

The etiology of anthrax, based  
on the life history of  
*Bacillus anthracis*

1876 • Robert Koch

Koch, Robert. 1876. Die Aetiologie der Milzbrand-Krankheit, begründet auf die Entwicklungsgeschichte des Bacillus Anthracis. *Beiträge zur Biologie der Pflanzen*, Vol. 2, No. 2, pages 277-310.

I. INTRODUCTION

SINCE THE DISCOVERY OF ROD-SHAPED bodies in the blood of animals dying of anthrax, there has been much effort

directed to attempts to prove that these rod-shaped bodies are responsible for the transmissibility of this disease as well as for the sporadic appearance of it. These studies have

sought to determine whether these bodies are the unique contagium of anthrax. Recently Davaine has carried out a number of inoculation experiments with fresh and dried blood containing the rods and has stated decisively that these rods are bacteria, and that only in the presence of these bacteria can a fresh case of anthrax be produced. The lack of proof of the direct transmission of the anthrax disease in man and animals is due to the ability of the bacteria to remain alive for a long time in dry conditions and to be transmitted through the air by insects and the like. It seems here that the mode of transmission of anthrax has been explained.

Nevertheless, these ideas of Davaine have found many opponents. Several workers have obtained experimental anthrax by inoculating blood containing bacteria, but have been unable to show the presence of bacteria in the blood of the diseased animals. Others have been able to induce anthrax by inoculation with blood which could not be shown to contain bacteria, but the diseased animals then had bacteria in their blood. Others have noted that anthrax is not derived solely from a contagium which is transmitted above ground, but that this disease is related in some way with conditions of the soil. . . .

These experiences cannot be explained by the hypothesis of Davaine, and because of this, many people feel that bacteria are of no significance for anthrax.

Since I have had the opportunity several times of examining animals which had died of anthrax, I performed a series of experiments which would clear up the uncertainties in the etiology of anthrax. Through these, I came to the conclusion that the theory of Davaine concerning the transmission of anthrax is only partly correct.

I could show that the rods in the anthrax blood were not so resistant as Davaine had believed. As I will show later, the blood, which contains only rods, keeps its ability to induce anthrax on inoculation only a few weeks in the dry state, and only a few days when moist. How is it possible then for an organism which is so easily destroyed to maintain itself as a dormant contagium for a year in soil and throughout the winter? If bacteria are really the cause of anthrax, then we must hypothesize that they can go through a change in life history and assume a condition which will be resistant to alternate drying and moisture. What is more likely, and what has already been indicated by Prof. Cohn is that the bacteria can form spores which possess the ability to reform bacteria after a long or short resting period.

All of my experiments were designed to discover this developmental stage of the anthrax bacterium. After many unsuccessful experiments, I was finally able to reach this goal, and thus to find a basis for the etiology of anthrax.

Since the life history of the anthrax bacterium offers not only botanical interest but also much light on the heretofore uncertain etiology of the soil-related infectious diseases, I am publishing now the most important results of my experiments, although my work is still in progress.

## II. LIFE HISTORY OF *Bacillus anthracis*

It has not been possible for me to observe the multiplication of the bacteria directly in the animal. But it can be inferred that this occurs from the inoculation experiments which follow. I have used the mouse as my experimental animal, as it is simple to use. . . . In most experiments I inoculated them at the base of the tail, where

the skin is loose and covered with long hair. . . . I have made a large number of inoculations in this way, using fresh anthrax material, and in every case I have had a positive result, and I believed therefore that the success of the inoculation could be used as an indication of the life or death of the bacilli inoculated. I will show through later experiments that this idea is true.

