The etiology of tuberculosis

1882 • Robert Koch

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THE DISCOVERY OF VILLEMIN THAT tuberculosis can be transmitted to animals has been confirmed a number of times, but has also been opposed on seemingly good grounds, so that up until recently it has not been possible to state for certain whether tuberculosis is an infectious disease or not. Since then, Cohnheim and Salomonsen, and later Baumgarten, have achieved success by inoculation in the anterior chamber of the eye, and Tappeiner has been successful with inhalation. These studies have shown without a doubt that tuberculosis must be counted amongst the infectious diseases of mankind.

If the importance of a disease for mankind is measured from the number of fatalities which are due to it, then tuberculosis must be considered much more important than those most feared infectious diseases, plague, cholera, and the like. Statistics have shown that 1/7 of all humans die of tuberculosis. . . .

The nature of tuberculosis has been studied by many, but has led to no successful results. The staining methods which have been so useful in the demonstration of pathogenic microorganisms have been unsuccessful here. In addition, the experiments which have been devised for the isolation and culture of the tubercle virus * have also failed, so that Cohnheim has had to state in the newest edition of his lectures on general pathology, that "the direct demonstration of the tubercle virus is still an unsolved problem."

In my own studies on tuberculosis I began by using the known methods, without success. But several casual observations have induced me to forego these methods and to strike out in a new direction, which has finally led me to positive results.

The goal of the study must first be the demonstration of a foreign parasitic structure in the body which can possibly be indicted as the causal agent. This proof was possible through a certain staining procedure which has allowed the discovery of characteristic, although previously undescribed bacteria, in organs which have been altered by tuberculosis. . . .

The material for study was prepared in the usual manner for the study of pathogenic bacteria. It was either spread out on cover slips, dried, and heated, or cut into pieces after dehydration with alcohol. The cover

* [The word "virus" as used here means "infective agent."]

slips or pieces were placed in a dye solution which contained 200 cc. distilled water with 1 cc. of a concentrated alcoholic solution of methylene blue. They were shaken and then 0.2 cc. of 10% potassium hydroxide added. This mixture should not give a precipitate after standing for days. The material to be stained should remain in this solution for 20–24 hours. By heating this solution at 40°C. in a water bath, this time can be shortened to ½ to 1 hour. The cover slips are then immersed in a freshly filtered aqueous solution of vesuvin for 1–2 minutes, and then rinsed in distilled water. When the cover slips are removed from the methylene blue, the adhering film is dark-blue and strongly overstained, but the treatment with vesuvin removes the blue color and the films seem light brown in color. Under the microscope the structures of the animal tissues, such as the nucleus and its breakdown products, are brown, while the tubercle bacteria are a beautiful blue. Indeed, all other types of bacteria except the bacterium of leprosy assume a brown color. The color contrast between the brown colored tissues and the blue tubercle bacteria is so striking, that the latter, although often present in very small numbers, are quite easy to find and to recognize.

The tissue slices are handled differently. They are removed from the methylene blue solution and placed in the filtered vesuvin solution for 15–20 minutes and then rinsed in distilled water until the blue color has disappeared and a more or less strong brown tint remains. After this, they can be dehydrated with alcohol, cleared in clove oil and can be immediately examined under the microscope in this fluid or first placed in Canada balsam. In these preparations the tissue components are brown, and the tubercle bacteria are a most distinct brown.

Further, the bacteria are not stained exclusively with methylene blue, but can take up other aniline dyes with the exception of brown dyes, when they are treated at the same time with alkali. However, the staining is not so clear as with methylene blue. Further, it can be shown that the potassium hydroxide solution can be replaced with sodium or ammonium hydroxide, which shows that it is not the potassium which is especially important, but the strongly alkaline properties of the solution which are necessary. . . .

The bacteria visualized by this technique show many distinct characteristics. They are rod-shaped and belong therefore to the group of Bacilli. They are very thin and are only one-fourth to one-half as long as the diameter of a red blood cell, but can occasionally reach a length as long as the diameter of a red cell. They possess a form and size which is surprisingly like that of the leprosy bacillus. . . . In all locations where the tuberculosis process has recently developed and is progressing most rapidly, these bacilli can be found in large numbers. They ordinarily form small groups of cells which are pressed together and arranged in bundles, and frequently are lying within tissue cells. They present in places a picture similar to that in tissue which contains leprosy bacilli. Many times the bacteria occur in large numbers outside of cells as well. Especially at the edges of large, cheesy masses, the bacilli occur almost exclusively in large numbers free of the tissue cells.

