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


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
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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 officinal 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 everything had been decolorized except the bacteria, and they had remained intensely colored. Such a preparation could be examined at this stage, but the technical difficulties are considerable, since it is difficult to focus on the bacilli. It is better to stain the background in a contrasting color, such as yellow, if the preparation is violet, or blue, if the preparation is red.

May I briefly point out the advantages of this method. The aniline acts more gently on the tissues than the 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, in this procedure three-fourths to one hour is sufficient to make a preparation. Even more important is that the preparations are intensely colored, and the bacilli seem to be significantly larger than in Koch's procedure. Also considering that the background is lighter, this makes it possible to view the bacilli at a lower magnification. I believe also that more bacilli appear on the preparations in my technique, which may be important for the statistics of sputum examinations.

I have been able to stain the bacilli in all basic aniline dyes, as well as bismarck brown, so it is apparent that the substance of the bacillus is not 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. The first of these is that the outer layer is permeable to dyes only under the influence of alkali.

A further point which I would
like to mention is the question of what information on the nature of the bacillus is revealed by the staining characteristics. Since I have found that the outer layer is completely impermeable to the action of strong mineral acid, it would seem that this condition would have a practical interest regarding the light it may throw on the question of sterilization or disinfection. It may be that all disinfecting agents which are acidic will be without effect on this bacillus, and one will have to be limited to alkaline agents.

I will now report the results of my examinations using this method. All of the cases which I examined were frank cases of Phthisis pulmonum. I examined in all 26 cases and in all of them the bacillus could be demonstrated. In the examination and preparation of these sputums, I took no special cares and made no special selection. In almost all cases it was sufficient to examine a single preparation, and in most only a single microscopic field. Only in one case was it necessary to examine both preparations.

Naturally I have convinced myself through control experiments with other lung diseases, that there were no bacilli present. As further proof I can indicate a certain case. I had asked a friend to send me phthisisitic sputums. I obtained one sputum in which I could find no bacilli. After questioning, I discovered that this sputum had been sent to me mistakenly from a man who did not have phthisis, but a perforated empyema.

The further question of what prognostic significance can be derived from this discovery cannot be answered without further experiments. I have found in certain acute cases a large number of bacteria, while in other more chronic cases, I have found a smaller number. On the other hand I have found a large number of bacilli in cases which are only progressing slowly.

Comment

This paper presents the first staining procedure for the tubercle bacillus which makes use of its acid-fast characteristics. The Ziehl-Nielsen method currently used differs in detail but not in principle from Ehrlich's method. This paper also illustrates an early stage in Ehrlich's scientific development. He attempts to consider what properties of the tubercle bacillus are revealed by this remarkable acid-fastness and considers briefly the practical implications for disinfection. We will see later (page 176) how he uses such considerations to develop the whole field of chemotherapy.