Partly in order to always have available fresh material, and partly to discover if the bacilli would change into another form after a certain number of generations, I inoculated a number of mice in series, one from the other, each time using a mouse which had just died as a source of the splenic material. The longest series of mice treated in this way was twenty, which therefore represented that many generations of the bacilli.\* In all animals the results were the same. The spleen was markedly swollen and contained a large number of transparent rods which were very similar in appearance and were immotile and without spores. This same type of bacillus could be found also in the blood, but not in so great a number as in the spleen. In these experiments it was shown, therefore, that a small number of bacilli could always develop into a significant mass of individuals of the same type . . . which appeared to reproduce by growing in length and then splitting after they had reached about twice the length of the individual bacilli. These results also indicate that it is highly unlikely that the bacilli would go through some change in form if a longer series of inoculations were made, and therefore it is unlikely that there is ultimately some alternation of generations. . . .

It will take us too far afield to consider whether or not the actual cause

\* [We know now that the number of generations of the bacteria would be much greater than twenty.]

of the death of the animals is due to the production of carbon dioxide in the blood through the rapid growth of the bacilli there, or, what seems more probable, that death is due to a metabolic product produced by the parasite through its utilization of proteins as nutrients, and that this metabolic product is poisonous to the animal. . . . †

[To study the life history of the bacilli away from the animal,] a drop of fresh beef serum or aqueous humor from the eye of a cow was placed on a microscope slide. Then a small piece of spleen which contained bacteria and which had been freshly removed from an infected animal was placed in this and a cover glass placed on top. The microscope slide was then placed in a moist chamber to keep the liquid from evaporating, and this was then placed in an incubator. . . .

These preparations were incubated for 15–20 hours at 35–37°. At the end of this time, in the middle of the preparation between the tissue cells could be seen many unaltered bacilli, although in smaller numbers than in fresh preparations. However, away from the tissue in the fluid, one could see bacilli which were 3–8 times longer and showed shallow bends and curvatures (Fig. 2). † The closer to the edge of the cover glass, the longer the filaments, and these finally reached a size which was a hundred or more times the length of the original bacilli (Fig. 3). Many of these long filaments had lost their uniform structure and transparent appearance, and their contents had become finely granulated with the regular appearance of strongly

† [Such a metabolic product, known today as a toxin, is usually associated in some way with most infectious diseases, including anthrax.]

‡ [This plate also contains the figures for the paper of Cohn, 1876: "Studies on the biology of the bacilli." See page 49 for the text of this paper.]

light-refracting grains (Fig. 3a). The filaments which lay right at the edge of the cover glass, where the gas exchange with the nutrient fluid was the best, showed the most extensive development. They contained completely formed spores which were imbedded in the substance of the filaments at regular distances and were somewhat oval, strongly light-refracting bodies. In this form the filaments revealed a remarkable appearance, which can best be compared with a string of pearls.

Many filaments had already lost their spores, which can be seen between them as small, free clusters (Fig. 4b). In favorable preparations

it is possible to see all of the stages from short bacillus rods, through long, sporulating filaments, to free spores, and this is proof that the latter arises from the former. . . . [Because these spores seemed to form most frequently at the edge, it occurred to Koch that they might not actually come from the *Bacillus*, but be due to contamination from the air, since his preparations were not pure, and he had observed micrococci and bacterium types from time to time. So he decided that the only way to be sure would be to observe the spore formation actually take place.]

