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 hydroscopic, 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.

3657

Dissociation of B. Anthracis.

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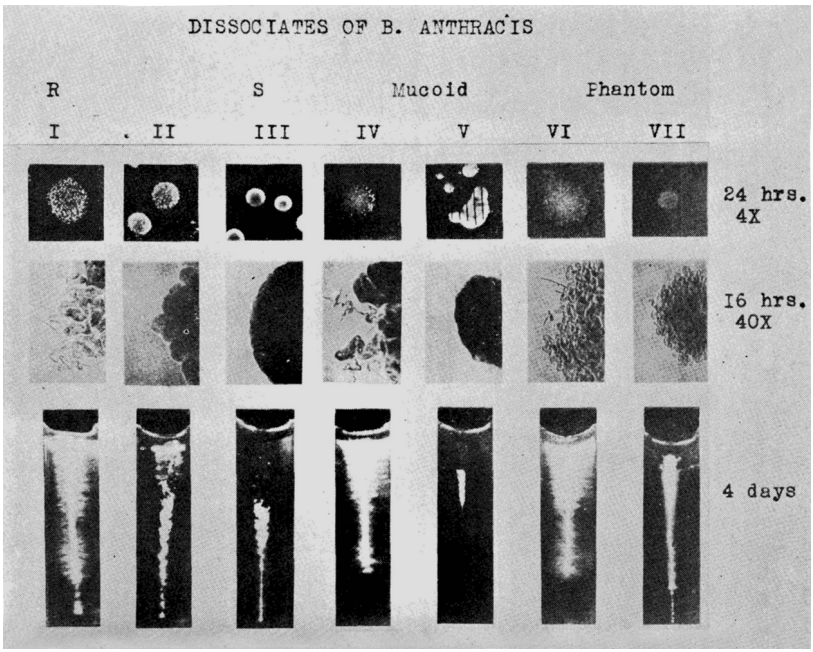
Occasional reports of atypical anthrax colonies have been made. These include such observations as those of Preisz,[1, 2] Markoff,[3] Wagner,[4] Gratia,[5] etc. Varying significance has been attached to

such atypical forms. Preisz noted these peculiar colonies in vaccine cultures and believed them to be contaminations. After reproducing these colony forms from pure line cultures weakened by growth in broth at 42.5° C., he concluded that they were variants arising from the "weakened" culture. Other workers have generally referred to these modifications merely as variations. Markoff stressed the external environment as influencing variation. Wagner suggested a possible analogy between his "teratologische forms" and the "sogenannten Q-Formen in der Typhus-Coli Gruppe."

In the spring of 1926 we obtained, by plating from broth, a dissociation of an apparently "normal" laboratory stock culture of *B. anthracis* (maintained continuously in the laboratory since 1888) into two colonial types: one was the usual flat, dull anthrax colony, the other a small, round, slightly rough colony. The latter resembled the "B" colony described by Wagner. This dissociation has been repeated on the stock culture purified by over 50 successive single colony isolations.

As a result of these findings we became interested in the behavior of the anthrax bacillus in the light of the modern views on microbic dissociation.[6] Should we consider such atypical colonies as having split from the parent culture and having remained as rather fixed variants? Or was there a closer genetic relationship?



During the last year we have studied further this phenomenon. As a result of aging these first two colony types under various conditions (in plain broth and in broth plus normal rabbit serum, each in 10 cc. and in 300 cc. volumes) we have, in the course of these experiments, noted from time to time the appearance of still other colony forms. At present we are able to report seven distinct colony types, all of which have been reisolated and all of which have been caused to revert to the usual form of anthrax colony and culture. Some of the essential colonial and cultural differences between these forms are shown in the accompanying plate.

Type I gives the growth characteristics of anthrax usually described. A markedly flat, gray, rough colony with the Medusa head appearance. A stab culture in gelatin gives the typical "inverted fir tree" growth.

Type II was our first atypical colony. It forms a smaller, less rough, more raised growth with a less dull surface than that observed in Type I. The colony border is nearly destitute of the typical medusa appearance of Type I. The "inverted fir trees" growth in gelatin is abbreviated.

Type III gives a small, smooth colony with a glistening surface. The border is entirely lacking in Medusa appearance. The gelatin stab gives an abbreviated "inverted fir tree" growth.

Type IV colony is similar to Type I except that it is mucoid. With the aid of a wire it can be made to produce threads 2 to 3 cm. long. This viscosity gives the colony a glistening surface as contrasted to that of Type I.

Type V gives a colony similar to that of Type III with respect to smoothness, but even more markedly mucoid than Type IV colonies. As the mucoid character increases with age the growth becomes transparent. After 16 to 18 hours adjacent colonies tend to coalesce into a slimy mass. In the photograph the markings of a glass scale placed below the plate are visible through the colony.

Type VI colonies are characterized by an extremely faint growth on plain agar. These "phantom colonies" are similar to those described by Soule[7] as occurring in his dissociation of *B. subtilis*. Type VI gives the "inverted fir tree" growth in gelatin stabs.

Type VII reveals colonies similar to those of Type VI but characterized by a diminished diameter of the colony, a more regular border and slightly heavier growth. The growth in a gelatin stab resembles a compact and dense "inverted fir tree".

The direction of the dissociative reactions have not been fully ascertained. However, from the work so far completed there appear to be certain trends of transformation which are more or less con-

stant. Type I may give rise to Type II which then may give rise to Type III. The reverse of this also appears to occur. Starting with Type II we may obtain either Types I or III. Types IV and V, when streaked on agar of a pH of 7.0, tend rapidly to lose their mucoid character and to revert to Type I. The reversion has often been observed as taking place in a single passage on such a medium. Agar having a pH of 7.8 to 8.0 is best suited for maintaining these types.

The relative virulence of the various culture types has also been studied. As a result of over one hundred guinea-pig inoculations, and a similar number of mouse inoculations, it appears that Type I is the most virulent form studied, while Type II stands second. Types III, VI and VII have a lower virulence while Types IV and V have a very low pathogenicity and usually fail to kill guinea-pigs or mice.

Dissociation in the animal body (guinea-pigs and mice) usually occurs when Types IV and V do produce a fatal issue; and under these conditions Types I or II are recovered, along with the type inoculated. A similar dissociation *in vivo* may or may not occur when the inoculum comprises Type VII culture.

Finally, regarding the possible identification of these culture types with the chief culture dissociates now recognized in other bacterial species, we believe that Type I represents the R form of *B. anthracis*. This conclusion is based on general cultural characteristics, and more especially on its stability. Further, all types here represented, tend finally to revert to Type I under conditions of aging. Type III, in view of its cultural characteristics, appears to represent the S form of the anthrax bacillus. Type II, judged by cultural characteristics and virulence, is an intermediate between the R and S. Until further work has been done we prefer not to attempt to correlate further the last four types described to the dissociation scheme.

This is a preliminary report.

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[1] Preisz, H., *Centralbl. f. Bakteriol., I. O.*, 1904, xxxv, 280.

[2] Preisz, H., *ibid.*, 1911, lviii, 510.

[3] Markoff, W. N., *Z. Infekt. d. Haustiere*, 1912, xii, 137.

[4] Wagner, G., *Centralbl. f. Bakteriol., I. O.*, 1920, lxxxiv, 386.

[5] Gratia, A., *Compt. rend. Soc. de biol.*, 1924, xc, 369.

[6] Hadley, P., *J. Infect. Dis.*, 1927, xl, 1.

[7] Soule, M. H., personal communication.