

THE INFLUENZA BACILLUS.<sup>1</sup>I.—PRELIMINARY COMMUNICATION ON THE  
EXCITING CAUSES OF INFLUENZA.

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The following results are based on the accurate examination of 31 cases of influenza, in 6 of which a necropsy was made. A complete report will be published as soon as possible.

1. In all the cases of influenza a bacillus of a definite species was found in the characteristic purulent bronchial secretion. In uncomplicated cases of influenza these tiny bacilli were found in absolutely pure cultures, and mostly in immense quantities. They were very frequently situated in the protoplasm of the pus corpuscles. If the influenza had attacked persons whose bronchial tubes were already otherwise diseased—as, for example, phthisical patients with cavities—other micro-organisms besides the influenza bacilli were found in the expectoration in variable quantity. The bacilli may penetrate from the bronchial tubes into the peribronchitic tissue, and even reach the surface of the pleura, where, in two cases examined *post mortem*, they were found in pure cultures in the purulent exudation.

2. These bacilli were found exclusively in cases of influenza. Very numerous control examinations proved their absence in ordinary bronchial catarrh, pneumonia, and phthisis.

3. The presence of bacilli kept equal pace with the course of the disease; with the cessation of the purulent bronchial secretion the bacilli began to disappear.

4. I had already seen and photographed similar bacilli in the same enormous quantities two years ago, during the first epidemic of influenza, in preparations of the sputum of patients suffering from the disease.

5. The influenza bacilli appear as very tiny rodlets, of about the thickness of the bacilli of mouse septicæmia, but only half the length of these. One often sees three or four bacilli strung together in the form of a chain. They stain with some difficulty with the basic aniline dyes. Better preparations are obtained with dilute Ziel's solution and with hot Loeffler's methylene blue. In this way it can be seen almost, as a rule, that the two ends of the bacilli take the stain more intensely, so that forms are produced which can only with great difficulty be distinguished from diplococci or streptococci. In fact I am inclined to believe that some of the earlier observers also saw the bacilli described by me, but that, misled by their peculiar behaviour with regard to staining agents, they described them as diplococci or streptococci. They cannot be stained by Gram's method. In hanging drops they are immobile.

6. These bacilli can be obtained in pure cultures. On  $\frac{1}{2}$  per cent. sugar agar the colonies appear as extremely small droplets, clear as water, often only recognisable with a lens. Their continued culture on this nutrient medium is attended with difficulties, and up to the present I have not succeeded in carrying it beyond the second generation.

7. Numerous inoculation experiments were made on apes, rabbits, guinea-pigs, rats, pigeons, and mice. Only in apes and rabbits could positive results be obtained. The other species of animals showed themselves refractory to influenza.

8. In view of these results I consider myself justified in pronouncing the bacilli just described to be the exciting causes of influenza.

9. It is very probable that infection is produced by sputum charged with the germs of the disease; and the disinfection

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tion of the sputa of patients suffering from influenza is therefore urgently required as a prophylactic measure.

*Addendum.*—Dr. Kitasato has succeeded in cultivating the influenza bacilli to the fifth generation on glycerine agar.

II.—ON THE INFLUENZA BACILLUS AND  
THE MODE OF CULTIVATING IT.

By DR. S. KITASATO.

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GENTLEMEN,—It is, perhaps, remarkable that in the case of a disease which in the last few years has attacked hundreds of thousands of persons, the specific exciting causes have, in spite of extremely numerous investigations, only lately been discovered. The cause, in my opinion, lies in the extreme difficulty of cultivating the tiny bacillus here before you; and, without pure cultures, a bacteriologist cannot, of course, come before the public with a new specific micro-organism.

The difficulty of obtaining cultures of specific bacteria present in the sputum depends chiefly on the great contamination of them with micro-organisms from the mouth, etc. The latter, in consequence of their more luxuriant and abundant growth, can, on our artificial nutrient media, completely overgrow and hide the particular parasites sought for. This occurs all the more easily the longer the specific parasitic micro-organism in question takes to form colonies, as in fact happened in the case of the tubercle bacillus.

With the view of avoiding the obstacles standing in the way of a successful cultivation, Privy Councillor Koch has devised a method which has not yet been published, which enabled him many years ago, and myself again quite recently, to obtain pure cultures of tubercle bacilli directly from the sputum, and which has also been followed by me in the pure cultures of tubercle bacilli here before you. The method to which I have just referred will be published in full detail in an early number of the *Deutsche medicinische Wochenschrift*.

With regard to the characteristics of the pure cultures of influenza bacilli here before you, I may emphasise the following points: On a sloping surface of set glycerine agar the individual colonies present themselves as extremely small points like droplets of water, recognisable during the first twenty-four hours only with the aid of a lens, so that macroscopically a test tube containing them can scarcely be distinguished from a sterile one. The individual colonies are, as has been said, so unusually small that they may easily be overlooked, and it may thus have happened that previous investigators have overlooked them.

If a culture obtained from such a colony is placed on a new nutrient agar medium, numerous small colonies arise on the moist agar surface, as may be seen in this tube. A particularly remarkable point about them is that the colonies always remain separate from each other, and do not, as all other species of bacteria known to me do, join together and form a continuous row. This feature is so characteristic that the influenza bacilli can be thereby with certainty distinguished from other bacteria.

The possibility of continued cultivation is now demonstrated, and the tubes here before you already form the tenth generation in pure cultures. On gelatine they do not grow, as they do not generally multiply at a lower temperature than 28° C., which is the temperature at which gelatine solidifies. In *bouillon* they grow scantily. In the first twenty-four hours single white particles are seen swimming in the *bouillon*, the intervening fluid being perfectly clear. Later, they sink to the bottom, and there form a white woolly mass filling the end of the test tube, whilst the supernatant *bouillon* remains entirely clear—a proof that we have to deal with an immobile bacillus. In conclusion, I may remark that I have accurately studied with the microscope and by culture for a long time back the sputa of tuberculosis in respect to all the micro-organisms occurring therein besides the tubercle bacillus, and also the sputa of pneumonia, bronchitis, etc.; but the present bacillus, so extraordinarily characteristic in its cultures, and so easy to be recognised, has not come within my experience except in influenza patients.