As soon as the peak of the tubercle eruption has passed, the bacilli become rarer, but occur still in small groups or singly at the edge of the tubercle mass, with many lightly stained and almost invisible bacilli, which are probably in the process of

dying or are already dead. Finally they can disappear completely, but this complete disappearance occurs only rarely, and then only in such sites where the tuberculosis process has stopped completely. . . .

Because of the quite regular occurrence of the tubercle bacilli, it must seem surprising that they have never been seen before. This can be explained, however, by the fact that the bacilli are extremely small structures, and are generally in such small numbers, that they would elude the most attentive observer without the use of a special staining reaction. Even when they are present in large numbers, they are generally mixed with finely granular detritus in such a way that they are completely hidden, so that even here their discovery would be extremely difficult. . . .

On the basis of my extensive observations, I consider it as proven that in all tuberculous conditions of man and animals there exists a characteristic bacterium which I have designated as the tubercle bacillus, which has specific properties which allow it to be distinguished from all other microorganisms. From this correlation between the presence of tuberculous conditions and bacilli, it does not necessarily follow that these phenomena are causally related. However, a high degree of probability for this causal relationship might be inferred from the observation that the bacilli are generally most frequent when the tuberculous process is developing or progressing, and that they disappear when the disease becomes quiescent.

In order to prove that tuberculosis is brought about through the penetration of the bacilli, and is a definite parasitic disease brought about by the growth and reproduction of these same bacilli, the bacilli must be isolated from the body, and cultured so long in pure culture, that they are freed from any diseased production of the animal organism which may still be adhering to the bacilli. After this, the isolated bacilli must bring about the transfer of the disease to other animals, and cause the same disease picture which can be brought about through the inoculation of healthy animals with naturally developing tubercle materials.

The many preliminary experiments which helped to solve this problem will be passed over, and only the final method will be described. The principle of this method is based on the use of a solid, transparent medium, which can remain solid even at incubator temperature. The advantage of a solid medium for bacteriological research in the production of pure cultures has been discussed by me in an earlier paper.* This same procedure has led to the solution of the difficult problem of the pure culture of the tubercle bacillus and is further proof of the value of this method.

Serum from cow or sheep blood, which is obtained as pure as possible, is placed in cotton-plugged test tubes and heated every day for six days, one hour per day at 58°C. Through this procedure it has been possible in most cases to completely sterilize the serum. This serum is then heated for a number of hours at 65°C., until it has solidified completely. The serum appears after this treatment as an amber-yellow, perfectly transparent or lightly opalescent, solid gelatinous mass. When this is placed for a number of days in the incubator, no bacterial colonies develop. . . . In order to obtain a large surface for the culture, the serum is allowed to harden while the test tubes are in a slanted position. . . .

* [See page 101.]

On this solidified blood serum, the tuberculous materials are placed in the following manner.

The simplest way, and one which is almost always successful, is by the use of an animal which has just died of tuberculosis, or by the use of an animal suffering from tuberculosis which is killed for this purpose. First the skin of the breast and abdomen is laid to the side with a flamed instrument. Then the ribs are cut in the middle with a flamed scissors and forceps, and a portion of the ribs are removed without at the same time opening the abdominal cavity. The lungs are then to a great extent uncovered. The instruments used here are now discarded and freshly sterilized ones taken up. Single tubercles or particles about the size of a millet seed are quickly cut out of the lung tissue and immediately carried over to the surface of solidified serum in a test tube, with the use of a flamed platinum wire. Naturally the cotton plug should only be exposed to the air for the shortest possible time. In this way, a number of test tubes, perhaps 5–10, are inoculated with tuberculous material. Such a large number are prepared because even with the most careful manipulations, not all test tubes can remain free of accidental contamination. . . .

These test tubes are now placed in an incubator and are kept there for a long time at 37–38°C. In the first week, no noticeable changes take place. Indeed, if bacteria develop in the first days, either around the inoculum or away from it, these usually white, gray, or yellowish droplets, which often bring about the liquefaction of the serum, are due to contamination, and the experiment is a failure.

The growth of the tubercle bacilli can first be seen by the naked eye in the second week after seeding, ordinarily after the 10th day. They appear as very small dots, dry and scale-like. This growth arises from the material inoculated, and if the tubercle has been spread around extensively on the surface, then a large amount of growth ensues, while if the tubercles have remained in small patches, then the bacterial growth is less extensive. If there are only very few bacilli in the inoculum, then it is hardly possible to free the bacilli from the tissue and have them growing directly on the nutrient medium. . . . With the help of low magnification, 30–40 power, the colonies of the bacilli can already be seen at the end of the first week. . . .

