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Specific Aggregation of Streptococcal Proteins Adsorbed on Oil Droplets.*

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Emulsions of oil droplets in solutions of certain streptococcal proteins have proven to be a means of transforming precipitative reactions into agglutinative reactions. Type-specific aggregation, by means of antistreptococcal antisera, of oil droplets emulsified in solutions of Lancefield's "M" substance are of particular interest.

The solutions used have been solutions of the proteins extracted from *Streptococcus pyogenes* (Group A β-hemolytic streptococci) at neutrality[1] as well as the acid-soluble type-specific protein (Lancefield's "M") from *Streptococcus pyogenes.*[2, 3] The antisera used were rabbit sera prepared by injecting rabbits with living cultures of Type 1 and Type 6 streptococci, and by injecting rabbits with a solution of the neutral extractable proteins.

The experimental technic employed follows:

Preparation of the antigen. 0.5 cc of olive oil was emulsified in 20 cc of distilled water. This emulsification was accomplished by putting the material in the magnetostriction oscillator of Chambers† and Gaines and treating with sound waves.[4, 5] To this emulsion was added the protein solution. This was prepared by dissolving 25 mg of protein in 5 cc of distilled water and carefully adjusting the pH to 7.4 (brom thymol blue indicator). The protein solution and emulsion were then shaken for 15 minutes in a Kahn-test shaker and allowed to stand overnight in the refrigerator. Examination of such an emulsion under the dark field microscope showed many minute droplets of oil homogeneously dispersed in water.

Macroscopic Test. To 0.2 cc of antiserum dilution was added 0.2

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1 Sevag, M. G., Lackman, D. B., and Smolens, J., *J. Biol. Chem.*, 1938, **124**, 425.

2 Lancefield, R. C., *J. Exp. Med.*, 1928, **47**, 91.

3 Zittle, C. A., *J. Immunol.*, 1939, **37**, 1.

† We wish to acknowledge the assistance of Dr. Leslie Chambers in preparing the oil emulsions.

4 Chambers, L. A., and Gaines, N., *J. Cell. Comp. Physiol.*, 1932, **1**, 465.

5 Flosdorf, E. W., Chambers, L. A., and Malisoff, W. M., 1936, **58**, 1069.

cc of a 1:3 dilution of the antigen. After thorough shaking, the tubes were placed in the 56°C bath overnight. In the morning the tubes were centrifuged and read. In reading the test it must be borne in mind that agglutinated oil droplets will be found in a creamy layer at the surface of the liquid. The degree of reaction is evaluated by the degree of clearing of the underlying liquid and the compactness of the creamy layer obtained upon centrifuging.

Slide Test. It was found to be possible to set up the above test as a microscopic test. In this test a large drop of antigen and of antiserum, diluted to a suitable concentration, were mixed upon a slide. The reaction was very rapid and striking; if positive, the oil droplets were seen to aggregate into large clumps. This reaction is best observed with a dark field microscope; however it may be observed with the high dry objective and when the reaction has progressed sufficiently, the aggregated oil droplets may be seen with the unaided eye.

A refinement of this test which permits of continued observation and of preservation is as follows: a coverslip was attached to a slide by means of paraffin placed on two edges of the slip; a drop of antigen dilution and of serum dilution were allowed to flow from opposite sides under the coverslip and meet in the center; the coverslip was then sealed on all sides with paraffin and the preparation examined with the dark field microscope. An advantage of this method was that negative controls could be observed in the same preparation, since the reaction occurred only where the 2 reactants met, thus leaving the homogeneously dispersed globules of oil on one side and the serum on the other side.

A sample result is shown in Table I and Fig. 1.

The results obtained show that it is possible to adsorb the neutral extractable protein and the acid-soluble type-specific protein of

TABLE I.
Slide Agglutination.

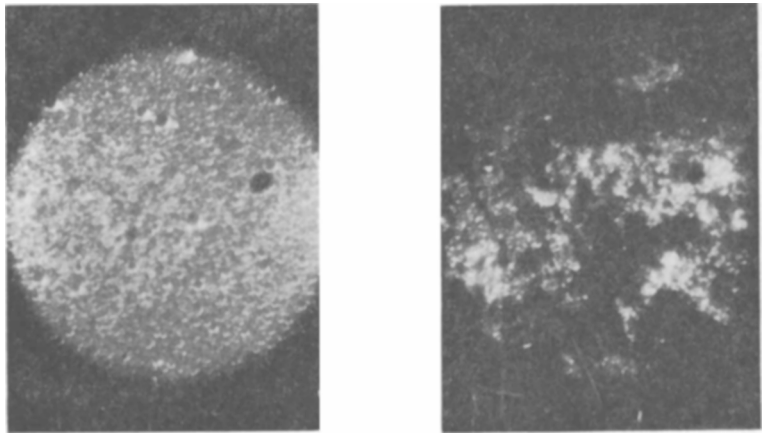
Antigen: Olive oil emulsion of Type 1 and Type 6 acid-soluble protein (Lancefield's "M," type-specific).
Antisera: Rabbit sera against Type 1 and Type 6 whole streptococci.

Antiserum	Antigen	Dilutions of antiserum						
		1/2	1/4	1/8	1/32	1/64	1/128	1/256
Type 6	Type 6 "M"—oil	4	4	4	3	1	1	0
" 6	" 1 " "	1	0	0	0	0	0	0
" 1	" 6 " "	0	0	0	0	0	0	0
" 1	" 1 " "	2	4	3	2	1	0	0
Normal rabbit serum	" 6 " "	0	0	0	—	—	—	—
Normal rabbit serum	" 1 " "	0	0	0	—	—	—	—

Examples of

A negative reaction

A positive reaction



Protein adsorbed on oil emulsion and normal rabbit's serum

Protein adsorbed on oil emulsion and immune rabbit's serum

FIG. 1.

Streptococcus pyogenes upon droplets of olive oil in emulsion, and that such droplets react with appropriate antiserum in much the same manner as an agglutinogen.

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Further Studies of the Agent in Intestines of Normal Mice Which Induces Encephalomyelitis.

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It has been shown[1] that normal mice, as well as those affected by spontaneous encephalomyelitis (Theiler's disease—now commonly called "poliomyelitis of mice"[2, 3]), harbor in their intestinal contents an active agent which, after cerebral transfer to normal mice, induces

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1 Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 434.

2 Theiler, M., *Science*, 1934, **80**, 122; *J. Exp. Med.*, 1937, **65**, 705.

3 Iguchi, M., *Kitasato Arch. Exp. Med.*, 1939, **16**, 56.