Although I had imagined that such an experiment would be very difficult

#### Plate—Koch and Cohn

Figs. 1-7. ANTHRAX BACILLUS (*Bacillus Anthracis*). Fig. 1. Anthrax bacilli from the blood of a guinea pig. The bacilli appear as transparent rods, occasionally with beginnings of division, or bent. (a) white blood cells. (b) red blood cells. Fig. 2. Anthrax bacilli from the spleen of a mouse, after three hours in a drop of aqueous humor. The bacteria have lengthened into filaments . . . Fig. 3. From the same preparation as Fig. 2, after ten hours. The bacilli have grown to long filaments, which often are intertwined. (a) in isolated filaments strongly refractile bodies appear regularly spaced. Fig. 4. The same culture as Fig. 3, after 24 hours. (a) oval spores appearing like beads on a string have developed in the filaments. (b) many filaments are undergoing decomposition and releasing free spores, which occur singly or in clumps. Fig. 5. Spore germination. Fig. 5a and 5b at different magnifications. The spores elongate into cylindrical bodies, with the refractile area remaining at one pole. This body becomes smaller, breaks up into 2 or more parts, and finally disappears completely. Fig. 6. Diagram of the method of culture of anthrax bacilli. The fluid containing the bacteria is in the hollow of a hanging drop slide, covered with a cover glass which is ringed with olive oil to prevent evaporation. The slide is placed on a warm stage heated to body temperature, so that observations can be made during incubation. The bacilli are suspended in a drop of fresh

aqueous humor. Even with the naked eye it is possible to see the developing masses of filaments. Fig. 7. Appearance of the epithelial layer from the skin of a frog, in which a piece of infected spleen from a mouse had been placed. The layer consists of large, nuclear cells (a); in occasional cells many short, occasionally bent or crooked *Bacilli* (b) have been taken up, which have developed further within the cells and later escaped; (c) disrupted cell; (g) free spiral *Bacilli*; (e) blood cells of the frog. In addition, unaltered *Bacilli* are visible. Figs. 8-10. HAY BACILLUS (*Bacillus subtilis*). Fig. 8. *Bacilli* in long parallel rows of filaments, which had formed an iridescent film on the surface of a boiled hay infusion after 24-48 hours. Between the parallel rows are seen short motile rods, or rods in the process of elongating. Fig. 9. Spore formation in the segmenting filaments of the hay *Bacillus* after 3 days. Fig. 10. *Bacillus* filaments encased in slime . . . at the left end of the figure can be seen the beginning of the formation of chains of spores in the filaments. Fig. 11. Portion of a mass surrounded by slime, in which the formation of chains of spores is complete, and the single *Bacillus* filaments have become indistinct; the spores however are still arranged in parallel rows and are bound together by the slime. MAGNIFICATIONS, Figs. 1-7, 8 and 10, 650X; Figs. 5b and 9, 1650X; Fig. 11, 900X. [The handwriting on the figure was placed there later by some interested reader.]



to perform, it actually proved quite simple. . . .

[The preparations were so arranged that they could be observed under the microscope while being incubated continuously.]

Observations every 10–20 minutes revealed that the bacilli at the beginning were somewhat thicker and seemed to be swollen and hardly showed any changes in the first two hours. Then they began to grow. After 3–4 hours they had already lengthened 10–20 times; they began to curve, to push against each other or to cross each other and make a network. After a few more hours the individual filaments were already so long that they covered several microscope fields. . . .

If the free end of a filament was observed continuously for 15–20 minutes, it was quite easy to observe its lengthening and perceive the remarkable spectacle of actually watching the bacillus grow. It was therefore possible to obtain direct evidence of the further development of these filaments. After only 10–15 minutes the contents of the strongest and most luxuriantly growing filaments were finely granular, and soon the small, refractile grains were cut off in regular sequence. These enlarged in the space of several more hours into the strongly refractile, oval-shaped spores. Gradually the filaments disintegrated, fragmented at the ends, and the spores became free. . . . In this condition the preparations could remain for weeks without changing. . . .

Observations were made to obtain a complete picture of the life history of the *Bacillus anthracis* and to discover whether the spores passed through some intermediate form, such as a swarm spore, or passed directly into a bacillus. In order to do this it was necessary to discover conditions which would permit the spore to develop into the bacillus which would

allow for direct microscopic observations.

