organisms.

No. I. – A tube of solidified serum containing tubercle bacilli. This was the ninth cultivation from the lung of a man who died of phthisis on January 23rd, 1882, and this tube was inoculated on July 25th, 1882.

Experiment X, August 28th, 1882 – The growth was scraped off the surface of the serum and rubbed up in a pure mortar with boiled distilled water. The following injections were then made, a purified syringe being employed for each experiment: -

(a) One or two drops injected into the anterior chamber of the left eye of a black rabbit.

September 23rd, 1882 – This rabbit has well-marked tuberculosis of the iris and is thinner.
October 8th, 1882 – The cornea has now become opaque, and the iris can no longer be seen.
November 12th, 1882 – Large cheesy mass projecting from the eye, more especially at the seat of inoculation. Animal rather thin, but not apparently ill.
November 26th, 1882 – Animal killed to-day. Lived ninety days. Left eye converted into caseous mass. (For detailed description of the eye in this and all the following cases, see Mr. Jennings Milles' report, which will appear later). Slight enlargement of the glands in the neck, chiefly on left side. No caseation. Lung full of tubercular masses, more especially at the bases where these masses are confluent. All the masses have commencing caseation in the centre. No cavities in the lungs. Bronchial glands not enlarged. No other organs apparently affected.
December 31st, 1882 – Examined microscopically. *Lungs* full of tubercular masses which, however, contain few bacilli. No other micro-organisms.
Liver, spleen, and kidneys healthy.

(b) One or two drops injected into the left eye of a brown and white rabbit.
September 23rd, 1882 – Left eye completely converted into a caseous mass. Animal thin. Killed September 30th, 1882. Lived thirty-three days.

On microscopical examination the *liver*, *spleen*, and *kidneys* were found to be healthy. In the *lungs* I found in several sections only two small tubercles. These contained very few bacilli which were only in epithelioid cells apparently in alveoli. The tubercles were in immediate contact with masses of lymphatic tissue, one being close to a bronchus, and the other close to a large vessel.

Mr. Milles found the eye converted into a tuberculous mass, containing few tubercle bacilli, and also another form of bacilli staining blue.

(c) The point of the needle of the syringe was introduced into the left eye of a brown rabbit, but the piston was not moved.
September 9th, 1882, died. Lived twelve days.
There was no immediate appearance of tubercle of the iris, and on examination of the various organs microscopically, they were found healthy with the exception of the liver, which was full of gangrinæ.

(d) Nine drops of the mixture of cultivation and boiled distilled water were injected into the abdominal cavity of a white guinea-pig.

This animal died September 15th, 1882. Lived eighteen days. Lumbar lymphatic glands enlarged, and some of them caseous. Tubercles in liver, spleen, and lung; none in kidney.

Liver examined on October 24, 1882. There were tubercles in the substance of the liver, of all sizes and in all stages of growth. The bacilli were few or in moderate numbers, on an average four to six, in a thin section of a tubercle. The liver cells

could be distinctly traced into the interior of the tubercles in the lobules of the liver, and the bacilli were generally in these cells. I also found them in several places, apparently in liver cells where no tubercle yet existed, but, as a rule, the nucleus of these cells had subdivided, and a commencing tubercle was apparent. I also saw several bacilli, apparently in intercellular spaces, close to the surface of the capsule.
Lung – In the lung the tubercles were not so numerous nor so far advanced. The bacilli were few in number.
Spleen – In the spleen there were a good many tubercles and a considerable number of bacilli.
Glands – More or less completely converted into tuberculous masses, with caseation here and there. Numerous bacilli.

Experiment XI., October 30th, 1882 – Portion of cheesy material from the eye of rabbit *b*, Experiment X., inoculated under the skin of the axillary region of a guinea-pig, and a piece of lung containing what I thought might be a tubercle introduced under the skin of the abdomen.

A tuberculous sore formed in the axillary region, and the axillary glands became enlarged. I had intended to kill the animal about the middle of December, as it was getting thin and evidently tuberculous, but it disappeared on December 8th.

Other two animals were inoculated at the same time, but one (a rabbit of the same brood as Experiment III) died three days later, and the other, a guinea-pig, was stolen on October 8th.

The result in the one case showed that the tuberculous material in the eye could produce tuberculosis when re-inoculated.

Experiment XII., November 28th, 1882 – Inoculation of animals with tubercles from the lungs of the black rabbit (*a*, Experiment X.).
A rabbit had portions of the tubercles put into the eyes. The result is that the animal has now (January 28th, 1883) tuberculous iritis, and apparently general tuberculosis, but as the experiment has reference to points not alluded to in the present report, I will not give the details of it in the meantime.

A guinea-pig was inoculated subcutaneously in the groin with tubercle from the lungs. There is now (January 28th, 1883) a tuberculous sore and enlarged glands in the groin, and the animal is getting thin.

February 12th, 1883 – This animal died to-day. Tuberculous sore, tuberculous glands, tubercle in lungs, liver, spleen, intestines, and kidneys.

Another guinea-pig and two rabbits were also inoculated at the same time, but these died in two, eight, and fourteen days respectively, before there was time for the development of tubercle. In the animal which lived fourteen days there was apparently commencing tuberculosis of the iris in the neighbourhood of the piece of tissue inoculated, but it has not yet been examined microscopically.

This experiment likewise shows that the tuberculosis induced by the injection of bacilli in Experiment X. was re-inoculable in the same manner as tubercle produced by the inoculation of tuberculous material.

No. II. – A tube of solidified-serum containing the same bacilli as No. 1. (the ninth cultivation from the lung of a man who died on January 23rd, 1882).

Experiment XIII., November 2nd, 1882 – Growth scraped off surface of serum and rubbed up with boiled distilled water as usual. A little of the pure material injected into the right eye of three rabbits. The piston in these syringes did not fit tightly, and almost no fluid was injected. Into the left eye the following material was injected:-

Into No. 1 – One part of the fluid containing bacilli mixed with one part of 1 to 1000 watery solution of bichloride of mercury. The bichloride of mercury solution was allowed to act for eight minutes before the mixture was injected.

No. 2 – One part of the fluid containing bacilli and one part of a one per cent. solution of resorcin in water. The resorcin solution acted for nine minutes before injection.

No. 3 – One part of the fluid containing bacilli and one part of a five per cent. watery solution of carbolic acid. The carbolic acid solution acted for twelve minutes before injection.

Result in No. 1 – This animal was killed on December 15th, 1882. Lived forty-three days. Up till a fortnight previously there was no appearance of tuberculous iritis in either eye, but when killed it was found that the left iris was tuberculous, the right eye being buphthalmic but not tuberculous. In the left eye the tuberculosis had apparently developed from the neighbourhood of the point of inoculation; the upper part of the iris was completely infiltrated with tuberculous material, and there were nodules round about; the lower part of the iris was almost free.

There were a few tubercles in the lungs, but the other organs were apparently healthy.

January 10th, 1883 – On microscopical examination all the organs except the lungs were found to be healthy. In the lungs there were a few tubercles, and these were very young. They only contained a few bacilli, and these were always in epithelioid cells. These cells were apparently derived from the alveolar epithelium, filled up the alveoli, and there was also increase of the inter-alveolar tissue.
Mr. Milles reports tubercles in the left eye. Tubercle bacilli few.

Result in No. 2 – On November 23rd, 1882, there was tubercular iritis in the right eye, but the left was still clear. On December 10th, 1882, the cornea of the right eye had become opaque. Iris could not be seen. Rabbit looked ill. There was tubercular iritis in the left eye.

January 7th, 1883 – Animal died to-day. Lived sixty-six days. Both eyes highly tuberculous. Cornea opaque and protruding, and neither iris could be seen. Cheesy glands under the jaw. Lungs excessively tuberculous; tubercle of kidneys, but not large or numerous. Apparently none in the spleen or other organs.

January 21st, 1883 – Microscopical examination. *Lung* very tuberculous; numerous spots of caseation. Few bacilli. In one or two places at the margin of cheesy matter there are a good many bacilli, but as a rule they are few. A few commencing tubercles in the *liver* with few bacilli. Tubercles in *spleen*, but few bacilli. Also tubercles in the cortical part of the *kidney*. Here the bacilli were rather more numerous than in the other organs, and some were seen among masses of cells in the kidney tubercles.

Mr. Milles found tubercles in both eyes. Tubercle bacilli few.

Result in No. 3 – Tubercular iritis developed in both eyes, but considerably later in the left than in the right (about twelve days). Examined on January 28th, 1883 (after eighty-seven days); both eyes now converted into tuberculous masses. Animal thin and undoubtedly tuberculous.

February 2nd, 1883 – Rabbit died. Extensive tuberculosis of lung. Tubercles in spleen and kidneys. Also a few in the liver.
Microscopical examination – *Lungs*; tuberculous masses with bacilli in parts in masses, and in parts in moderate numbers. *Spleen*; large and small tubercles. Bacilli numerous and very numerous in the cheesy parts, in the young tubercles in moderate or considerable numbers, and there in the epithelioid cells. *Liver*; numerous small tubercles containing bacilli in moderate or considerable numbers. *Kidney*; large and small tubercles in the cortical part containing bacilli in considerable numbers and numerous.

No. 4 – Three or four minims of the fluid containing bacilli were injected into the abdominal cavity of a guinea-pig, and the right eye was also inoculated. Tubercular iritis developed in the eye. On January 28th, 1883, eye completely tuberculous. Tuberculous sore on abdomen at seat of injection, and the neighbouring lymphatic glands greatly enlarged. Animal thin.