The growth of the culture ceases after several weeks, and a further increase probably does not occur because the bacilli have lost their own power of movement,* and only spread because of the slow reproduction of the bacilli, being pushed forward on the surface, and because of the slow growth of the bacilli, this spread can only occur to a small extent. In order to keep such a culture going, it must be brought onto a new medium 10–14 days after the first inoculation. This is done by removing several of the small scales with a flamed platinum wire, and transferring them to a fresh, sterilized serum slant, where the scales are broken up and spread out as much as possible. Further scaly, dry masses then develop which coalesce and cover more or less of the surface of the serum, depending upon the extent of the seeding. In this way the culture can be continued.

The tubercle bacilli can also be cultured on other nutrient substrates, if the latter possess similar properties to the solidified serum. They are able to grow on a solidified gel which remains solid at incubator temperature,

* [The tubercle bacillus is not motile.]

prepared by adding agar-agar * to a meat infusion or peptone medium. However, on this medium the bacilli form only irregular small crumbs, which are not nearly so characteristic as the growths on blood serum.

Originally I cultivated the tubercle bacilli only from lung tubercles of guinea pigs which had been infected with tubercular material. Therefore the cultures from various sources had first to pass through the intervening stage of the guinea pig before they were obtained in pure cultures. In this way there was a possibility for error, in the same way as in the transfer of a culture from one test tube to another. This might occur through the accidental inoculation of other bacteria into the animal, or through the appearance in the guinea pig of spontaneous tuberculosis. In order to avoid such errors, special precautions are necessary, which can be deduced from observations on the behavior of this spontaneous tuberculosis.

From hundreds of guinea pigs that have been purchased and have occasionally been dissected and examined, I have never found a single case of tuberculosis. Spontaneous tuberculosis develops only occasionally and never before a time of three or four months after the other animals in the room have been infected with tuberculosis. In animals which have become sick from sponstaneous tuberculosis, the bronchial glands become quite swollen and full of pus, and in most cases the lungs show a large, cheesy mass with extensive decomposition in the center, so that it occasionally resembles the similar processes in the human lung. . . . Animals that have been inoculated with tuberculosis show a completely different picture. The place of inoculation of the animals is in the abdomen. close to the inguinal gland. This first becomes swollen and gives an early and unmistakable indication that the inoculation has been a success. Since a larger amount of infectious material is present at the beginning, the infection progresses much faster than the spontaneous infection, and in tissue sections of these animals, the spleen and liver show more extensive changes from the tuberculosis than the lungs. Therefore it is not at all difficult to differentiate the artificially induced tuberculosis from the spontaneous tuberculosis in experimental animals. From a consideration of these facts, it can be concluded that the development of tuberculosis in an experimental animal is due to the action of inoculated material, when a number of guinea pigs are purchased and inoculated at the same time in the same way with the same material, and kept separated from other animals in their own cage, and when they show the development of the characteristic tuberculosis symptoms of inoculated animals in a short period of time.

In this way, a substance can be tested for its virulence by inoculating four to six guinea pigs with it, after making use of all precautions, such as previously disinfecting the site of inoculation, using sterile instruments, etc. The results are uniformly the same. In all animals which are inoculated with fresh masses containing tubercle bacilli, the small inoculation site has almost always coalesced on the next day, then remains unaltered for about eight days, then forms a little nodule which may enlarge without breaking open, although it most often changes into a flat, dry abscess. After about two weeks, the inguinal glands and axillary glands on the side where the inoculation has occurred enlarge

* [Koch did not at this time seem to be aware of the superiority of agar as a solidifying agent.]

until they are the size of peas. From then on the animals become progressively weaker and die after four to six weeks, or are killed in order to exclude the later development of spontaneous tuberculosis. In the organs of all of these animals, and most especially in the spleen and liver, the recognizable changes due to tuberculosis occur. That these changes in the guinea pigs are only due to the inoculation of material containing the tubercle bacilli, can be see from experiments in which inoculation was performed with scrofulous glands or fungus masses from joints, in which no tubercle bacilli could be found. In these cases, not a single animal became sick, while in the animals inoculated with bacilli-containing material, the inoculated animals always showed an extensive infection with tuberculosis after four weeks.

