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Effect of Specific Antibody on the Capsule of Anthrax Bacilli.

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The characteristic effect exerted by the type specific immune serum on the capsule of pneumococci, a phenomenon observed by Neufeld, induced several investigators (Sabin,[1] Armstrong[2] and Logan and Smeall[3]) to work out a new rapid method for typing pneumococci. Apart from its practical importance this reaction is of theoretical interest, since it contributes direct proof to the previous supposition that the antipneumococcus immune sera induce in a specific way the alteration of the bacterial capsule. Tulczynska[4] demonstrated that this phenomenon occurs also in case of other encapsulated bacteria, pneumobacillus and streptococcus. Similar experiments performed by her with anthrax bacilli gave however very inconclusive results.

It seems somewhat striking that whereas studies on anthrax contributed perhaps most to the elucidation of the basic facts of immunity, and that the relation of capsule formation to bacterial virulence was first demonstrated with this microorganism, yet our present knowledge on the effect of immune serum on the capsule of the anthrax bacilli is considerably less than in many other bacteria.

Our previous work[5, 6] revealed the existence of an antibody produced by immunization with encapsulated anthrax bacilli and probably acting on the bacterial capsule.

The strain used for the major part of our study produced abundant capsule on agar media. Its 24 hours' growth was very moist and sticky and it killed rabbits with typical symptoms of anthrax infection when a 1/1000 part of a 24 hour agar slant culture was injected subcutaneously. The strain was isolated from the "Carbozoo" vaccine.[7]

Fig. 1 represents a wet India ink preparation showing that almost all of the bacilli are surrounded by well developed capsules.

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1 Sabin, *J. Inf. Dis.*, 1930, **16**, 469.

2 Armstrong, R. R., *Brit. Med. J.*, 1931, **1**, 214; 1932, **1**, 187.

3 Logan and Smeall, *Brit. Med. J.*, 1932, **1**, 188.

4 Tulczynska, R. E., *Z. f. Hyg. u. Inf.*, 1933, **114**, 769.

5 Tomesik, J., and Szongott, H., *Z. f. Immunitätsforsch*, 1933, **77**, 86.

6 Tomesik, J., and Bodon, G., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 118.

7 Mazzucchi, M., *La Clinica Veterinaria*, 1931, **9**, 3.

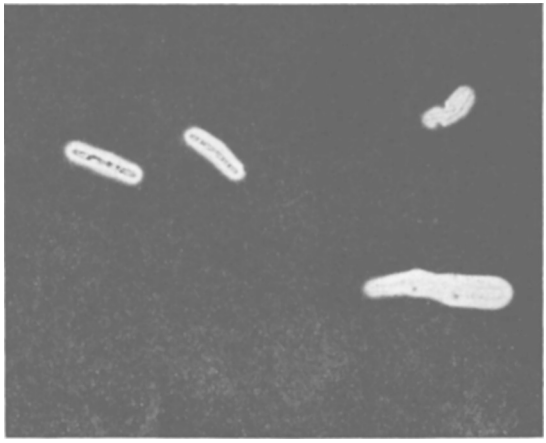

FIG. 1.

No trace of capsule was observed however, when the bacteria were suspended in physiologic salt solution and examined in hanging drop. The result did not differ, when one loopful of different dyes was mixed with the suspension. These observations conform with our previous knowledge, that the capsule of the anthrax bacillus is not visible in hanging drop because its refraction is similar to that of the surrounding fluid.

One loopful of different sera was then added to the bacterial suspension. Several normal as well as non-specific immune sera have been tried, but none of them caused any change in the microscopic picture. The anthrax sera studied here were divided again in 2 groups: (1) sera containing only antipolysaccharide precipitins and

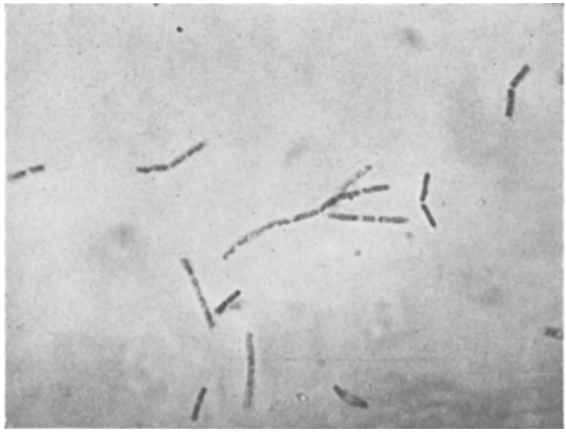

FIG. 2.