February 18th, 1882 – Killed to-day. Tubercles in lungs, liver, and spleen.

No. III – Tube of serum containing tubercle bacilli. This was the fifth cultivation from a case of perlsucht which died March 20th, 1882. The inoculation was made from a nodule on the diaphragm.

Experiment XIV., November 2nd, 1882 – The bacilli were rubbed up with boiled distilled water as usual. A little of the pure material was injected into the right eyes of three rabbits. Into the left eyes the following materials were injected:-

No. 1 – One part of this fluid containing bacilli was mixed with one part of a 1 per 1000 watery solution of bichloride of mercury. This mixture was allowed to stand for twelve minutes, and then injected into the left eye of No. 1.

No. 2 – One part of the fluid containing bacilli was mixed with one part of a one per cent. watery solution of resorcin. This stood fourteen minutes, and was then injected into the left eye of No. 2.

No. 3 – One part of the fluid containing bacilli was mixed with one part of a five per cent. watery solution of carbolic acid. This stood fifteen minutes, and was then injected into the left eye of No. 3.

Result in No. 1 - On November 23rd, 1882, it was found that there was a well-developed tubercular iritis in the right eye, but apparently nothing in the left. On December 10th, 1882, the left eye was beginning to show appearances of tubercular iritis; the right eye had become converted into a caseous mass. This animal died on January 7th, 1883. Lived sixty-six days. Right eye highly tuberculous, and converted into a caseous mass. . In left eye tubercular iritis, but cornea still clear. Cheesy glands on each side below the jaw. Very extensive tuberculosis of the lungs. Tubercles in liver, kidneys, and spleen. Tubercles in large intestine and omentum. Enlarged and caseous mesenteric and lumbar glands. Tubercle of bones of the skull, but no tubercle in the brain or meninges.

Microscopical appearances: January 21st, 1883:-
Lung – Full of tubercular masses leaving very few healthy alveoli. These masses contain enormous numbers of bacilli. There is not much caseation. At the margin of the confluent masses, and throughout the lung generally, the alveoli are filled with epithelioid cells and contain numerous bacilli. (Plate II, Fig. 12). In one place part of the wall of a bronchus has become tuberculous, and the bacilli are seen growing luxuriantly in the epithelium lining it. In many of the alveoli around, the epithelioid cells are swollen and generally contain bacilli, and in many parts of the lung bacilli are seen in alveolar epithelium which is apparently almost healthy.

Kidney – Contains several large tubercular masses in the cortical part which are full of bacilli. No tubercles in the medullary part. At the margin of the masses the bacilli are seen in the kidney tubules both in the canal and in the epithelium (Plate II., Fig. 9), and in one case I found a small cluster of bacilli in the interior of a tubule at a considerable distance from any cheesy mass.

Liver – Here the process is not nearly so advanced as in the other organs. There are only small tubercles in the shape of small nodules containing epithelioid cells and numerous bacilli. On looking over the sections many liver cells are seen to contain bacilli where no tubercle yet exists. Some bacilli are, I think, also in the capillaries, but it is difficult to determine whether they are in capillaries or in the adjacent liver cells, they lie so close to the edge of the cells.

Spleen – Contained tubercles and numerous bacilli.

Intestine – There are nodules in the mucous tissue containing numerous bacilli. The tuberculous masses extend around the intestinal glands, but the bacilli do not seem to grow in the glandular epithelium. I only once saw bacilli in the interior of a gland duct.

Mr. Milles found tubercles in both eyes containing tubercle bacilli in enormous numbers.

Result in No. 2 – This animal was killed on November 26th, 1882. Lived twenty-four days. Tubercular iritis in both eyes, but most advanced in the right in which the cornea is becoming opaque. Nothing seen in the organs.

On microscopical examination of the organs they were found to be healthy.

Mr. Milles found tubercles in both eyes containing large numbers of bacilli.

Result in No. 3 – This animal was killed on December 15th, 1882. Lived forty-three days. Both eyes very tuberculous, but right most advanced. (Tubercular iritis appeared about a week earlier in the right than in the left eye.) Cornea vascular in both except at the centre, and the pupils cannot be seen. A considerable number of minute tubercles in both lungs, but only one or two with commencing caseation. A few tubercles in the liver and also in the spleen which was enlarged. Other organs healthy.

Microscopical examination on January 15th, 1883, showed a few tubercles in *lungs*, *liver,* and *spleen*. They were fewest in the liver, where only one or two were seen. In the *lung* they were present in considerable numbers, and showed the ordinary appearances of tubercles. The bacilli were found only in the epithelioid cells, and were numerous in all the tubercles. In one alveolus there was a large giant cell filling it up entirely and containing bacilli. (Plate I., Fig. 7.)

About three minims of the fluid containing bacilli were injected under the skin of the abdomen of a guinea-pig. This animal was killed December 21st, 1882. Lived forty-nine days.

A sore was found at the seat of puncture, and a cheesy patch in the neighbourhood. There were large cheesy glands in the left groin, and smaller glands, not cheesy, in the right groin. There were one or two grey points in the spleen. Nothing apparently in the liver or other organs. Calcareous and cheesy glands below the stomach.

January 27th, 1883 – Examined microscopically; the organs were found to be healthy. The inguinal glands were tuberculous and cheesy, and contained bacilli in moderate numbers.

Two minims were injected into the abdominal cavity of a guinea-pig. Animal still alive on January 28th, 1883. On examination a large tuberculous sore and enlargement of the neighbouring lymphatic glands were found.

February 7th, 1883 – This guinea-pig was killed and there were found a tuberculous sore at the seat of injection, enlarged and cheesy inguinal glands, extensive tuberculosis of spleen and liver, a good many tubercles in the lungs.

I have inoculated a number of animals with tuberculous material chiefly with the view of obtaining tubercles for cultivation purposes; but as these experiments illustrate the constant presence of bacilli in tubercle, I may mention them here.

Experiment XV., April 9th, 1882 – A guinea-pig was inoculated subcutaneously with a piece of diseased synovial membrane from the amputated knee-joint of a patient who had strumous disease of the knee and phthisis.

June 1st, 1882 – This animal was killed, and the spleen, lungs, and liver were tuberculous. On microscopical examination these organs were found to contain tubercles and tubercle bacilli in considerable numbers.

Experiment XVI., June 17th, 1882. Two guinea-pigs were inoculated subcutaneously with portions of a tuberculous lung (Mrs. M.'s) which contained a considerable number of bacilli. One of these died August 12th, 1882, during my absence, and was not examined. The other died August 16th, 1882, and was found to have a tuberculous sore on the back, enlarged axillary glands and tubercles in liver, lungs and spleen.

September 27th, 1882 – Examined microscopically. A number of large and small tubercles in the *lung* containing a considerable number of bacilli. Several tubercles in the *spleen* with few bacilli, and also young tubercles in the liver with few bacilli. In the liver there were appearances which indicated that those liver cells which contained bacilli might develop into giant cells.

Experiment XVII., August 17th, 1882 – A portion of the spleen of this guinea-pig was inoculated subcutaneously into a rabbit. An abscess had been induced on the side of this rabbit in February, 1882, by means which need not now be detailed, as they belong to a series of unpublished experiments on another subject. This abscess burst, and there remained a cavity containing a cheesy mass. This cheesy mass still remained when the animal was inoculated with tubercle, but the rabbit was apparently in perfect health. This animal died on October 25th, 1882. Lived sixty-nine days. On post-mortem examination there was a small ulcer at the point of inoculation and the neighbouring glands were cheesy. There were numerous small, cheesy nodules in the lungs. Other organs apparently healthy.

December 21st, 1882 – On microscopical examination, small tubercles were found in the lungs, and also largish masses with commencing caseation. Tubercle bacilli few. Other organs healthy.

Experiment XVIII., August 28th, 1882 – (a) Phthisical sputum containing a moderate number of tubercle bacilli was injected subcutaneously into a rabbit and a guinea-pig.

The result with regard to the rabbit was, that on September 23rd, 1882, a sore was found at the seat of injection, and a cord extending down towards the inguinal region. Inguinal glands on that side enlarged. On October 7th the same condition was found, and the animal was getting thin. The rabbit was stolen on October 8th.

In the case of the guinea-pig, a sore formed at the seat of inoculation, and the glands in the neighbouring inguinal region became enlarged. The animal was killed on December 2nd, 1882. Lived ninety-six days. The left inguinal glands and the lumbar glands were enlarged. There was extensive tuberculosis of the spleen, liver, mesentery, and lungs. The kidneys were apparently healthy. There was not much emaciation. Almost all the tubercles had cheesy centers.

In the uterus there was found an almost full-grown fœtus. The placenta and the organs of the fœtus were apparently healthy.

On microscopical examination of the adult animal, the following appearances were found:-
Lungs – large and numerous tubercles with cheesy centres.
Spleen – very tuberculous.
Kidney – healthy.
Liver – small tubercles.
Gland (*Inguinal*) completely tuberculous and in parts cheesy.

There were bacilli in all the tubercles, but they were few in number, except in the gland, where they were numerous. Section of the ulcer of the skin showed cheesy masses underneath containing a considerable number of bacilli.

Careful microscopical examination of the placenta and the organs of the fœtus showed no tubercles and no bacilli.

(b) Phthisical sputum from another source, containing rather fewer bacilli, was inoculated subcutaneously into a rabbit and a guinea-pig. The rabbit died three days later, but on microscopical examination no cause was found.

In the case of the guinea-pig, a sore formed at the seat of injection (middle of the back), and the inguinal glands on both sides became enlarged.

