A STUDY OF THE CULTIVATION OF THE TUBERCLE BACILLUS DIRECTLY FROM THE SPUTUM BY THE METHOD OF PETROFF.

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The purpose of this paper is to confirm the work of Petroff[1] in a recent paper on the cultivation of the tubercle bacillus and to add a little from my own experience with the method.

Briefly, as outlined by Petroff, the sputum is mixed with a 3 per cent sodium hydroxide solution and incubated for a period of thirty minutes at 37° C. This is neutralized to sterile litmus paper with normal hydrochloric acid, centrifugalized, and the sediment inoculated on a veal-egg medium to which gentian violet in the dilution of 1 to 10,000 has been added.

From the standpoint of the general pathologist, since I had had variable results from antiformin, it seemed to me that if this method were available many avenues were opened up for the study of this organism in pure culture. It was therefore tried out in a series of twenty-five known positive sputa. At the same time other media, concerning which I hope to report later, were used as controls.

Many tubes were planted from each case, some with a platinum loop, and others by means of a sterile pipette. The latter is very satisfactory for inoculating a series of tubes rapidly with considerable amounts of material.

My results in the use of this method at first were discouraging, but, as often, the fault or faults were mine and not the method's.

[1] Petroff, S. A., A New and Rapid Method for the Isolation and Cultivation of Tubercle Bacilli Directly from the Sputum and Feces, *Jour. Exper. Med.*, 1915, xxi, 38.

Out of the twenty-five cases, from the last series of seven planted on twenty-one tubes I was able to secure ten tubes of gentian violet-egg-veal media with a pure culture of tubercle bacillus. Since my purpose was to test the method not so much for its rapidity as for its availability in isolating tubercle bacilli from generally contaminated material I think it may be stated that the method offers unlimited possibilities.

My failures may be grouped under three headings: drying, no growth, and contamination. Drying of this medium in the incubator and even at room temperature is very marked, so that it may be necessary to add a few cubic centimeters of sterile water to unused media kept only for a short time. Petroff also advises leaving the freshly inoculated tubes in the incubator for a few days until the moisture carried from the centrifuge tubes is absorbed and then paraffining the tubes. This has eliminated the question of drying. Secondly, no growth took place where known positive organisms existed. From my experience the reason for this probably was the neutralization of the sodium hydrate solution. As the neutral point is reached the liquid assumes a milky appearance. It would seem that it is better to stop just here, leaving the solution possibly a little alkaline in reaction. Thirdly, contamination is somewhat harder to explain. Contamination usually resulted in liquefaction of the medium. In my experience it occurred mostly in tubes without gentian violet, although some of the latter also suffered. Here (aside from the question of technique, for some of my contaminations, *e. g.,* with subtilis and spores, were due to errors in technique) there still remain some organisms which this method will not eliminate. These contaminations in some instances were due, in spite of care, to the frequent daily examinations of many of the tubes. The temperature of the incubator is an important point in successful cultivation; the maximum of range should be between 37° and 38° C.

As to the rapidity of growth much cannot be stated as yet. At the end of three weeks' incubation, however, well marked colonies appear which will continue to grow at room temperature. They start as small pin points, becoming multiple small, raised, grayish yellow points about 1 mm. in diameter at the end of two months.

Where they become confluent they preserve their original colonies, growing upon one another.

Microscopically these cultures bear out the statement, which is often made, that the tubercle bacillus has no morphology. It is acid-fast, resisting 30 per cent hydrochloric acid for at least thirty seconds; no pleochromatic forms are encountered. It occurs as rods, straight, curved, long, short, broad, thick, and thin; as small, large, and mostly single cocci; occasionally it resembles a streptococcus; forms similar to the diphtheria bacillus are seen, but larger, dumb-bell-shaped, clubbed, barred, and granular. The beaded appearance of the original organism from the sputum is conspicuous by its absence. Branching forms are encountered and, aside from the question of overlying, a Y-shape is the most frequent. The predominating type is a slightly curved, short, moderately thin, solid rod with denser granules at one or both ends.

CONCLUSIONS.

1. Probably in the majority of cases it is possible, with scrupulous technique, to isolate the tubercle bacillus from contaminated material such as the sputum, by the method outlined by Petroff.

2. It is necessary to use several tubes.

3. Neutralization, drying, temperature of the incubator, and contamination after the growth has started are important points to be noted in the process.

4. The colonies start as pin points, becoming larger and confluent, but still preserving individual groups of a dry appearance which simulate the medium closely in color.

5. The bacillus of tuberculosis in young culture is tinctorially acid-fast and of a polymorphous morphology.