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Alcohol Soluble Specific Substances of B. Diphtheriae and of Streptothrix.

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The alcohol soluble specific substance of *B. diphtheriae* was studied, and an effort made to demonstrate such antigens in different micro-organisms, especially organisms related to *B. tuberculosis* and *B. diphtheriae,* and higher pathogenic fungi. The organisms were grown on 0.2 per cent dextrose broth, washed, dried, extracted twice with acetone and twice with alcohol. The immune sera were obtained from rabbits injected with suspensions of the micro-organisms. The titer of the diphtheria immune serum I and II in the complement fixation was 1:160 with the alcoholic extract, 1:160 with the bacillary suspension, and 1:160 with diphtheria broth. The titer with the alcoholic extract dropped in the course of one year to 1:40 and 1:15. The titer of the streptothrix serum I was 1:40 and that of II, 1:10 with alcoholic extracts, while with suspension of streptothrix serum II reacted stronger (1:300) than serum I (1:80). In a few months the titer of serum I dropped 1:15. No alcohol soluble antigens could be demonstrated in the case of *B. xerosis, trichophyton* and yeast, although the sera reacted with the suspensions of the respective organisms. With two strains of *actinomyces* no sera giving complement fixation were obtained. The negative results do not exclude the possibility that other, perhaps freshly isolated strains, would incite antibody production against the alcohol extracts.

The alcohol extract of *B. diphtheriae* gave positive complement fixation neither with tubercle bacillus nor with streptothrix immune sera. The diphtheria immune serum reacted in a dilution 1:10 with suspension of tubercle bacillus and in a dilution 1:30 with alcoholic extract of the same, but reacted neither with the suspension nor with the alcoholic extract of streptothrix.

In the Castellani experiment, employing filtration through paper pulp, the alcoholic extract absorbed the antibodies for itself and for the bacillary suspension. Since it is quite probable

that the bacillary suspension contains other antigens than those present in alcoholic extract it is quite possible that the antibodies corresponding to the different antigens are combined with each other. Potent sera suitable for the Castellani experiment have not yet been obtained by the injection of watery extract free from alcohol soluble antigens.

Dilutions of the alcoholic extract 1:10 and 1:100 gave precipitation with the undiluted immune sera in the ring test. Diphtheria toxin (received through the courtesy of Dr. C. Shore, Laboratory of Hygiene of North Carolina) gave no positive complement fixation with our immune sera, nor did an antitoxic serum with the alcohol extracts, although the broth from which the growth was removed by centrifugalization, without appreciable toxicity, was more potent antigen than the bacillary suspension or the alcoholic extract.

The alcohol soluble antigen is readily soluble in methyl alcohol, in acetone and in weak alkali; insoluble in ether, in weak acids, and in water. The potency of an alcoholic extract, measured by the smallest amount of dry material giving complement fixation (2 units of complement in the volume of 0.6 cc.) was increased by the following procedure of purification: The acetone or alcohol extract was evaporated *in vacuo* at 45° C., extracted with ether, dissolved in methyl alcohol, precipitated by HCl, dissolved in weak Na_2CO_3 or in NaOH, the precipitation repeated twice, the final precipitate dissolved in methyl alcohol or in weak NaOH. The potency of the different purified preparations is quite uniform.

	Unit for the complement fixation test	P. Content	N Content
	mgm.	Per cent	Per cent
1. Acetone extract	0.001	0.15	
2. Purified preparation from 1	0.0004		
3. First alcoholic extract	0.0030		
4. Purified preparation made from 3	0.00045	0.68	6.7
5. Second alcoholic extract	0.0016	0.42	4.8
6. Purified preparation made from 5	0.00034		5.6
7. Purified preparation made from 5, dissolved in alkali	0.00046	0.5	

In two purified preparations, prepared from the second alcoholic extract, after boiling with $Ba(OH)_2$, no ether soluble substance (fatty acids) could be found, nor after treatment with 25 per cent H_2SO_4, could reducing substances be demonstrated. Pepsin and trypsin did not decrease the potency of a purified preparation. The addition of lecithin to the first alcoholic extract, when the mixture contains 99 per cent of lecithin expressed in dry material, enhances the potency four times.

The examination of the extract of streptothrix has been retarded because of the lack of a highly potent serum, and because of the rapid drop of potency of the serum available.

The smallest amount of the alcoholic extract giving complement fixation was 0.006 mgm. The activation of this extract by the addition of lecithin is very striking. The antigen and lecithin were mixed in alcoholic solution. In a mixture containing 0.2 per cent of the alcoholic extract an activation 200 times was observed.

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Local Hypersensitiveness in Tuberculous Guinea Pigs.

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The plan of the experiments described in this note was suggested to us by the papers of Lewis and Loomis[1] on allergic irritability.

Guinea pigs shortly after a tuberculous infection, during the interval when the tuberculin sensitiveness usually appears, were treated with egg white, and later the skin sensitiveness toward egg white and the appearance of precipitins and complement fixating antibodies in the blood serum were examined. Non-tuberculous guinea pigs were treated and observed in the same way.

Table I contains the observations with one series of animals. The other series, together with the detailed description of the observations, will be published later.

Six control guinea pigs were treated together with the series described in the table; three were treated like the guinea pigs 1-3