October 19th, 1882 – Animal died: lived fifty-two days. Besides the sore and the enlarged inguinal and lumbar glands there were tubercles in the spleen and also a few in the lungs.

November 14th, 1882 – Microscopical examination showed a few tubercles in the lungs and spleen and also very few in the liver. Bacilli few in the tubercles generally, only one or two in each section of a tubercle. A considerable number of bacilli in the enlarged lumbar glands.

Experiment XIX., October 31st, 1882 – a guinea-pig was inoculated with tubercular material from a phthisical lung. (two rabbits inoculated at the same time died before there was time for the development of tuberculosis.)

November 23rd, 1882 – The guinea-pig died. Lived twenty-three days. Enlarged cheesy inguinal glands and enlarged lumbar glands. Organs apparently healthy. On microscopical examination the various organs were found healthy, but the glands were tuberculous and contained in parts a considerable number of bacilli.

Experiment XX – Inoculation of three mice with portions of phthisical lung failed to produce tuberculosis, but the incisions in the skin were too large and the material may have slipped out.

I inoculated a mouse with bacilli grown on serum and produced tuberculosis, but as I have lost the notes I cannot give the particulars.

I have also examined a considerable amount of material both in Berlin and here.

In Berlin Dr. Koch placed the following tissues at my disposal, and the result of my investigation of them is as follows:-

1. Lung of a cow affected with perlsucht. Numerous caseous nodules with enormous numbers of bacilli; the bacilli are most numerous in the caseous part and in the giant cells. No other micro-organisms.
2. Persucht (nodule from pericardium). Masses of cells with cheesy patches, a few giant cells, enormous masses of bacilli both in the tissue and in the giant cells.
3. Perlsucht (lung of a cow). Tubercular infiltration with cheesy degeneration in patches; bacilli in places in enormous masses, very numerous inside the giant cells (which are few in number), where they are arranged circularly just inside the nuclei.
4. Tubercle of horse (gland). A large number of giant cells, and cheesy and non-cheesy tissue; bacilli very numerous, especially in giant cells and cheesy matter.
5. Tubercle of horse (lung). Tubercles in the lung containing bacilli in considerable numbers.
6. Tubercle of hen (spontaneous). Intestine: enormous masses of bacilli in the nodule surrounding the intestine.
7. Liver of first dog inoculated with bacilli at Christmas, 1881, and killed four weeks later (see No. 4, p. 278). Liver full of small tubercles without caseation; bacilli present in moderate and considerable numbers.
8. Rabbit inoculated in the eye with cultivation of bacilli from monkey tuberculosis. *Lung* full of tuberculous nodules in which the bacilli are numerous; *liver* contains numerous young tubercles with numerous bacilli; *spleen* tuberculous, bacilli numerous and very numerous.
9. Rabbit inoculated with old cultivation of bacilli from man. *Lung* full of tuberculous masses; bacilli present in parts in enormous numbers and masses, especially in alveoli. *Liver*; tubercles in and between the lobules, with bacilli in considerable numbers and numerous.
10. Phthisis cavernosus. Bacilli present in considerable numbers in the walls of cavities and in the alveoli, which were full of cheesy material.
11. Phthisis. Great development of fibrous tissue, almost no caseation; bacilli very few.
12. Acute miliary tuberculosis. Bronchial gland. This gland was cheesy and contained enormous numbers of bacilli both in the cheesy material and at the margin. In some places in the cheesy part there were circles of bacilli as if they had lain radially in giant cells.
13. Acute miliary tuberculosis. Bronchial gland. This was in the main caseous, and in three places there were enormous numbers of bacilli and there were also numerous bacilli throughout the cheesy part. Dr. Koch drew my attention to an artery surrounded by enormous masses of bacilli which infiltrated its walls and extended quite to its interior. The lumen of the artery was still patent, and thus the bacilli could enter the blood in great numbers. Koch looked on this as the source of the acute affection. There were also a number of plugs of micrococci in the vessels, but they had not spread into the tissue and did not seem to be causing inflammation Koch said that these entered by an ulcer in the tongue, and he thought that they probably hastened the fatal result

14. Acute miliary tuberculosis. Lung. Tubercles containing very few bacilli.
15. to 19. A number of specimens given to Dr. Koch by Prof. Weigert. No history, but probably from cases of acute tuberculosis.

Tubercle of thyroid. Tubercular masses in thyroid with a few giant cells and caseation; bacilli present in considerable numbers

Tubercle of bladder. Tubercular ulceration of bladder, the surface being cheesy; bacilli very numerous in the cheesy matter; but they diminish rapidly in numbers as one passes from the free surface.

Tubercle of suprarenal capsule. A number of tubercles with caseation in parts confluent; bacilli present in considerable numbers.

Tubercle of kidney. Tubercles in the cortical part of the kidney, bacilli in moderate and considerable numbers.

Tubercle of pons. Tubercle at the surface of the pons in the immediate vicinity of the pia mater; bacilli in moderate and considerable numbers

20. Tuberculosis of the tongue (Ehrlich's case). Numerous tubercles and tubercular infiltration, chiefly sub-mucous; in some there is commencing caseation and a good many large giant cells; the bacilli are numerous in many places, especially where there is commencing caseation.

21. Tuberculous testicle (extirpated). Tubercles between the septa containing very few bacilli.

22. Cervical gland (extirpated). Scrofula; very extensive caseation; at the margin of the caseous part there are bacilli, but very few.

23. Scrofulous glands. Extensive caseation; bacilli very few.

24. Scrofulous axillary gland. Gland full of caseous masses; bacilli extremely few.

25. Synovial disease of finger amputated by Dr. Koch in 1878. Contains a considerable number of giant cells; bacilli very few.

I have also obtained the following materials in London and examined them with the view of ascertaining the presence of tubercle bacilli or other micro-organisms and their relation to the morbid process: -

26. Mrs. M. A rapid case of phthisis. Large caseous masses in the lung, and the alveoli in the neighbourhood full of cheesy material; bacilli in considerable numbers in the alveoli and also at the margin of the cheesy masses.

27. D. Also a rapid case of phthisis. Small cavities lined with caseous matter containing a considerable number of bacilli and surrounded by inflammatory material.

28. W. Acute phthisis. Large cavity at right apex; small cavity at left apex; both lungs infiltrated with grey and partly cheesy tubercles; on microscopical examination these tubercles contained very few bacilli.

29. Lung from a chronic case of phthisis. Very extensive tracts of fibrous tissue, with one or two caseous spots, very few bacilli, and only in the cheesy parts.

30. A.P. Phthisis of about eight months' duration. Large cavities throughout left lung; small cavities and tubercular masses in upper part of right lung. Chiefly the so-called fibrous phthisis with here and there cheesy masses. In one place in the centre of a cheesy patch there were enormous masses of bacilli (Plate I., Fig. 2), and around, in the cheesy matter, the bacilli were numerous. Near this mass, also in the midst of the cheesy patch, there was a piece of inflammatory tissue, apparently the remains of the wall of an alveolus, quite isolated from the walls of the patch. In one or two other cheesy parts the bacilli were in considerable numbers in the centre. There were no bacilli except in the cheesy matter. Throughout the fibrous tissue were numerous giant cells, but in none of them could I see bacilli. These cells were apparently developing into blood-vessels. In some the processes almost joined each other (Plate II., Fig. 8), and in one I saw a distinct central space which apparently contained red blood-corpuscles.

31. S.D. Phthisis. History unknown. Large fibrous masses with caseation, and at the margin alveoli filled with cheesy matter.; bacilli very few; some giant cells only containing pigment granules, but no bacilli.

32. Case of very rapid phthisis (thirteen weeks). Masses chiefly caseous; bacilli very few, chiefly in the cheesy matter and two or three in giant cells.

33 to 35. Three cases of potter's phthisis, varying in rapidity from several months to three or four years. The rapid case showed a large amount of cheesy degeneration, and in the caseous masses and immediately adjacent, epithelioid tissue. The bacilli were present in considerable numbers (Plate II., Fig. 11). In one alveolus filled with large epithelioid cells I saw several bacilli in almost every cell. No bacilli in the fibroid parts.

In the most chronic case there was great development of fibrous tissue and only a few cheesy masses and alveoli filled with cheesy material. In the latter the bacilli were present in moderate numbers. There were a number of giant cells in the fibroid tissue, but these only contained pigment. No bacilli.

In the intermediate case there was more cheesy matter and the bacilli were present in considerable numbers in it, but absent in the fibrous tissue and in the giant cells. In one part in the cheesy matter at the margin of a small cavity I found enormous masses of bacilli (Plate I., Fig. 3).

36. Miner's phthisis. I did not get the history. Large masses of pigment in the trabeculæ and great thickening of the trabeculæ; fibrous tissue well developed

and very vascular; in some alveoli the epithelium is increased in amount and the cells large, and in parts there are patches of embryonic tissue with plenty of vessels; no caseation; not at all tubercular in appearance; no tubercle bacilli.

For the opportunity of examining the last four cases I am indebted to Dr. Heron, to whom they were sent by Dr. G.S. Hatton, of the North Staffordshire Infirmary.

37. Lung from a case of acute tuberculosis. Commencing caseation in the nodules; bacilli few; in some of the neighbouring vessels there were plugs of micrococci, but these had no relation to the tubercles.

38. Case of acute phthisis. Patient had suffered from lupus for years, and the lupus was still extending about the nose. Strumous ulcer in neck; enlarged glands; small cavities at apices of lung; lung infiltrated with small tubercles; tubercular ulceration of intestine; enlargement of mesenteric glands in the vicinity; other organs healthy.

