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On a Specific Substance of the Cholera Vibrio.

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Differences in opinion (Levaditi,[1] Prausnitz[2]) as to the alcohol solubility of the specific substances of the cholera *Vibrio* called for an experimental investigation of the subject.

While we were unable to detect such substances in extracts made with ether or hot absolute alcohol, they were present in 75 per cent alcoholic extracts of the bacilli, previously washed with saline, and extracted with ether and hot absolute alcohol. On cooling, a sediment separated from the solution which reacted with anti-cholera immune serum, up to dilutions of 1-500,000 of the antigen. This product in a 1 per cent solution gave distinct protein reactions. On hydrolysis with dilute hydrochloric acid, the solution reduced Fehling's reagent and gave a crystalline osazone. During the hydrolysis the fluid became turbid, and an insoluble product settled. The hot 75 per cent alcoholic extract was put through a Berkefeld filter, and the solution of the sediment, which appeared on cooling, was injected into rabbits. After 3 injections of 2 mg. each of the substance, precipitating and agglutinating antisera were obtained.

For further separation of the specifically reacting substance, the extraction was made first with a smaller, and then a larger volume of hot 75 per cent alcohol, and the sediment from the second extract only was used. To a 10 per cent solution, four volumes of N/10 NaOH were added, and after centrifuging, the solution was precipitated with alcohol. The precipitate was then redissolved in water, neutralized, some Na_2CO_3 added, centrifuged, and the solution precipitated with alcohol, after acidifying with acetic acid.

The precipitate obtained is a white powder, soluble in water. That the specific substance contains carbohydrates is indicated by the formation of reducing sugar upon hydrolysis. Also the insoluble substance mentioned above separated, after heating with dilute acid. A 1 per cent solution gave no biuret reaction.

The purified product had very slight antigenic activity, if any. With immune sera it reacted in a dilution of 1-500,000. In higher concentrations precipitation appears immediately, leading to the formation of heavy flakes. An analysis of this product gave the following values, calculated for ash free substance, C. 49.05; H. 7.17; N, 4.34; P, 1.67; no S. Other preparations gave similar figures.

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.

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On the Alcohol Soluble Specific Substance of B. Smegmae.

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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