All efforts to obtain the further development of the spores in distilled water or in well water failed. In serum or aqueous humor, the results were equivocal; bacilli developed without question, and these formed filaments and spores, but their number was small and it was not possible to observe the transformation of single spores into bacilli. Finally I arrived at a procedure which was successful. Preparations were used which revealed under the microscope only a pure culture\* of *Bacillus anthracis*, and which contained mostly free spore masses. The spores were allowed to dry on a cover glass . . . and then a drop of aqueous humor was placed on a microscope slide and the cover glass was laid on it so that the mass of spores was wetted by the fluid. These preparations were placed in the moist chamber and incubated at 35°.

After a half hour the remains of the filaments began to disintegrate, and after 1½ to 2 hours they had disappeared.

Already after 3–4 hours the development of the spores could be seen. . . .

By careful examination at high magnification, it appeared that each spore was oval-shaped and was imbedded in a round transparent mass, which appeared like a small, light ring surrounding the spore. The spherical shape of this ring could be easily seen by rolling the spore in various positions. This material first lost its spherical shape, lengthened itself on one side in the direction of the long axis of the spore and became like a long oval. The spore remained in one of the poles of the cylindrical shaped body. Soon the transparent covering

\* [This term will receive a more precise meaning in one of Koch's later papers (see page 101). It is not at all certain that he really had a pure culture at this time.]

became longer and filamentous, and at the same time the spore began to lose its strongly refractile characteristics. It became quickly pale and smaller, broke apart into many pieces, and finally completely disappeared. In Fig. 5 is pictured a mass of spores showing the conversion to filaments.

Later I was able to observe in the same preparation and same drop of aqueous humor the appearance of bacilli from the spores and then later a second generation of spore-containing filaments. . . .

It may be assumed that when these spores in some way reach the blood stream of a sensitive animal, a new generation of bacilli will be produced. In order to prove this assumption, the following experiments were performed. . . .

By the inoculation of mice with material rich in spores, or with material with few spores, the interesting fact was discovered, that in the first

case, with many spores, the mice died after 24 hours, while in the latter case, the mice did not die from anthrax until after three or four days. I have repeated these experiments many times. Such substances containing spores were dried and allowed to stand for a while. When moistened with water and injected, they had not lost their ability to produce anthrax. . . .

On the other hand I have inoculated mice with spore masses which had come from cultures in glass cells and which I had ascertained by microscopic examination to have been derived from completely pure cultures of *Bacillus anthracis*, and every time the inoculated animals died of anthrax. It follows, therefore, that only a species of *Bacillus* is able to cause this specific disease, while other schizophytes have no effects or cause entirely different diseases when inoculated. . . .

### Comment

This was the first proof that a specific microorganism could cause a specific disease in an animal, and although the proof was not perfect, it was good enough for most people. This work is all the more remarkable when it is recalled that Koch was a mere country doctor, with no formal research training. It is not exactly clear what prompted him to begin work on anthrax, but the choice was quite fortuitous. The disease occurs most often in animals, less frequently in man, and produces characteristic symptoms. The organism can be transferred readily to mice by inoculation, and this made it possible for Koch to study the disease in the laboratory. This is an extremely important point, since laboratory study can be carried out under reproducible conditions, and avoids the complications which beset Semmelweis and Lister. The causal organism of anthrax is a very large bacillus, easily seen under the microscope. In infected animals it occurs in very large numbers in the blood stream, sufficient to

be seen by direct examination of blood. The organism has a quite characteristic morphology, making a microscopic identification reasonably certain. In addition, it forms spores. Koch's observation of these spores and of the development of spores from bacilli and bacilli from spores is an important observation in fundamental bacteriology. It confirmed rather nicely the work of Cohn (see page 49) and indicated the medical importance of spore-forming organisms. This was especially noteworthy since Cohn had shown that bacterial spores were highly heat-resistant. Finally, Koch's observations were so thorough and so accurate that his work has remained completely valid up until the present. The success of this work brought Koch to the attention of the German medical world. He soon received the opportunity to move to Berlin, where he was given adequate facilities to further his work. His most important discoveries were yet to come (see pages 96, 101, 109, and 116).