(2) those containing both antipolysaccharide and antiprotein precipitins. The effect of 10 different sera belonging to the first group had been studied with completely negative results, that is, the capsules did not turn visible after the addition of these sera. (Fig. 2).

An entirely different picture was observed when we employed 5 sera belonging to the second group. The addition of one loopful of these sera caused agglutination as reported previously. Apart from this the bacteria not included in the large clumps showed a characteristic change. The capsule showed up 1 or 2 minutes after the addition of the serum as a bright body with brown color and with a sharply defined outline toward the fluid. (Fig. 3).

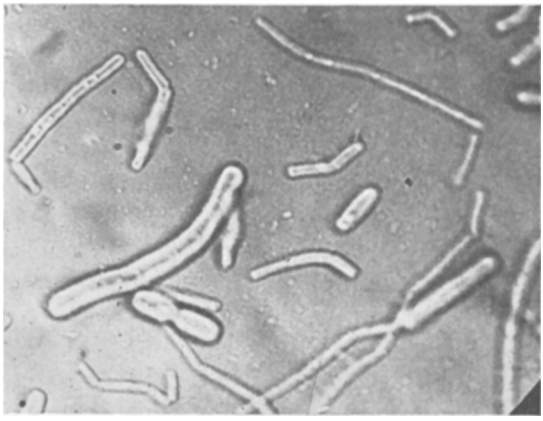

FIG. 3.

The method we adopted finally to show the effect of this immune serum on the capsule was the following: Two loopfuls of the 24 agar culture of the encapsulated bacilli were suspended in 0.5 cc. salt solution. One loopful of this fairly homogeneous suspension was mixed on a cover-slip with the same quantity of Loeffler's methylene blue as well as of the immune serum containing P precipitins. The cover-slip containing this mixture was then inverted and placed over a hollow ground slide and sealed with vaseline. The capsule became visible as a rule after a few minutes owing to its changed refraction. In many instances it was stained as a pink body around the light blue bacilli due to the metachromatic effect of this stain.

In comparing the thickness and the appearance of the capsule with that in the India ink preparation, we had the impression that the visibility of the capsule was not due to a layer of immune serum surrounding and covering it, but rather to a specific reaction which

changed the refraction and the staining properties of the capsule proper.

Altogether 12 different encapsulated and 9 other anthrax strains were examined in this way. We never failed to observe the closest parallelism in demonstrating the capsule production in India ink preparation and in our specific capsular reaction.

The correlation of the agglutinability of our strains and of the capsular reaction was also complete. The capsular reaction therefore has to be regarded as a visible sign of the union of anticapsular antibody with the capsular substance. The agglutination must be in consequence a secondary reaction following the specific alteration of the capsular material.

We believe that it would be of great importance to study in infected animals the protecting rôle of an antibody exhibiting such a marked specific action on the capsule of anthrax bacilli.

Summary. Anthrax immune serum containing P precipitin exerts a specific effect on the capsule of anthrax bacilli. Following the addition of a small quantity of this immune serum to the suspension of encapsulated anthrax bacilli, the capsular material is specifically affected and becomes visible in unstained preparates. This is the final proof of the existence of a separate antibody in specially prepared immune serum acting in a specific way on the capsule of anthrax bacilli.

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Stability of Toxin Producing Attribute of Scarlet Fever Strains of Streptococci.

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Several reports have appeared concerning the stability of the toxin producing power of scarlet fever strains of streptococci. Organisms dried on swabs or in cultures have been found to retain the toxin producing attribute (Jettmar,[1] Tunnicliff[2]). Tunnicliff[3] reported also that filtrates of certain different appearing colonies of

[1] Jettmar, M. H. v., *Z. f. Hyg. u. Infektionskr.*, 1927, **107**, 265.

[2] Tunnicliff, R., *J. Infect. Dis.*, 1927, **41**, 272.

[3] Tunnicliff, R., *J. Infect. Dis.*, 1931, **48**, 511.