Apex of lung – Fibrous tissue with caseation; bacilli as a rule very few, but in several of the cheesy parts and adjoining epithelioid cells they were present in moderate numbers.

Intestine – Masses of epithelioid cells surrounded by leucocytes in the sub-mucous tissue; in the epithelioid part bacilli were present in considerable numbers; in some of the masses caseation had occurred, and there were a few bacilli among the cheesy matter.

Mesenteric Gland – For the most part cheesy and containing a moderate number of bacilli around the margin of the caseous part.

Nothing in liver, spleen, or kidney. I could not find any bacilli in the ulcer of the skin.

39. Acute phthisis. Patient admitted moribund, and no history obtained. Alveoli full of cheesy matter containing bacilli in moderate numbers and few.

40. Acute phthisis. Cavities in both lungs with grey granulations around, but getting few towards base; bacilli few except at the margins of cavities, where they were in places in considerable numbers.

41. Disease of the synovial membrane of the knee-joint (the case from which the animal in Exp. XV. was inoculated). A considerable number of giant cells, some containing a bacillus; bacilli few.

42. Case of old phthisis supposed to have been cured. Round, fibrous masses, apparently old tubercles; no caseation; no tubercular tissue; no bacilli.

43. Synovial degeneration of the ankle-joint (amputation). Bacilli present, but extremely few, and only in giant cells.

44. Case where there was apparently a primary tuberculosis of the small and large intestine. Enlarged and caseous mesenteric glands and slight affection of apices of both lungs. Lungs examined: tuberculosis with few bacilli. Three glands examined: all cheesy, and contain bacilli in considerable numbers and numerous.

(Since this report was handed in I have examined the lung and liver from a case of acute tuberculosis, and found tubercles with the same histological appearances as in artificial tuberculosis; bacilli few. – Also the spleen, lung, and intestinal ulcer from another case of acute tuberculosis; bacilli few except in intestine, where they were in considerable numbers. – The cheesy material from three strumous joints (two knees and one hip) previously unopened, containing few bacilli. – The diseased synovial membrane from one of these joints; bacilli present, but very few. – And the lungs, spleen, and cheesy gland from a case of monkey tuberculosis; bacilli few and in moderate numbers.)

In the present report I have not touched on my own cultivations. They are not sufficiently advanced to admit of a report, but I may state that I have cultivations of tubercle bacilli now growing and that I have failed to cultivate micrococci as described by Klebs, Toussaint and Schüller.

The experiments which I have performed on the induction of tuberculosis by inoculation of non-tuberculous substances, and which were done under the best hygienic conditions, with care as to complete isolation of the animals, and with special attention to sources of contamination of instruments &c., with tuberculous material, gave entirely negative results. I may summarise them here. In six cases setons of various kinds were introduced; in ten vaccine lymph, both from the calf and from man, was employed; in three pyæmic pus was injected into the eye subcutaneously, and into the abdominal cavity; and in six various materials (cork, hardened tubercle, and worsted thread) were introduced into the abdominal cavity. Not one of the twenty-five animals (the result in twenty-three being known) became tuberculous. In addition to these experiments I have cited a further fact against setons, viz. that I have often (in about fifty cases) stitched up wounds in rabbits and guinea-pigs with cotton thread, which was left in sometimes for weeks, and that not in a single case did tuberculosis follow. And I may also add all the cases in which experiments were done with Toussaint's material, micro-organisms being present (thirteen animals having been under observation for a sufficient length of time), without the production of tubercle in any case. Then I have, from time to time, found animals with cheesy patches in their livers, probably due to psorospermiæ, and though they contained this cheesy material in their bodies they did not become tuberculous. I have also in several cases, not yet published, excited abscesses by injection of lanthanum, of croton oil, and other irritants, these abscesses exhibiting the tendency to caseation so common in abscesses in rabbits, and in no instance has tuberculosis followed. In connection with this I may instance the rabbit mentioned in Experiment XVII., where a cheesy abscess had existed for months, and where it was not till the inoculation of the specific tuberculous material that tuberculosis occurred.

What then is the explanation of the contradictory results obtained by former observers? In the first place I may point out that where microscopical examination of all the organs has not been made, cheesy masses, not tubercular, might easily be taken for tubercles; and even where a microscopical examination was made the accuracy of the diagnosis would depend greatly on the methods of staining employed, and the views which the observer held as to what constituted a tubercle. I may call attention to some observations which I have made in this research: -

(1) The cheesy masses in rabbits' livers, due to psorospermiæ,[1] might easily be mistaken for tubercles, especially while the fibrous envelope around them is being formed, while it is more or less in the condition of granulation tissue. There, one would find a central cheesy mass surrounded by indifferent cells, which, on some of the views as to the structure of a tubercle, might be regarded as tuberculous. In one or two cases which I have seen I could easily understand this mistake, but I had no difficulty in coming to a conclusion, for the psorospermiæ stood out as red bodies among the cheesy material when the sections were stained by the fuchsin and the methylen blue in the manner required for demonstrating the tubercle bacilli (Plate I. Fig. 6). Further, in the lung of rabbit No. 1, Experiment XIII., there was, in addition to the young tubercles already mentioned, a body which was apparently a parasite. The body was the centre of a round mass which, till I examined it more closely, I thought was a tubercle. The centre of the body did not stain and was apparently caseous. Around it was seen a narrow, red coat with peculiar transverse striation, not complete all round, and here and there in the body were red spines resembling the hooklets of an echinococcus, though larger. At one end the body formed a sort of head, the outline of which was marked here and there by pieces of red-stained membrane (see Plate I. Fig. 4). Whatever it may be, had the method of staining not been that employed for tubercle bacilli it would have appeared as a cheesy mass in the centre of granulation tissue, and might have been mistaken for a tubercle.

(2) In the case of rabbit No. 2, Experiment IV., there were inflammatory masses in the heart and lungs which, on macroscopical examination, presented all the appearance of tubercles.

(3) I may also call attention to the changes produced in the lungs by the ova of the strongylus vasorum described by Laulanié, whose preparations I have seen. Here the strongylus is the centre of a nodule containing epithelioid cells surrounded by inflammatory tissue, but the nature of the nodule is easily made out by the presence of the strongylus in a blood vessel in the centre.

[1] According to Eimer the psorospermiæ found in rabbits' livers are encapsuled gregarinæ.

(4) There are generally present in the lungs of guinea-pigs small masses of lymphatic tissue in the immediate vicinity of large vessels or bronchi, and if the section is made at a place where these are present in considerable numbers (at the root of the lung) and of considerable size the observer who regards tubercle as a lymphatic growth may see tubercles in lungs where none exist.

While it is well to bear these facts in mind as possible explanations of many cases of supposed tuberculosis as the result of injury or inoculation of non-tuberculous material, it is not, I believe, the most frequent explanation. In Dr. Wilson Fox's paper he describes sores at the point of inoculation and enlarged glands in the vicinity implying true tuberculosis, while this was demonstrated in several instances by the fact that inoculation of the tubercles thus produced again caused tuberculosis. Now it has been shown that inoculation of the inflammatory masses produced in the lungs by inhalation or intravenous injection is not followed by tuberculosis. With regard to the early experiments on this subject it must, however, be remembered that at that time the communicability of tubercle by mediate contagion was not recognised, and as the precautions necessary for thorough disinfection of instruments, &c., had not yet been made out, the channels for the possible introduction of specific micro-organisms were left unguarded.

As to the particular form of organism associated with tuberculosis, three observers, Klebs, Schüller, and Toussaint, describe micrococci which they have been able to cultivate, and with the cultivations of which they state that they have produced the disease. Only one of these, Schüller, has seen the organisms in the tubercles thus produced, and in tubercular tissue generally. As I have only been able to see Toussaint's experiments and results, and as he has gone much further than the other observers, it will be sufficient if I discuss his work alone in detail.

Toussaint's method is, as has already been described, to cultivate organisms (micrococci) from the blood of tuberculous animals, and after repeated cultivations to inject the organisms into other animals. I received from Toussaint tubes containing these micrococci (Plate I. Fig. 5), which he said were still living, as, indeed, they proved to be. I have inoculated animals from them, and from the cultivations which I have obtained from them. The material obtained from Toussaint was injected into three rabbits, two guinea-pigs, one cat, and one mouse, and of these seven animals six were under observation for a sufficient length of time for the development, at least, of local tuberculosis, and in not a single instance did tuberculosis follow. I have also cultivated the micrococci obtained from him, and injected them into nine rabbits and three guinea-pigs; of these, four rabbits and three guinea-pigs were under observation for a sufficient length of time without the development of tuberculosis in any of the animals. The total result is that thirteen animals were inoculated with the micrococci with which Toussaint works, obtained from Toussaint himself, and in no case did tuberculosis follow. Had these thirteen animals been inoculated with bovine tuberculosis, which is the original source of Toussaint's tuberculosis, there is no doubt that they would all have become tuberculous.

I also obtained from Professor Toussaint a number of tuberculous organs from animals on which he had experimented; six of these were from three animals which

had been inoculated with micrococcal fluid of the seventh, - tenth, - fifteenth, sixteenth, twenty-first, and twenty-second generations respectively, and five were from two animals inoculated with tuberculous material. I thus had the opportunity of examining the result in five animals from Toussaint's series of experiments. In all of these tubercles were present, differing in no respect from those always produced by inoculation of tuberculous material. I have spent much time in searching for micrococci by all known methods, but I have found none. I had specimens of Toussaint's micrococci by me, and thus knew the size of the organisms, which do not differ in their relation to staining agents or in their grouping from other forms of micrococci with which I have worked for a long time. I can therefore say definitely that there were no micrococci in the organs given me by Toussaint, whether taken from animals which had been inoculated with tuberculous material or from animals which had become tuberculous after the injection of micrococcal fluid. On the other hand, the tubercle bacilli were present in all the organs, in some in large numbers, corresponding to the result found, as a rule, after inoculation of bovine tuberculosis (Plate I, Fig. 1).

