

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.

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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

still others are using the newer direct counting method, which is the most accurate. These various methods have never before been correlated experimentally and no comparison could be made in analyzing the various findings reported in the past.

In this report we shall describe the results obtained in an attempt to determine the number of tubercle bacilli present in one milligram of dry weight. The main object is to bring together all these various methods.

A homogenous type of tubercle bacilli described in the previous paper was used in this experiment. As stated before, this organism grows very readily in the broth and not on the surface like other acid-fast organisms. The suspension was made in physiological salt solution having a pH of 7.8. Phosphate buffer mixtures could not be used for the reason that the salt, being hygroscopic, no complete desiccation could be obtained, and the determinations were not constant. On the other hand, using saline of such a pH, suspensions are easily made and after several filtrations through cotton, approximately 90 per cent represents individual organisms which will not agglutinate spontaneously. One cubic centimeter of the suspension was desiccated to complete dryness. After the weight was determined it was compared with another fraction of the same thick suspension, which was diluted sufficiently so it could be counted by the new counting chamber. The determinations were repeated about six times, from which we calculated that one milligram of dry weight of tubercle bacilli represents approximately 300,000,000 tubercle bacilli. If we take into consideration that moist tubercle bacilli contain 85 per cent of water, then Calmette's figures of 40,000,000 organisms per milligram are about correct. By calculation, and his figures converted from moist to dry weight, the result will be somewhere near 266,000,000 organisms, which closely approaches our actual counting method.

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Dissociation of B. Anthracis.

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Occasional reports of atypical anthrax colonies have been made. These include such observations as those of Preisz,^{1,2} Markoff,³ Wagner,⁴ Gratia,⁵ etc. Varying significance has been attached to