a cloven hoof indicates to the observer the forms of the teeth, of all the big bones, thighs, shoulders, and of the trunk of the body, of the animal which left the mark."[1]

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THE VIRULENCE OF CULTIVATED ANTHRAX VIRUS.

Experimental studies on the artificial attenuation of the infectious properties of the bacillus of Anthrax by means of cultivation. By Dr. R. KOCH, Dr. GAFFKY, and Dr. LOEFFLER.

Mittheilungen aus dem Kaiserlichen gesundheits-amte. Zweiter band. Berlin, 1884. (Extract from the publication of the imperial board of health of Germany.)

PASTEUR'S announcement that the parasites of malignant Anthrax were capable of changing their characteristics when cultivated under certain conditions, and that when thus modified they could be used for protective inoculation, aroused the greatest interest among investigators. Such a statement could not be accepted without confirmation at the hands of other observers; and none were better fitted for this task than the Royal health commission of Germany, at the head of which stands Dr. Robert Koch.

The experiments, which were instituted under his direction, have been carried on for two years, and have shown, that, although the bacilli could be rendered harmless, their protective power was not so great as was expected.

The original communication of the French *savant* was not exact in the details by which the experiments were to be carried out, and Koch had to employ much time in preliminary studies. This cannot, however, be considered as lost, since many valuable facts have been obtained by it.

According to Pasteur, if the cultivations were kept at a temperature constantly maintained between 42° and 43° C., the virulence gradually decreased until the ninth day, when it was entirely lost. By removing a specimen on any day, and allowing it to germinate at a temperature of 37° C., its activity at that stage could be perpetuated, and thus any degree of virulence that was required could be preserved. Two such cultures of different strength were used for protective inoculation, the weaker of which was called the *premier*, and the stronger the *deuxième vaccin*.

Koch commenced his investigations in the following way. A mouse was injected with blood containing spores of the bacillus, which had been kept five years, and was known to be of great virulence.

The animal was killed at the end of twenty-four hours, and a minute quantity of the spleen was taken on the point of a platinum needle which had been sterilized by heat, and sown in a glass bulb containing twenty cubic centimetres of chicken-bouillon neutralized with sodic carbonate. The bulb was then sealed, and placed at once in a constant-temperature apparatus, which was kept between 42° and 43° C.

Samples were taken daily, and tested upon animals; but, contrary to the promised result, the growth was found to be as deadly for small animals on the ninth as on the first day. Further cultivation proved, however, that, in a period varying from eighteen to twenty-nine days, the infectious property was entirely lost, although the external appearance of the bacillus was unaltered. Thus far, Pasteur's assertion was substantiated, except in regard to the length of time. A portion of this was taken, and allowed to grow at an ordinary temperature for two years; and during this time there has been no evidence of a return of virulence, nor has the form changed. These bacilli are as immovable as the active ones; their ends appear sharply truncated; they form long filaments, in which are developed oval glancing spores. Vaccination with this entirely inactive form did not give immunity against inoculation with the virulent one.

Those of a slight degree of force were next tried. At the end of twenty-four days a culture was obtained which would kill mice, but not guinea-pigs or other small animals, but still did not render them safe. This particular form Koch speaks of as 'mouse anthrax.'

It was thought that perhaps this represented the second and the inactive form, the first vaccination of Pasteur. Accordingly, a sheep was tried, but it succumbed to the malignant form as quickly as ever. It was next proposed to use three or more preventive inoculations; and, accordingly, cultures from the fifteenth day were taken as the first, from the eleventh as the second, from the ninth as the third, and from the fifth as the fourth, and these were followed by the virulent form. In this manner seven sheep, seven rabbits, and eleven guinea-pigs were tried. At the end, all the rabbits and guinea-pigs, and five of the sheep, had died.