I have also attempted to grow micrococci from the blood of tuberculous animals by Toussaint's method, taking every precaution against accidental contamination, and I have entirely failed to do so.

From these facts I conclude that the micrococci described by Toussaint do not cause tuberculosis, and therefore his results must be due to some other agency. The constant presence in the organs of his animals of the bacilli described by Koch is, in my opinion, sufficient explanation of the occurrence of tuberculosis. How are Toussaint's results to be explained? He cultivates micrococci from the blood of tuberculous animals, and after injection of these micrococci he sometimes obtains tuberculosis. On this point I may throw out the following suggestions. The growth of micrococci in the materials inoculated may depend either on fault in experimentation, or, as I believe to be more likely from the constancy of the results, on the presence of large numbers of these organisms in the air of the room in which the experiments are done (*vide* Mr. Lister's and Professor Tyndall's experiences). The success of some of the inoculations may depend on two causes, either on growth of tubercle bacilli, as well as micrococci, in the fluids which yield successful results, or on contamination of the micrococcal fluid during injection. It may be that bacilli grow, though very slowly and imperfectly, in the fluids, and perhaps better in serum than in rabbit infusion; hence the greater success with the former. The growth of bacilli need not necessarily be very luxuriant, for Toussaint injects so much fluid that if they grow at all he would probably get some in ten drops of the fluid. On the other hand, however, Toussaint trusts greatly to carbolic acid as a disinfecting agent for the purification of the instruments employed in inoculations. Now, carbolic acid, though apparently effectual for the destruction of the ordinary forms of micro-organisms, as evidenced by the satisfactory results obtained from its use in aseptic surgery, has been shown to be ineffectual against the spores of bacilli, unless it acts for a long time. The bacillus of tubercle apparently produces spores, and there is no reason to suppose that these are less resistant than those of bacillus anthracis and other bacilli. Indeed, as will be seen from Experiments XIII. and XIV., a saturated watery solution of carbolic acid, even though it acts as long as fifteen minutes, is not sufficient to arrest the development of the tubercle bacilli, and therefore the washing of a syringe with carbolic acid is not such a certain means of disinfection as was formerly supposed. I think that imperfect

disinfection of the syringes employed for injection of the micrococci and of tuberculous blood, &c., is a possible explanation of the results which ought not to be left out of account.

In the researches of Klebs and Schüller a pure cultivation was not obtained, nor were the cultivations carried beyond the third generatioin, and Schüller was not always successful in producing tuberculosis by the injection of his cultivations. Schüller has seen micrococci in tubercles artificially produced, and he showed me drawings of them, but no-one else has been able to find them, although some of the best microscopists and those who have done most with micro-organisms (Koch, Weigert, &c.) have searched for them most assiduously. I have taken great care in examining the various sections which I have made, but I have not found micrococci in the tubercles in any case. In only two instances have I found micrococci at all. These were both cases of acute tuberculosis, and there the micrococci were not present in the tubercles, but in vessels which had no relation to the tubercles. They were present, either having got in through some ulcer, as in Koch's case, and though quite independent of the disease, probably hastened the death of the patient, or they may have been present simply as the result of lowered vitality on the part of the patient, as I pointed out some years ago might occur, though in that case I have never found them as plugs in the vessels.

In Koch's research, the results are much more definite than any previously obtained. He also cultivates micro-organisms from tubercle, but now it is no longer the fact that he only sometimes succeeds in causing tuberculosis and that the tuberculosis thus produced occurs as slowly, or more slowly, than inoculation of tuberculous material. The result of the inoculation of his cultivations is certain and the disease is more rapid in its commencement than after inoculation of tuberculous matter.

I have given so fully the details of my visit to him, and the result of the investigations which I have carried on with the materials obtained, that I need only refer very shortly to the facts. In Berlin I was able to see a large number of cultivations from a great variety of sources, all presenting the same appearances and containing tubercle bacilli alone. I examined some of these in Berlin and have examined those I brought back with me, and I find nothing but tubercle bacilli, no other micro-organisms, no remnants of the original tissue or caseous material. Indeed, the method of cultivation is such as soon to get rid of all the original cheesy material – a tubercle is crushed and at numerous points on the surface of the serum little masses are seen to appear. One of these masses is picked up and crushed out thoroughly over a large surface of fresh serum. Again fresh masses appear all over this serum and one of these is again taken and crushed out and so on. Thus the original cheesy material is very soon lost and nothing remains but the organisms which have developed from it. The serum in the tubes may be allowed to dry up, as occurred in one of my tubes, but still the bacilli grow when transferred to fresh serum and produce tuberculosis when inoculated into an animal.

The result on animals of inoculation with the bacilli cultivated in this way is certain, and tuberculosis always follows. I may refer to the cases which Dr. Koch inoculated in my presence (four animals) and to the animals which I have inoculated here (twelve animals having lived long enough) all of which became tuberculous and that more

rapidly than after inoculation of tuberculous material. The tubercles produced in these cases were infective, and caused tuberculosis in other animals.

On the examination of tuberculous material these bacilli are always found, though in varying numbers. They are always most numerous in bovine tuberculosis, and least numerous in human tuberculosis. I have examined a large number of organs of tuberculous animals and of cases of human tuberculosis, and in all of these, without exception, I have found tubercle bacilli, though the material has been got from many different sources. (I do not include among the latter cases the miner's lung and the case of supposed cured phthisis, neither of which were tuberculous). I have also examined a number of morbid growths for these bacilli, and have not found them.

The fact that the inoculation of the cultivated bacilli is so certain and rapid in its effects can only be explained on the supposition that in these cultivations we have the virus of the disease in a more or less pure state, and in large amount. But as the only thing which we see multiplying on the serum are these bacilli, and as they are also constantly present in tubercles, it is difficult to see what other conclusion can be come to than that they constitute the virus. This view is still further supported by former experiments on the inoculation of tuberculous material, for it has been found that the result of inoculation is most uncertain when strumous glands and lupus are employed, while it is most certain in the case of phthisical sputum and bovine tuberculosis. Now, in strumous glands there is plenty of cheesy material, plenty of tuberculous material (and the same is the case in phthisis), but there are extremely few bacilli, and the result is slow and uncertain. On the other hand, in bovine tuberculous material there are large numbers of bacilli, and the result is rapid and certain, while in sputum the bacilli are free, and here also the result is rapid. The certainty of the production of tuberculosis by inoculation of tuberculous material, and the rapidity of its occurrence, are in a direct ratio to the number of bacilli present in the original material and have no relation to the amount of caseous or other tuberculous matter introduced. And the most rapid and efficacious of anything which has yet been tried is the inoculation of the tubercle bacilli growing free on serum. Introduce the point of a syringe which contains fluid in which these bacilli are present into the anterior chamber of the eye without moving the piston of the syringe, and the result will, in all probability, be tuberculosis of the iris, which, however, will be slow in appearing, for but few bacilli were introduced. Inject a small quantity of the same fluid and the result is a rapid appearance of tubercles on the iris (see the case inoculated before me by Koch). Introduce the point of a syringe containing cheesy or other tuberculous material, but only few bacilli, and the chances are that an extremely small proportion of the animals would be infected, for it has been found that the introduction of a considerable amount of such material is extremely doubtful in its result, and when tuberculosis does occur it resembles the case where the point of the syringe was introduced and the piston not moved rather than the case where a small quantity of the fluid containing bacilli was injected. Again (see Experiments XIII. and XIV.), the effect of germicides, which have not acted long enough to penetrate dense masses or to destroy spores, is to delay the appearance of tuberculosis, because some at least of the bacilli (and probably the most active ones) have been destroyed. The conclusion is, I think, that we have before us in these bacilli, the virus of the acute tuberculosis caused in the lower animals by the inoculation of tuberculous material.

Professor Klebs in his letter makes two objections to Koch's facts, and apparently still upholds his views that a micrococcus and not a bacillus is the real cause of tuberculosis. The first of his objections is that these bacilli may be crystals. This objection is not a formidable one; for anyone acquainted with micro-organisms can tell on examination that this is an organism and not a crystal. (Plate II. Fig. 10.) Its appearance, the apparent presence of spores, its variable lengths, its frequent curved form, its arrangement, its behaviour with staining agents, and above all its growth on serum, demonstrate its living nature. It is difficult to conceive that a few crystals put on serum will grow and extend from one point with the rapidity and in the mode that these bacilli spread, and I know of no better test, in spite of Klebs's assertion to the contrary, for a living organism than its increase and multiplication when placed on a suitable soil. They are not any kind of fat crystals, for they are not found in cheesy matter which is not tubercular; and they vary greatly in number in tubercular material itself, sometimes only one being present in two or three fields of the microscope; sometimes a large number. The presence of motion is not a constant attribute of bacteria; indeed, if that were necessary to prove the bacteric nature of a rod, many bacilli such as the bacillus anthracis, would be looked on as crystalline. Nor is the observation of division under the eye of the observer necessary for arriving at a conclusion as to the presence of life, for there are but few organisms which have been seen to divide, and nevertheless, there are a great many microscopic bodies which we look upon as living. The best test that we possess is growth under suitable circumstances, and this proof Koch has supplied with regard to the tubercle bacillus. Klebs, however, though he starts this view, himself looks on these rods as bacilli.