Cultures of tubercle bacilli were prepared from guinea pigs which had been inoculated with tubercles from the lungs of apes, with material from the brain and lungs of humans that had died of miliary tuberculosis,* with cheesy masses from phthisistic lungs, and with nodules from lungs and from the peritoneum of cows affected with bovine tuberculosis. In all these cases, the disease processes occurred in exactly the same way, and the cultures of bacilli obtained from these could not be differentiated in the slightest way. In all, 15 pure cultures were made of tubercle bacilli, four from guinea pigs infected with ape tuberculosis, four with bovine tuberculosis, and seven with human tuberculosis.

In order to answer the objection that the nature of the bacilli was changed through the preliminary inoculation into guinea pigs, so that they became more similar, experiments were set up to cultivate tubercle bacilli directly from spontaneous cases in man and animals.

This was successful a number of times, and pure cultures have been obtained from the lungs of two people with miliary tuberculosis, as well as one with cheesy pneumonia, twice from the contents of small cavities in phthisistic lungs, once from cheese-like mesenteric glands, twice from freshly removed scrofulous glands, twice from lungs of cows with bovine tuberculosis, and three times from the lungs of guinea pigs that had suffered spontaneous tuberculosis. All of these cultures were quite similar and also resembled those that had been isolated through the preliminary guinea pig inoculation, so that the identity of the bacilli occurring in the various tuberculous processes cannot be doubted. . . .

Up until now my studies have shown that a characteristic bacillus is always associated with tuberculosis, and that these bacilli can be obtained from tuberculous organs and isolated in pure culture. It now remained to prove the most important question, namely, that the isolated bacilli were able to bring about the typical tuberculosis disease process when inoculated again into animals. . . .

The results of a number of inoculation experiments with bacillus cultures inoculated into a large number of animals, and inoculated in different ways, all have led to the same results. Simple injections subcutaneously, or into the peritoneal cavity, or into the anterior chamber of the eye, or directly into the blood stream, have all produced tuberculosis with only one exception. Further, the infection was not limited to only isolated nodules,

* [An acute, systemic form of the disease.]

but depending upon the size of the inoculum, large numbers of tubercles were produced. . . .

A confusion with spontaneous tuberculosis, or an accidental infection with tubercle virus in the experimental animals, is excluded for the following reasons: (1) Spontaneous tuberculosis or accidental infection cannot develop in so short a time into the extensive eruption of tubercles experienced here. (2) The control animals, which were handled in exactly the same way as the inoculated animals, remained healthy. (3) The typical picture of miliary tuberculosis does not occur when guinea pigs or rabbits are injected with other substances. . . .

All of these facts taken together lead to the conclusion that the bacilli which are present in the tuberculous substances not only accompany the tuberculosis process, but are the cause of it. In the bacillus we have, therefore, the actual tubercle virus.

Comment

The scientific world quickly recognized the importance of this work of Koch, and it was widely acclaimed. We must consider it his masterpiece and the culmination of all the work he had done before. We can see the evolution of his work clearly through the last four papers. This evolution is all the more remarkable when we remember that in 1876, only six years previously, Koch published his first work on anthrax. In those six years he developed a series of new techniques, and it was these techniques which enabled him to discover the tubercle bacillus.

Several properties of the tubercle bacillus make it an organism that is extremely difficult to work with, and it is remarkable that Koch achieved such quick success in his experiments. The organism is extremely tiny, being at any rate a tenth the size of the anthrax bacillus. It is very difficult to stain successfully, due to a waxy layer on its cell surface. Further, it is a very slow-growing organism and requires several weeks for good growth on solid media. Thus Koch had to be extremely persistent in his work. If he had thrown out his cultures after one week, he would have been unsuccessful. It was necessary to have patience and a faith that tuberculosis was an infectious disease.

Koch was also fortunate that the strain of tubercle bacillus that is pathogenic for humans can be transferred so readily to guinea pigs. Without an experimental animal which showed characteristic symptoms upon inoculation with tuberculous material, his work would have been much harder. He might have cultured the organism successfully, but the actual proof that this organism was the causal agent for tuberculosis would have been much more difficult. It should be noted that in this paper he does not have a final proof that the organism he has isolated in pure culture is really the cause of human tuberculosis. This could only be done by making inoculations in humans. Since this cannot be done, we can only infer that the isolated organism causes the human disease. Such a dilemma is always with the investigator of human diseases. He must learn to live with it