

3655

Microbic Dissociation: II. The Tubercle Bacillus.

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In a previous communication¹ we called attention to the observation made on microbic dissociation of the tubercle bacillus. Two types of acid-fast organisms, a bovine and an avian type, were described, both of which dissociated into two colonies varying in structural characteristics, *i. e.*, one was rough and irregular, while the other was perfectly smooth and appeared very much like moth balls. The bovine tubercle bacillus (B 1) is a culture of moderate virulence which has been in our possession for many years, and the avian culture was obtained from outside sources.

Further investigations have been made by subculturing the single colonies, with the hope of stabilizing their cultural characteristics. At present we feel that various colonies obtained by subculturing are more stable than those ordinarily obtained with other organisms. In all probability this is due to the fact that the tubercle bacillus develops very slowly, taking weeks for visible colonies to appear. In other words, dissociation may be in proportion to the rate of the multiplication of the organisms.

In this communication we shall describe two distinct acid-fast organisms isolated from the sputum of a patient suffering from pulmonary tuberculosis. These organisms were isolated by the author's method, *i. e.*, equal parts of sputum and 4 per cent sodium hydroxide were mixed, shaken well and incubated for 15 minutes at 37.5° C., then centrifuged and the sediment neutralized with normal hydrochloric acid and inoculated on the surface of Petri plates of gentian-violet-egg medium, which lately has been modified, using the dye in dilution of 1:20,000 instead of 1:10,000. This slight modification of the culture medium seems to be more favorable. Growth appeared after 4 weeks of incubation. Several distinct types of colonies could be fished out and were subcultured.¹

From this direct seeding 2 colonies were obtained, one perfectly round, smooth and moist which we shall designate as No. 1, and the other, No. 2, dry, rough and very irregular.

Colony No. 1 (Plate No. 1) appeared round, moist, opaque and was easily suspended in salt solution. It grew well in the bottom of glycerol broth and synthetic media, rendering them diffusely turbid in 4 to 5 days. It was impossible to cultivate this organism on the

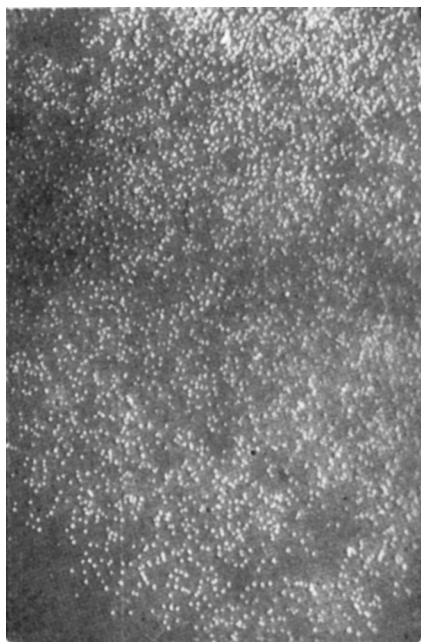


PLATE 1.

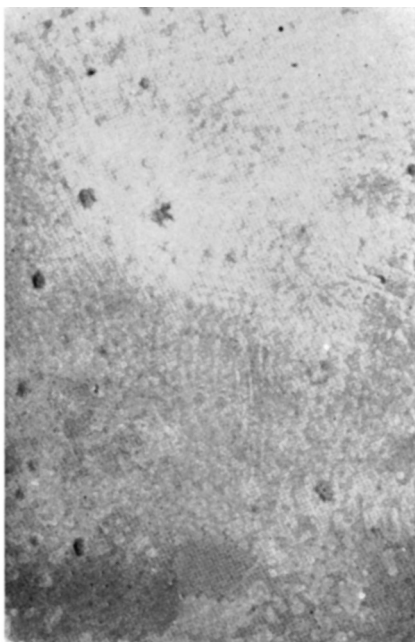


PLATE 2.

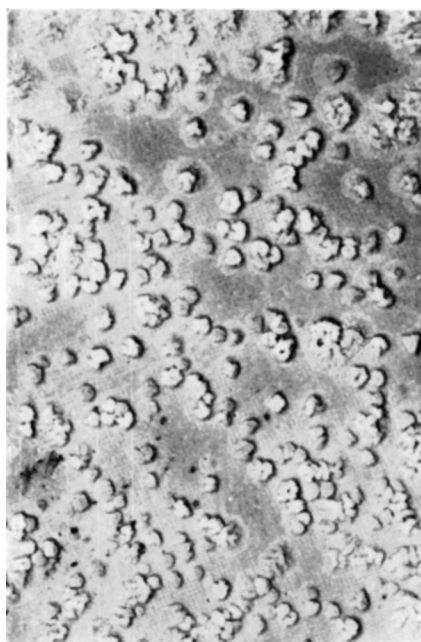


PLATE 3.

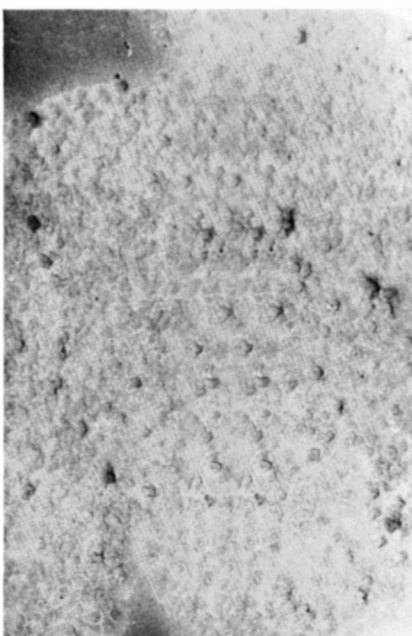


PLATE 4.

All plates actual size.

surface of the fluid medium, as is customary with most acid-fast organisms. When the broth cultures were examined by dark field illumination, only single organisms with an occasional pair could be seen, which stained well by the Ziehl-Neelsen method. There is a probability that this organism can further be dissociated into two distinct types of colonies.

The other colony, No. 2, which was dry, wrinkled, and irregular, was very difficult to suspend in saline. It grew best on the surface of the glycerol broth and had all the cardinal characteristics of other acid-fast organisms. It could further be dissociated into 2 or perhaps 3 types of colonies. One of the colonies (Plate No. 2) was small, flat, with irregular border, very dry and difficult to emulsify. The second colony (Plate No. 3) was also dry but very much more wrinkled, raised and not spreading, while the third colony (Plate No. 4), in all probability, is intermediate.

To our knowledge this is the first time two distinct cultural types of acid-fast organisms have been isolated from a single sputum. One grows homogeneously on fluid media, while the growth of the other on the same medium has the ordinary surface growth and appearance considered characteristic of acid-fast organisms.

This is a preliminary report.

¹ Petroff, S. A., *Proc. Soc. Exp. Biol. and Med.*, 1927, xxiv, 632.

3656

Correlation of Weight to Counting Method in Determining Number of Tubercle Bacilli.

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In testing the degree of immunity set up by vaccination, either with living or with dead tubercle bacilli, in experimental animals, the lack of uniformity in the selection of the dosage in all probability was responsible to some extent for the discrepancies reported by various investigators.

In calculating such dosage, 3 different methods are in use. Calmette and some of his followers select a dosage based on the calculation that one milligram of moist weight contains 40,000,000 tubercle bacilli. Others in Germany and in this country select their dosage represented in dry weight, using from .001 to .01 milligram, while