That Koch does grow the cause of tuberculosis in the lower animals all who have seen his work or done similar work are prepared to admit, even Klebs and Schüller, who have described other organisms, but in his letter Klebs makes a second objection. He says that he has seen, even in Koch's cultivations, finely granular material which appears to possess the characters of micrococci. He does not say how he observed this, whether it was in looking at the organisms as they lay on the serum, or whether he spread out the bacilli on a glass and stained them and these round bodies as well. We cannot, therefore, be quite certain what has been seen. If one looks at the organisms as they are growing on a slide covered with solidified serum, one of course sees granular matter; but this is the granular matter of the coagulated serum. On the other hand, I have examined the masses of bacilli when squeezed out between cover-glasses dried and stained, and I can say that I have failed, even though I have taken the greatest care in the preparation and in the search, to find any finely granular matter in any way resembling micrococci. Further, micrococci growing on serum present quite a different appearance to the cultivations of these bacilli. They grow as whitish soft masses, and much more rapidly. One cannot attach much importance to granular matter of an indefinite nature. Bad lenses, bad illumination, and many other causes will show granular matter where none exists. Unless the granules have a definite form and arrangement, or stain well, one cannot speak of them as micrococci.

Aufrecht, in 1881 (Pathologische Mittheilungen), describes the centre of the tubercle as composed not of cheesy matter, but "of very small micrococci, of micrococci united in twos and threes to form short chains, and of very short rods." He now considers these short rods to be the same as Koch's bacilli. This however, is not the case, for he says that in sputum they may be stained by a ½ per 1000 watery solution of fuchsin; Koch's tubercle bacilli cannot be stained by this solution.

Leaving these questions, we must now consider what is the gain as regards human pathology from all the researches which have been carried on on tuberculosis, and especially from the demonstration of the tubercle bacillus. All that has as yet been absolutely *proved* is that a variety of materials in man which we class together as tuberculous, produce, when inoculated into rabbits, guinea-pigs, and other animals, acute tuberculosis, and that this also occurs from the inoculation of bovine tuberculosis. Koch's researches further demonstrate that this result is due only to the tubercle bacilli which were present in the materials inoculated. It therefore remains for inquiry in what relation these bacilli stand to the morbid processes in man and in cattle in which they are found.

In man we have the disease termed acute miliary tuberculosis, which resembles in every respect – histological structure, tendencies and presence of bacilli – the acute tuberculosis produced in animals by the inoculation of tuberculous material. It will I think be admitted that the two diseases are identical, and as they are identical their cause must be the same, viz. the tubercle bacillus. We may therefore say definitely that the tubercle bacillus is the cause of acute tuberculosis and that scrofulous glands, degenerated (strumous) synovial membranes of joints, phthisical lungs, (in short, all those materials obtained from man which, inoculated into animals produce acute tuberculosis), contain in them bodies (bacilli) which, if they entered the circulation in sufficient numbers, would give rise to acute tuberculosis. It has been demonstrated by several observers that probably in all cases of acute tuberculosis a place can be found where these bacilli get into the circulation. Ponfick was the first to touch this question, and he showed that in acute tuberculosis, especially in children, tuberculous changes which he regarded as the source of the infection of the blood, were found in the thoracic duct. These however are only found in a small number of cases of acute miliary tuberculosis. Weigert and Mügge found that in many cases of acute tuberculosis there were tubercular masses in the walls of the pulmonary veins and these Weigert considers as the primary source of infection. It is not however always the pulmonary veins which are the seat of this infection. Weigert has found that the walls of the innominate vein have become affected from the bronchial glands, and he has also found masses in the splenic vein, &c. Koch's case of acute miliary tuberculosis of the bronchial gland in which the wall of an artery was involved (No. 12 p. 292) also in his opinion shows a third mode in which this general infection may take place.

The relation of the tubercle bacilli to localised tuberculous processes in man (phthisis, scrofulous diseases of the glands, joints, &c.) is much more difficult to understand. The only one of these processes which I have had the opportunity of studying fully is phthisis, and in order to make clear the conception which I have formed of the relation of bacilli to this disease, I must point out one or two facts which I have observed as to the mode of distribution of these organisms in the tissue and their relation to the histological elements of the tissue.

In studying artificial tuberculosis in animals we have now a better opportunity of deciding what a tubercle is, and what parts of a tubercle may be looked on as essential histological elements, for we have the cause of the nodule – the bacillus – before us, and can study its precise relation to the nodule. I have, therefore, taken advantage of my opportunities to study this matter, and I have directed my attention to tubercles in

the lungs and liver. I have not had time as yet to study the origin of the cells in tubercle of other organs, but the structure of tubercle and the relation of bacilli to the elements of the nodule is the same in all the organs which I have examined (lung, liver, spleen, kidney, lymphatic gland, and intestine). The description which I give here applies specially to the lungs.

Two distinct structures have been described, and may be readily recognised in a tuberculous lung, viz. nodules of lymphatic tissue in close proximity to the vessels and bronchi, and nodules which are largely made up of epithelioid cells. If a case of commencing tuberculosis of the lung be examined, it will be found that bacilli are only present in the latter nodules; and, indeed, it is rare, even in the latter stages, to find them in the former, and in that case epithelioid cells will be found as well. The bacillus being the cause of this disease, only the nodules containing epithelioid cells are tuberles. The others are, I believe, merely the normal lymphatic tissue which exists in considerable quantity, more especially in the guinea-pig's lung, probably hypertrophied by absorption of irritating materials, the products of the growth of the bacillus.

Not only are the bacilli present solely in nodules containing epithelioid cells, but it will be found, on careful investigation, that they are only present in or among the epithelioid cells themselves. Of course I speak here of young tubercles, and of tubercles where the bacilli are in moderate or considerable numbers. Where there are enormous masses of bacilli they may be found in the outer part of the tubercle, even though in this case they are generally confined, in the first instance, to the epithelioid tissue. Where the bacilli are few, one need only look for them in epithelioid cells. (Plate II., Figs. 12 and 13.)

Surrounding the epithelioid cells, which are always in the central portion of the tubercle and make up the greater part of it (though after a time leucocytes penetrate among them), we have what I consider to be simply inflammatory tissue, but what is sometimes spoken of as lymphatic tissue. I have not been able to convince myself of the existence of the so-called reticulum of tubercle, certainly not of the existence of a delicate reticulum resembling that found in lymphatic glands. It seems to me that the appearance of a reticulum is explicable as follows: - The inflammation set up by the development of the epithelioid cells is in the first instance but slight. The normal fibrous tissue surrounding the epithelioid mass becomes infiltrated with leucocytes, or it may be that new fibrous tissue is in some cases formed, and the fibrous tissue containing leucocytes presents a reticular appearance somewhat resembling course lymphatic tissue.

As the tubercle gets older, it is found that the epithelioid cells at the centre undergo cheesy degeneration, and they can only be seen, if present at all, at the margin. In this case the bacilli are present in the caseous mass, but they are best seen at the margin of it where epithelioid cells still exist, though they may also then be found penetrating into the inflammatory tissue.

In tubercle we also find giant cells in which bacilli are generally present, sometimes in considerable numbers. These giant cells I have distinctly traced to epithelioid cells, especially to epithelioid cells containing bacilli, for where several bacilli are present in cells all gradations may be found between the single nucleated cell and the

multinucleated giant cell. In inoculated tubercle, giant cells of large size are not common, I presume because time is required for their formation, while in inoculated tubercle the process is too rapid and degeneration occurs early.

As to the origin of these epithelioid cells I can only speak from a study of the process in the lungs and the liver. In the lungs I am satisfied that the great majority are derived from the alveolar epithelium. The bacilli escape from the blood-vessels or lymphatics and get into the alveolar epithelium, where they grow and cause multiplication of the epithelial cells, till the alveolus becomes completely filled with these cells and infiltrated leucocytes. Around this mass the walls of the alveolus become inflamed and thickened, and form the granulation tissue surrounding the epithelioid mass (Plate II., Figs. 12 and 13). The idea of some is that the epithelioid cells are derived from leucocytes, but in cases where the epithelioid mass has become caseous, bacilli may be present in the tissue around which is granulation, not epithelial tissue, and these granulation cells do not become epithelioid. It is hardly conceivable that these bodies possess the property of converting leucocytes into epithelioid cells; it is surely much more likely that they grow by preference in epithelioid cells already existing and lead to their multiplication. I will not assert that the epithelioid cells found in tubercles in the lung are always derived from alveolar epthelium; they may also be derived from the endothelium of the blood and lymphatic vessels. Nevertheless I believe that the great majority are alveolar cells. In the case where Koch injected bacilli into the veins of a rabbit in my presence, I found that many of the masses of epithelioid cells were undoubtedly derived from the alveolar epithelium, but there were others in which I could not trace this mode of origin, and in which they must have been derived from the lymphatic or blood-vessels. In lymphatic glands their source must be from one of the latter, I believe from the lymphatic endothelium. Schüppel is inclined to regard them in this case as generally derived from blood-vessels, but this I cannot admit, for if the bacilli were present in the blood-vessels there seems no reason why the disease should be arrested in the glands even for a time, as, however, is found to be the case.

In the liver I have frequently found the bacilli in liver cells at the margins of the tubercles, and where tubercles were commencing to form; and even where the tubercles are older, direct continuity between the epithelioid cells and the liver cells can frequently be traced, especially by the use of Ehrlich's method of staining with hæmatoxylin, rubin s. and orange. I have also seen the liver cells, in which bacilli were present, with two nuclei, and apparently giant cells are often formed by multiplication of the nuclei in liver cells or by fusion of liver cells. In the latter case regular tubes may be formed, which on transverse section would appear as Langhans' cells with a circular arrangement of nuclei around the wall.