In order to determine whether there might not be some other difference, specimens of the vaccinating-material, as furnished by Pasteur through his agent, were purchased, and proved

[1] *Proc. am. assoc. ad. sc.*, xiv. 146.

in regard to their power. The first corresponded to the 'mouse anthrax' (that is, a culture from the eighteenth to the twenty-fourth day), while the second vaccination corresponded to the ninth day. Six sheep were inoculated with these; and out of these, one died after inoculation with the malignant form. As a result, it can be stated, that, after the most careful protective vaccination, an unconditional immunity against infective inoculation is not reached in all cases. Koch thinks that Pasteur's perfect success must be due to the fact that the malignant anthrax used by him was not so virulent as he himself employed.

The cause of the diminution of virulence is regarded by Pasteur as due to the action of oxygen; by Koch, on the other hand, as due to the effects of temperature alone, even so small an amount as a few tenths of a degree C. causing a marked variation in the length of time required to render the bacillus perfectly harmless. Chauveau's experiments also point in the same direction; for while it took from three to four weeks, at a temperature of 42.5° C., to reach the desired result, it could be attained in a few days at 43° C., and in a few hours at 47° C., while a few minutes sufficed if a temperature of from 50° to 53° was used. The lower, however, the temperature, the more surely is the attenuation preserved in later cultivations. When developed in the bodies of animals for several successive generations, Koch found that there was an occasional tendency, on the one hand, for a weaker form to become more powerful; and, on the other, for a stronger to become weaker. But, as a rule, the degree was preserved unaltered, as in artificial cultures.

The scientific fact that sheep could be rendered safe against inoculated anthrax was confirmed by these experiments. The question then arises, How do the vaccinated animals conduct themselves against natural infection? As is well known, different kinds of animals differ in this respect. Cattle, for example, are very refractory to artificial inoculation, while they are very often attacked from a natural source. Pasteur regards the natural source of infection as much less liable to produce the disease than the artificial. His method of placing a number of vaccinated animals in a meadow, in which notorious cases had occurred, is capable of such great errors that it cannot be relied upon for scientific accuracy.

What is the most common way in which natural infection occurs? One method analogous to inoculation is from the bites and stings of insects, who leave, at the same time, spores of the bacillus, which may be attached to their bodies. Another is by the inoculation of scratches in the mouth, caused by sharp particles of fodder.

Koch believes, however, that the intestine itself is the common place of entrance for the parasite, but only when in the condition of spores.

To show this, a portion of the spleen of an animal who had died from anthrax was put inside a small ball of potato, and placed on the back part of the tongue of a sheep. In this way any danger of wounding the mucous membrane of the mouth was avoided. (Since spores are never formed within the body, by taking a portion of the organs, as above, it was known that it was the bacilli alone that were introduced.) Every experiment failed, even after enormous doses, and thus proved that the bacilli are destroyed in the stomach, and are therefore not in a condition to produce intestinal anthrax. When, however, the bacilli were allowed to produce spores, and these were given, every animal died. The examination after death showed that the spores had developed in the intestinal tract, and the bacilli had invaded the body from these. It is therefore in the highest degree probable that the introduction of spores with the food is the most common source of natural infection. The amount would never be so great at one time as was here used; but, if smaller doses were repeatedly given, the picture of an ordinary epidemic could be nearly reproduced.

Cattle could not be obtained for experiment; but an examination made on a cow who had died from natural infection showed similar lesions in the intestines to those found in the sheep.

Animals with a single stomach could not be infected in this way.

Finally the effects of protective inoculation were tried. Ten sheep were taken: five of these were vaccinated with material obtained from Pasteur, of two different strengths; and five, according to Koch, with cultures of the fifteenth, eleventh, ninth, and fifth day. They were then fed with spores. As a result, two of the first series died, but none of the second.

From these few yet unimpeachable experiments, Koch concludes, that, for a certain number of animals, absolute immunity can be obtained; but he doubts whether a simple vaccination, with only two different degrees of attenuation of the virus, is sufficient to give perfect protection.