

“Observations on the Influence of certain Culture Fluids and Medicinal Reagents in the Growth and Development of the *Bacillus tuberculosis*.” By C. THEODORE WILLIAMS, M.A., M.D., F.R.C.P., Physician to the Hospital for Consumption, Brompton. Communicated by Sir JOSEPH FAYRER, K.C.S.I., F.R.S. Received December 28, 1883. Read January 24, 1884.

(Abstract.)

The object of the inquiry is to determine the conditions under which the *Bacillus tuberculosis* of Koch grows and multiplies, and to examine its behaviour under the influence of certain medicinal agents and reputed antiseptics.

The sputum of patients in advanced phthisis was used for experiment, on account of its abundance, the number of tubercle bacilli found in it, and its being easily preserved from decomposition when protected from the air. It was spread on cover-glasses, due precautions being taken to ensure a uniform thickness of the film, and the staining process used was that known as the Weigert-Ehrlich modification of Koch's original method.

Between 200 and 300 specimens were thus prepared and examined with a Zeiss microscope with Abbé condenser, under an F. objective ( $=\frac{1}{1\frac{1}{2}}$  immersion lens) and No. 2 ocular, giving a magnifying power of 550 diameters, this being the field generally used for counting the bacilli, and higher powers, up to 1390 diameters, were employed for investigating the structure of the bacilli.

The methods adopted to ascertain the increase or diminution of the bacilli were:—1st, to count the numbers present in a series of fields of the microscope, at least six, and often twelve, being counted, and in doubtful cases the whole slide was carefully gone over before a conclusion was arrived at; 2nd, to note the length of the bacilli and the presence or absence of well-marked divisions in these, preceding their multiplication; 3rd, to observe whether the bacilli are isolated or in groups.

In every case a standard for comparison was first taken from the sputum, and the number of bacilli counted; the rest of the sputum was divided into portions of 20 to 30 minims, mixed with solutions of various medicinal and other agents, and then kept in a Page's incubator at a uniform temperature of 38° C., for periods of from forty-eight hours to eight days. The following cultivation fluids were used:—

Syrup solution of the strength of 2 drachms of syrup to 1 ounce of water.

Hay infusion.

Pasteur's solution (without sugar).

Beef solution, 1 ounce of meat to 2 ounces of water.

„ „  $\frac{1}{2}$  ounce of meat to 2 ounces of water.

Pork broth (Klein).

Also distilled water and the subjoined medicinal agents in solution were mixed, in generally equal proportions, with the sputum, and kept at the same temperature as above.

Solutions of quinine in strengths varying from 2 grs. to the ounce to 10 grs. to the ounce.

„ „ arsenious acid,  $\frac{1}{2}$  gr. to the ounce and 1 gr. to the ounce.

„ „ boracic acid, 1 part in 30 and 1 part in 15.

„ „ iodine, 1 part in 12.

„ „ perchloride of mercury, 1 gr. to the ounce.

The results were as follows :—

*Syrup*.—Cultivation was carried on for eight days. The tubercle bacilli were found in diminishing numbers in the first forty-eight hours; they afterwards gradually disappeared, and during the last three days none were detected. On the first day they were of fair length, and some groups were noted.

*Hay Infusion*.—Four days' cultivation. Bacilli were present in the first twenty-four hours, but afterwards disappeared entirely.

*Pasteur's Solution*.—Eight days' cultivation. Bacilli diminished in numbers at first and then remained stationary for some days. They showed signs of increase on the eighth day.

*Beef Solution*.—Two strengths were experimented on during seven and eight days. In the stronger solution the bacilli increased enormously, both in numbers and in length, and in some fields of the microscope it was impossible to count them. Groups of five and ten were not uncommon, but those of greatest length appeared isolated. Some were long and slender, without cross markings, others shorter and slightly broader with well-marked spores. The bacilli were most abundant in the neighbourhood of the meat *débris*, round which they swarmed like ants on sugar. As the solution became decomposed, the number of bacilli decreased, but about the seventh or eighth day, they were seen increasing by division. The *Bacterium termo* and other bacilli were also present. A weaker solution of beef gave the same results, though to a less marked extent.

*Pork Broth*.—Four days' cultivation. The results were different, for the bacilli did not increase in numbers, but rather declined, though they did not totally disappear from the solution.

*Distilled Water*.—Two to three days. The sputum of three different patients was mixed with distilled water, and in each case no growth or multiplication of bacilli was observed.

*Sulphate of Quinine*.—A large number of experiments were made

with this agent, and resulted in demonstrating that the number of bacilli decreases rapidly under its influence, and that the bacilli in the sputum after being mixed with quinine could not be cultivated even in the beef solution before mentioned, thus showing that this drug not only arrests the growth of the bacillus, but destroys its power of multiplication. All the solutions of quinine appear to possess this property, but the stronger ones to the greatest extent.

*Arsenious Acid.*—This exercised no destructive influence on the bacilli, which increased sixfold in both strong and weak solutions. The rods were generally short, with occasional long ones, and but few spores, and the groups were scanty in number.

*Boracic Acid.*—The bacilli increased more abundantly in solutions of this than in arsenic, and displayed many groups of 2, 5, 7, and 10. Rods in couples, arranged at an angle of  $45^{\circ}$ , were common. They were of fair length and many contained spores. Multiplication by division was proceeding.

*Iodine.*—This drug reduced the numbers very considerably, and in many fields of the microscope it was difficult to find any bacilli, in others a few were present, but these showed no spores or indications of growth.

*Perchloride of Mercury* caused no diminution, but rather a marked increase in the number of the bacilli. They were longer and with more spores than in the standard solution.

The tubercle bacillus is characterised by great durability of structure, as evidenced by its not being destroyed by the strong acids used in the various processes for its detection, and by its little tendency to decomposition. It does not multiply in distilled water, but does so largely in beef solutions. Arsenic, boracic acid, and perchloride of mercury do not interfere with its development, but rather promote it. Quinine and iodine (especially the former) appear to entirely arrest its growth and destroy its power of multiplication.

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