The origin of the epithelioid cells from the endothelium of blood-vessels has been described by Lauliané in connection with the strongylus vasorum previously mentioned, and I saw it distinctly in the specimens which he showed me.

Giant cells are epithelioid cells from any of the sources mentioned which have grown rapidly, apparently as the result of the presence of bacilli in them. In the lung they are generally derived, like the ordinary epithelioid cells, from the alveolar epithelium. I have seen this distinctly, and in one case I found nothing in an alveolus but one large giant cell containing a number of bacilli (Plate I, Fig. 7). This cell may have arisen from fusion of several cells or from the disappearance of the other epithelioid cells.

The fact that giant cells generally contain a large quantity of pigment seems also to indicate that they were originally alveolar cells.

Tubercle has been regarded as a non-vascular tissue due to obliteration of the vessels in the centre. This is not the case, for, from the facts just mentioned, it will be seen that vessels were never present in the centre. At the same time, however, the accumulation of epithelial cells leads to pressure on the vessels outside, and blocks them. When this accumulation of epithelium has gone on to a certain extent caseous degeneration of these cells sets in, and thus the centre of the tubercle becomes converted into a cheesy mass. Where the process is rapid the giant cells also disappear, as is generally the case in tuberculosis artificially induced. When the accumulation of epithelioid cells is great, the pressure on the surrounding tissue leads to its atrophy and degeneration, and thus neighbouring cheesy masses come to communicate with each other. Where the process is slow and the accumulation of epithelioid cells is not very great, the granulation tissue surrounding them may become organised into fibrous tissue more or less perfect. It may be also that the caseous matter becomes absorbed, leaving only the giant cells in the midst of the fibrous tissue, or it may be, as in the case cited before, that all the cells of an alveolus have become fused together to form a giant cell, or the cells disappear except the giant cell, and thus, in any case, we have a giant cell left in the midst of fibrous tissue. As this giant cell contained bacilli in the first instance it may still contain them, and thus is explained the fact pointed out by Koch, that bacilli are very frequently present in giant cells, and that, in many cases where there is only fibrous tissue and giant cells, the latter are the only parts where one finds bacilli. What the further fate of these giant cells may be is difficult to say, but in one case I have seen distinct indications as if they were going to develop into blood-vessels (Plate II., Fig. 8).

From these facts it is evident that if we want a typical histological element for tubercle we must take the epithelioid cells, because they are always present in tubercles, and because the bacilli are always found in them in the first instance. They will not, however, be always found, as caseation sets in early and destroys them. In that case, if the process is slow, giant cells, may still be seen, the only remnants of the original epithelioid cells.

The structural definition of a tubercle must therefore run somewhat as follows: A nodule, composed of a central mass consisting in the main of epithelioid cells, or in its place a cheesy mass, surrounded by more or less inflammatory tissue, with or without the presence of giant cells. The absolute diagnostic mark is, however, the presence of the tubercle bacillus. A group of granulation cells without epithelioid cells, or without cheesy matter or giant cells to indicate the previous presence of epithelioid elements, is not a tubercle. On the other hand, I know of no morbid structure except tubercle which contains the same histological elements, arranged in the same way, and possessing the same tendencies. It is not, however, always necessary for a tubercle to be a nodule. If there are plenty of epithelial cells, or if it occurs where there are no pouches, as there are in the lung, it may be diffuse (see remarks on appearance in Koch's *Dog's Liver*, p. 278). In any case, as I have just said, the absolute diagnostic mark of a tuberculous process is the presence of tubercle bacilli.

With regard to phthisis, the divisions and subdivisions of this disease are too numerous and varied to be of importance for the present question. We may take the

two extremes – the rapid phthisis or caseous pneumonia, and the chronic or fibroid phthisis. If we examine the former cases we find alveoli distended with caseous material, or, in parts where the process is less advanced, with epithelioid cells, and the trabeculæ surrounding these thickened and converted into inflammatory tissue. In this case the bacilli are found in moderate or even in considerable numbers, in the caseous material and epithelioid cells filling the alveoli. By and by we find that the walls of the alveoli disappear, and thus irregular cavities are formed, containing caseous material surrounded by epithelioid cells and inflammatory tissue. In this case the bacilli are most numerous and sometimes in enormous masses, at the free margin of the cheesy material (Plate I., Fig. 3). They are also present, though not generally so numerous, in the epithelioid cells at the line of junction of the caseous mass with the surrounding tissue (Plate II., Fig. 11). It does not follow that in every section one will find many bacilli; in some they are very few, but the examination of a considerable number of sections will generally result in the discovery of considerable numbers at the free margin of the caseous material or in alveoli.

In fibroid phthisis the bacilli are, as a rule, extremely few, but here and there, if a cavity exists, or in the centre of a caseous mass (Plate I., Fig. 2), one may find considerable numbers. They may also be found in the giant cells, which are generally pretty numerous among the fibrous tissue, but this is very rare. As a rule, with the exceptional cases I have just mentioned, bacilli are extremely few in fibroid phthisis; but nevertheless, if a sufficient number of sections be carefully examined, one or two will be found here or there at the margin of, or in, the caseous masses.

The following is what the foregoing facts lead me to suppose to be the sequence of events in phthisis. The tubercle bacilli which reach the lung[1] by inhalation develop in the epithelioid cells lining an alveolus, this alveolus becomes filled with cells, neighbouring alveoli become infected, and the same process goes on in them. The further result will depend on the number and rapidity of growth of the bacilli, and on whether the patient is a good soil for their development. If they develop well, we have caseous pneumonia, if they grow slowly and with difficulty, we have fibroid phthisis. In the former case the alveoli become distended early with epithelioid cells, this leads to inflammation of the walls of the alveoli, the cells soon undergo cheesy degeneration, and the pressure of the masses leads to atrophy or sloughing of the walls of the alveoli. (In the latter case elastic tissue will be found in the sputum). Infection of neighbouring parts of the lung occurs both by continuity, and also by partial coughing up and re-inhalation of the bacilli into other parts of the lung. In this rapid phthisis, fibrous formation around the alveoli only takes place imperfectly, and the lung rapidly breaks down.

[1] I do not imply that bacilli when inhaled must grow in the epithelioid cells; they may pass on and be caught in the bronchial glands, or they may not grow at all. I only refer to what occurs in cases where phthisis develops. I do not think that the development of phthisis is merely a question of soil, but it seems to me that the lung must in addition be prepared, so to speak, for the reception of the bacillus, as may be the case if congestion or slight inflammation be present at the time of the inhalation of the organism.

In the case of fibroid phthisis the bacilli are few, and grow with difficulty. Thus fibrous formation occurs extensively, and giant-cells are caught in this tissue in the manner formerly described. Nevertheless, in parts the process may be more rapid, and there cheesy masses are formed which may lead to breaking down of the lung and the formation of cavities.

On this view we have an explanation of several facts. First, we have the rarity of acute general tuberculosis in connection with phthisis, even though bacilli are present in the lungs. One reason of this is probably that the bacilli can hardly be said to enter the body; they are separated from the circulation by the layer of granulation and fibrous tissue. This is a fact which can be readily observed.

Secondly, we have the explanation of their presence in the sputum even before physical signs are marked or indeed have become evident at all. It is now stated that the number of bacilli in the sputum is a means of forming a prognosis as to the rapidity of the disease. According the views I have just stated this would be a very likely thing, for the presence of large numbers in the sputum would indicate either an affection of numerous alveoli, or a large amount of caseous material, i.e. extensive affection of one part of the lung, and hence rapid death.

It will also be seen that the relation which was early maintained between cheesy pneumonia and what is ordinarily understood by tuberculosis of the lung, a relation denied by some, does in fact exist, and that the difference between the two lies in the mode of the spread of the disease. Caseous pneumonia arises from inhalation of the virus into the alveoli, while miliary tuberculosis and the eruption of nodules over the lung in acute phthisis are due to infection by the blood vessels in the first case, or by the lymph channels in the second. No doubt, however, the second case is also sometimes due to inhalation of tuberculous sputum into other parts of the lung during some mishap in coughing.

Against the view that phthisis is due to these bacilli might be urged the fact that the bacilli found in the lung after death are often very few in number. This fact is undoubted in some cases, and it is certainly difficult to understand how a few bacilli can set up such extensive changes. Not only are few bacilli found in phthisis, but there are also few in the tubercles in acute tuberculosis and in other tuberculous processes in man. Indeed, in tuberculous material in man, except in the case of the glands, as the bronchial and mesenteric glands, bacilli are few. Now, if the notes which I have given in the experimental part be carefully gone over, it will be seen that in all cases (with one exception) where material obtained from man was employed for inoculation I have noted the number of bacilli as few and moderate – not numerous; while in the case of inoculation of perlsucht the bacilli are, as a rule, numerous. (In the glands, however, they are numerous in both cases.) This did not strike me for a considerable time, but after I noted it I went over the cases again and found it to be the fact. Indeed, in some instances, it was surprising what an extensive tuberculous process was set up in guinea-pigs and rabbits with very few bacilli in the tubercles, even where cultivations of bacilli had been inoculated. The difference in the number of the organisms present in the tubercles produced in rabbits by inoculation of bacilli from man and in those from bovine tuberculosis has been almost constant in my experiments; but I am not inclined to lay it down as an axiom, for in two cases inoculated by Koch with bacilli from man I find them very numerous in the tubercles

produced. (See No. 9, p. 291 and No. 5, p. 279.) In the first of these cases the sections I used were thick, for I used a machine to which I was not accustomed, and that may to some extent account for the large numbers found, but in the last case I used Williams's machine and the sections were thin. This is therefore a point which requires further investigation, but I must say my results are very striking. Leaving out of account, however, this possible difference in energy between the bacilli in man and in perlsucht, which, were it proved, would be most important in connection with the question at issue, we have, nevertheless, the fact that very extensive tuberculous processes may be found in animals with only very few bacilli, and that in cases where we are sure that the bacillus was the only agency at work.

