

The etiology of tuberculosis [Koch's postulates.]

1884 • Robert Koch

Koch, Robert. 1884. Die Aetiologie der Tuberkulose. *Mittheilungen aus dem Kaiserlichen Gesundheitsamte*, Vol. 2, pages 1-88.

IT WAS FIRST NECESSARY TO DETERMINE if characteristic elements occurred in the diseased parts of the body, which do not belong to the constituents of the body, and which have not arisen from body constituents. When such foreign structures have been demonstrated, it is further necessary to ascertain if these are organized and if they show any of the characteristics of independent organisms, such as motility, growth, reproduction, and fructification. It is further necessary to determine the relationships of these structures to their surroundings, the behavior of the neighboring tissue substances, the distribution of the foreign substances in the body, their appearance in the various stages of the disease, and such similar circumstances, which would allow one to conclude with greater or lesser probability that there is a causal relationship between these structures and disease. The facts obtained in this study may possibly be sufficient proof of the causal relationship, that only the most sceptical can raise the objection that the discovered microorganism is not the cause but only an accompaniment of the disease. However, many times this objection has a certain validity, and then it is necessary to ob-

tain a perfect proof to satisfy oneself that the parasite and the disease are not only correlated, but actually causally related, and that the parasite is the actual direct cause of the disease. This can only be done by completely separating the parasite from the diseased organism, and from all of the products of the disease which could be subscribed to a disease-inducing influence, and then introducing the isolated parasite into healthy organisms and induce the disease anew with all its characteristic symptoms and properties. An example will clarify the above statements. If the blood of an animal dying of anthrax is examined, one finds in it a large number of regular, rod-shaped, colorless, immotile structures. It is not directly evident that these rods are plant-like, since in fact they were first taken by many to be non-living, crystalline structures. Only when it was possible to watch these structures grow, form spores, and then form new rods from the spores, could it be concluded with certainty that they were living and belonged to the class of lower plants. Further, if the rod-containing blood of an animal which had died of anthrax was inoculated in an extremely minute quantity into another animal,

Koch • The etiology of tuberculosis [Koch's postulates]

117

this second animal always died of anthrax and its blood contained the characteristic rods, the so-called anthrax bacilli. However, this has not proved that through the inoculation of the rods, the disease was transmitted, because not only the rods were inoculated, but also the other formed and unformed elements of the blood. In order to decide whether it is the bacilli or some other substance of the anthrax blood which causes anthrax, the bacilli must be isolated from the blood and inoculated by themselves. The most certain way of isolating the bacilli is through continued pure culture. For this purpose, a small amount of blood containing bacilli is placed on a solid medium on which the bacilli are able to grow, such as nutrient gelatin, or boiled potato. On these they begin to reproduce quickly and soon are present in large numbers, while the other substances of the blood, the red and white cells and the blood serum, remain unchanged. After two or three days when the bacilli have formed a dense mass of sporulating filaments, a very small amount of this white mass is taken and streaked again on nutrient gelatin or boiled potato. The bacilli reproduce again in the same way as before and form again a dense white covering on the potato, and already in this second culture there are no traces under the microscope of the other elements of the blood. In the same way the culture is transferred a number of times. After the third or fourth transfer, one can consider the bacilli to be completely free from the original blood substances that were inoculated with them. Now if the culture is transferred twenty or fifty times, then it can be assumed with complete certainty that the bacilli no longer are

associated with even the slightest amount of disease products from the body. They cannot even have captured these products within their own cells, since the original seeded bacteria are long since gone and the many generations of offspring have taken their nutrients for the production of their necessary substances from the potato itself. The anthrax bacilli in pure culture in this way have therefore no relationships with the first organisms that came out of the blood, or with the disease products which belong to the metabolism of the animal. In spite of this, they are able to induce fatal anthrax as soon as they are inoculated into a healthy animal. The inoculated animal dies just as fast, and with the same symptoms, as one inoculated with fresh anthrax blood, or one which succumbs to spontaneous anthrax. Also, in its blood appear the characteristic anthrax bacilli in countless numbers. From these facts no other conclusion can be drawn than that the anthrax bacilli are the actual cause of this disease, and not merely an attendant phenomenon or symptom. . . . These conclusions are so certain, that no one will dispute them, and the anthrax bacillus will be looked upon by the scientific world as the causal agent of ordinary, typical anthrax infection in both our domestic animals and in man himself.

The process outlined above, which has been successful in proving the parasitic nature of anthrax, and which has led to inescapable conclusions, has been used as the basis for my studies on the etiology of tuberculosis. These studies first concerned themselves with the demonstration of the pathogenic organism, then with its isolation, and finally with its reinoculation.

