

An explanation of the results of the immunizations could be given, if there exists in the dilute alcohol extract a complex antigen containing protein, and the specifically reacting non-immunizing substance.

Substances apparently similar to that described were found in another *Vibrio*, and in bacilli of the typhoid group (in collaboration with Dr. J. Furth).

¹ Levaditi, C., and Muttermilch, S., *Compt. rend. Soc. Biol.*, 1908, lxiv, 406, 844, 1151; *ibid.*, xlv, 26.

² Prausnitz, C., *Centr. Bakt.*, 1911, lix, 434.

3318

On the Alcohol Soluble Specific Substance of *B. Smegmae*.

J. FREUND. (Introduced by L. Dienes.)

From the Von Ruck Research Laboratory for Tuberculosis, Asheville, N. C.

It is of interest to find out if the close serological relation between the alcohol soluble substances of tubercle bacillus and that of *B. smegmae* is connected with a similarity as to their chemical characteristics, as far as they can be established.

The smegma strain used was the same as employed in previous work in this laboratory. It is not impossible that between the different strains of smegma bacillus there is a variation as to their serological properties. The methylalcohol extract was obtained after previous extraction of the dried bacilli with ether (twice) and ethylalcohol (twice). The immunserum was obtained from rabbits injected with suspension of the bacilli. The titer of the serum in the complement fixation was 1:300 with the bacillary suspension and also with alcohol extract.

In the Castellani experiment made with tuberculosis and smegma immunsera, no difference was found between the corresponding extracts, as they absorbed the antibodies for each other from both of the sera.

The specific substance is soluble in ethylalcohol and in ether, and insoluble in acetone, like the specific substance of alcoholic extract of tubercle bacillus. The crude methylalcohol extract was purified with acetone. The extraction of the evaporated extract, with acetone, seemed to enhance the potency of the preparation; the antigen

unit of the crude extract 0.00015 mgm. after extraction with acetone, and, solution in ether was 0.00005 mgm. Preparations obtained after two acetone precipitations of the ether solution of the substance lost something of their potency. They were markedly more potent than the original crude methylalcoholic extract (antigen unit 0.00015 mgm.). None of the acetone solutions has shown specific effect. In most of the preparations the antigen unit is somewhat smaller than the antigen unit of the corresponding preparation made from tubercle bacillus. The difference might be caused by the difference in sera used. One preparation, the ether solution, after the first acetone treatment, is markedly more potent than the others. It is not known to what factor is due the loss of potency of the preparations in the course of repeated precipitation by acetone.

The smallest amount of the crude methylalcoholic extract giving complement fixation is nearly the same, using Serum 597 II, obtained with a fast-growing avirulent strain of tubercle bacillus. It is eight times higher with Serum R obtained with a virulent strain of *B. tuberculosis*. As to the significance of this difference we refer to another publication in this PROCEEDINGS.¹ The serum obtained with the smegma bacillus, with the antibodies corresponding to the alcohol soluble antigen, is nearer the Serum 597 II, than to the Serum R obtained with a virulent strain.

The P. content of various purified preparations varies between 1.3 to 1.8 per cent, and the N content of a preparation purified with acetone but not washed thoroughly with water, was 1.4 per cent. The content of fatty acids (ether soluble substances after hydrolysis) of a preparation purified with acetone was 40 per cent.

The chemical characteristics of the extracts of smegma bacillus reported show close resemblance to the extracts of *B. tuberculosis*. The chemical study of the extracts of smegma bacillus is not sufficiently advanced to enable us to estimate the significance of the differences between the two extracts.

¹ Dienes, L., and Freund, J., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 27.