The views which I have just put forward with regard to phthisis are not at all opposed to the fact that inoculation of rabbits, &c., is generally followed by general tuberculosis, for the mode of infection differs in the two cases. Where tuberculosis is artificially produced, the bacilli are placed at once in direct communication with the lymphatic and blood vascular systems, while in the natural infection of the lungs by inhalation the bacilli grow outside the body and a barrier of granulation tissue is formed which prevents their entrance into the vessels. This is, however, not the only reason of the difference between the two; there must also be a special predisposition on the part of rabbits, &c., to general tuberculosis. For when the inoculation is made into the eye, the process is at first local and afterwards becomes general. This may, indeed, be due to some anatomical peculiarity of the part, but I think that it is most likely in the main a question of soil, or what is termed predisposition. That certain organs and tissues are better soil than others for the growth of parasites is well known, and can easily be shown with regard to tuberculosis; the apices of the lungs are better soil than the bases, epithelioid cells are better soil for the bacillus than granulation cells or cheesy matter, and so on. Now, it does not necessarily follow that the quality of the soil is the same in man and animals. In cattle the bacilli prefer the surfaces of the serous membranes to an extent which they only very rarely do in man. In man, again, the pia mater is a common seat of tubercles in acute tuberculosis; in rabbits and guinea-pigs the presence of tubercles in the pia mater is, I believe, unknown; certainly they have not been found in my experience. Other facts might be mentioned, but what I have said is sufficient evidence of the difference between tuberculosis in man and rabbits, dependent partly on the mode of infection but chiefly on difference in soil.

My views as to the nature of phthisis and its production by bacilli receive direct confirmation from the inhalation experiments of Tappeiner (Virchow's *Archiv*, vol. lxxiv., 1878). He caused dogs to inhale phthisical sputum, which was sprayed into their cage for a certain time once or twice a day and for a number of days in succession. As a result he produced tuberculosis in eleven cases. Now, in seven of these eleven cases the tubercles were limited to the lungs, and I see that he describes some of them as desquamative pneumonia with tubercles. In one case there were a few nodules in the kidneys, and in two a few in the liver and kidneys. In only one case was there a typical acute tuberculosis. Here the method of infection somewhat resembled what we must suppose to be the natural method in phthisis, though in Tappeiner's dogs a much larger quantity was administered, and that much more rapidly than probably occurs in nature. And hence the process was more diffuse and rapid than in man. Nevertheless, acute tuberculosis only occurred in one case, and a few nodules in three others. In seven the process remained limited to the lungs just as I have described in the case of phthisis. That in a few instances the bacilli did get into

the body need not excite surprise, when the amount and continuance of infection are taken into account. That this may also occur in man is well known (see Klebs' list, Virchow's *Archiv*, vol. xlv.).

A consideration of all the facts has led me to the conclusion that tuberculous processes in the lungs are due to the tubercle bacilli, and, so far as I know, to them only. By a tuberculous process I mean one where there is a proliferation of epithelium, caseous degeneration of this proliferated epithelium, and inflammation round about, these changes being progressive. It has been supposed that inhalation of dust of various kinds may give rise to phthisis. That the inhalation of dust will lead to inflammatory changes is very likely, that it may lead to proliferation of epithelium which may subsequently degenerate is possible, but that the process will be progressive and extend beyond the seat of irritation is not probable. That the changes set up by the presence of gritty particles may, however, prepare the lung and render it a fit soil for the implantation of bacilli is very probable, and in this way a true tuberculous process may supervene, not due to the original gritty substances but to the bacilli which came afterwards. I have only had the opportunity of examining three cases of potter's phthisis and one of miner's phthisis. In the former there was, histologically, a true tuberculous structure, and there the tubercle bacilli were found. In the case which was labelled miner's phthisis, but the details of which I did not obtain, there was fibrous formation, the fibrous tissue being very vascular, and there was no appearance, histologically, of tuberculous structure, nor were any bacilli present.

As to the intestinal ulcerations which often occur in phthisis, and which are supposed to be due to swallowing sputum, I have only examined two cases, and there I found tubercle bacilli in the wall of the ulcer bearing the same relation to epithelioid cells and caseous matter as elsewhere.

As to heredity of tubercle I would call attention to the case of the guinea-pig, which was highly tuberculous and which had an almost fully developed fœtus in its uterus (Experiment XVIII., p. 289). The fœtus and placenta were healthy and free from tubercles.

It has often been urged that the milk of tuberculous cows is infective. This may be the case when the mammary glands become tuberculous and the mode in which the bacilli might get into the milk is well illustrated by the appearances which I found in the kidney of rabbit No. 1, Experiment XIV. p. 286. There not only were bacilli present in the tubercular mass, they were also found in large numbers in the epithelium of the kidney tubules, both in the immediate vicinity of the mass and at some distance from it. I have not yet had an opportunity of examining an early tubercle of the kidney, but, from what I have seen, I think it quite likely that the epithelium of the tubules may, in some cases, be the primary seat of the bacilli in the kidney, just as the alveolar epithelium is in the lung. In that case bacilli would be present in the urine, not merely when there were marked tubercular masses in the kidney, but also where the disease was but slightly advanced, here again resembling the case of the lung. From analogy I suppose that the same is the case with the mammary glands, and that bacilli might be present in the milk even though the disease of the gland is not sufficiently far advanced to be noticeable.

The accompanying Plates were drawn by Mr. Edgar Thurston by means of Beale's neutral tint reflector at the standard distance of ten inches, and are faithful reproductions of the specimens. In all the specimens the bacilli are red.

Plate I

Fig. 1. – Section of lung from a rabbit which became tuberculous after being inoculated by Professor Toussaint with the micrococci with which he works. Centre of a tuberculous mass which has become caseous, inclosing a large pulmonary vessel. The tubercle bacilli have penetrated through the wall of this vessel and are seen growing in considerable numbers in its interior. From this point the blood would be constantly supplied with numerous bacilli and thus generalisation of the disease would rapidly occur. X 330. (The optical apparatus employed was Hartnach's No. 7 objective, Zeiss's No. 2 eyepiece, Abbe's condenser.)
Fig. 2. – Section of lung from a case of fibroid phthisis showing a cheesy mass with a group near its centre consisting of numerous bacilli. (See p. 284.) X 100. Hartnach's No. 4, Zeiss's No. 2 eyepiece, Abbe's condenser.)
Fig. 3. – Section of a lung from a case of potter's phthisis showing the margin of a cavity with masses of bacilli at the edge. X 100. Hartnach's No. 4, Zeiss's No. 2 eyepiece, Abbe's condenser.)
Fig. 4. – The structure in the lung referred to at p. 298. X 200. (Hartnach's No. 5, Zeiss's No. 2 eyepiece, Abbe's condenser.)
Fig. 5. – Toussaint's micrococci. X 950 Zeiss's 1/12th oil immersion, No. 4 eyepiece, Abbe's condenser.)
Fig. 6. – Psorospermiæ from the liver. Experiment I., No. 2. X 330. (Hartnach's No. 7, Zeiss's No. 2 eyepiece, Abbe's condenser.)
Fig. 7. – Alveolus filled by a giant cell containing several bacilli. Experiment XIV., No. 3. X 330. . (Hartnach's No. 7, Zeiss's No. 2 eyepiece, Abbe's condenser.)

Plate II

Fig. 8. – Section of fibrous part of lung from which Fig 2, Plate I was taken, showing giant cells, one of which seems to be forming a blood-vessel. No bacilli are present among the fibrous tissue or in the giant cells. The advantages of flooding the field with light by means of a condenser properly employed is well seen, for nothing but the stained bodies is visible, though in reality the main mass of the field consisted of dense fibrous tissue. X 330. . (Hartnach's No. 7, Zeiss's No. 2 eyepiece, Abbe's condenser.)
Fig. 9. – Section of kidney at margin of tuberculous mass showing kidney tubule containing a mass of caseous material with numerous bacilli. Experiment XIV., No. 1. X 330. . (Hartnach's No. 7, Zeiss's No. 2 eyepiece, Abbe's condenser.)
Fig. 10. – Tubercle bacilli. X 2,350. (Powell and Lealand's 1/25th oil objective N.A. 1.38)
Fig. 11. – Wall of cavity in case of phthisis showing the epithelioid tissue between the caseous mass and the inflammatory tissue. Bacilli in the epithelioid cells resembling somewhat the appearance of the bacilli in leprosy as regards their arrangement. X 330. . (Hartnach's No. 7 objective, Zeiss's No. 2 eyepiece, Abbe's condenser.)

Fig. 12. – Tubercles in the lung showing the alveoli full of caseous material and bacilli. X 330. . (Hartnach's No. 7, Zeiss's No. 2 eyepiece, Abbe's condenser.)
Fig. 13. – Tubercle in the lung showing epithelioid cells in alveolus surrounded by thickened wall of the alveolus. Three bacilli in the centre of the epithelioid mass. X 330. . (Hartnach's No. 7 objective, Zeiss's No. 2 eyepiece, Abbe's condenser.)