Comment

This paper presents for the first time the so-called "Koch's postulates." It should be noted that they are published in 1884, after Koch had completed his major work. They were therefore not guideposts for Koch's research, but formalizations of certain assumptions that he had adopted unconsciously in his earlier work. Furthermore, it is apparent that Koch would not have developed these postulates if the nature of the diseases he had worked with, especially

tuberculosis, had not forced him to do so. In his first work on anthrax there is no evidence of the organized concept we see in the present paper. Therefore, although he uses anthrax as the example to illustrate his present ideas, we cannot assume that he originally got the ideas while working on anthrax. Rather these ideas were forced upon him during his later work, and especially while working on tuberculosis.

A method for staining the tubercle bacillus

1882 • Paul Ehrlich

Ehrlich, P. 1882. Aus dem Verein für innere Medizin zu Berlin. Sitzung vom 1. Mai 1882. *Deutsche medizinische Wochenschrift*, Vol. 8, pages 269-270.

ONLY A FEW WEEKS AGO HERR Regierungs-Rath Dr. Koch reported on his highly significant work on the etiology and on the bacillus of tuberculosis. There is now a general duty to evaluate this extension of our knowledge in its relationships to diagnosis and therapy. I have been working in this direction and believed that this subject must be reexamined in its diagnostic significance. The main results of my experiments concerning a particular modification of the method will be presented here. It seems to me that these results indicate a certain simplification in the diagnostic procedures and at the same time give clues to certain characteristics of the tubercle bacillus.

As is known, the method of Herr Reg.-Rath Koch consists of staining dried preparations in a weakly alkaline solution of methylene blue. After 24 hours they are then treated with a solution of vesuvin. Then the preparation becomes brown, and under the microscope all of the substances are strongly brown, while the bacillus alone remains an intense blue. The principle of the method is that the methylene blue solution must remain alkaline. I have begun with this requirement for alkaline conditions and have attempted to substitute another alkali for that used by Koch. I have found that aniline is a suitable substitute.

Permit me now to describe my

Ehrlich • A method for staining the tubercle bacillus

method in a few passages. I have worked almost exclusively with dried preparations prepared from sputum, but I have also made control experiments which show that the method is also useful for tissue sections.

With a preparation needle I remove a small particle from the sputum and press it flat between two cover slips. Cover glasses of 0.10 to 0.12 mm. are the most suitable. Under these conditions it is quite easy to spread the drop of sputum equally thin throughout. The two cover slips are then pulled apart and one has two thin sheets, which are allowed to dry in the air. These preparations are not yet ready. It is expedient to fix the proteins. I ordinarily do this by keeping the preparations for one hour at 100-110°C. However, in actual practice, a more suitable procedure, which I have seen in operation at the Imperial Office of Health, is to take the dried preparations with forceps and pass them three times through the flame of a Bunsen burner.

For the staining I use water-saturated aniline oil. This is prepared by shaking water with an excess of aniline oil and filtering through a moist filter. To the water-clear liquid obtained is added dropwise an alcoholic-saturated solution of fuchsin or methyl violet until a distinct opalescence develops which indicates that the dye is saturated. The preparations are immersed in this liquid and within one-fourth to one-half hour they have become intensely colored. The differentiation of the tubercle bacilli proceeds only very slowly with vesuvin, so that it is necessary to use acid. I have used strong, even heroic, concentrations of acid. The most effective is to mix one volume official nitric acid and two volumes of water. Within a few seconds one can see the preparation fade under the acid treatment, yellow clouds are given off and the preparation becomes white. If one would examine the preparation at this stage, one would see that every part of the preparation had been decolorized except the tubercle bacilli, and they had remained intensely colored. Such a preparation could be examined at this stage, but the technical difficulties are considerable, it is difficult to focus on the bacilli, and it is better to stain the background with a contrasting color, such as yellow, orange, or red. The preparation is violet, or blue, if stained with aniline oil; if stained with fuchsin or methyl violet, the preparation is red.

May I briefly point out the advantages of this method. The aniline oil stains more gently on the tissues than does the strong alkali. In the latter, the slime especially is easily dissolved. A further advantage is the rapidity of the technique. While the Koch procedure takes 24 hours, this procedure three-fourths to four-fifths of an hour is sufficient to make a preparation. Even more important is the fact that the preparations are intensely colored and the bacilli seem to be significantly larger than in Koch's procedure, considering that the background is lighter, this makes it possible to differentiate the bacilli at a lower magnification. I believe also that more bacilli are stained on the preparations in my technique, which may be important for the statistics of sputum examinations.

I have been able to stain the tubercle bacillus in all basic aniline dyes, as well as in bismarck brown, so it is apparent that the substance of the bacillus is different in its staining characteristics from that of other bacterial species. However, the tubercle bacillus differs in its staining from all other fungi, and this is apparently due to the presence of an outer layer which possesses characteristic and specific properties. One of these is that the outer layer is permeable to dyes only under the influence of alkali.

A further point which I would like