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Collodion-Particle Agglutinative Tests in Detection of Antigen Derived from Tubercle Bacilli.*

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These studies have been devised to explore the range of antigens which react with anti-H37 tubercle-bacillus horse serum and to compare the delicacy of precipitin-tests with collodion-particle agglutination-tests for the detection of these antigens and their corresponding antibodies.

Materials. A single lot of anti-H37 tubercle-bacillus serum (designated as No. 5807 A and B) was used in all tests. It was prepared by the Mulford Biological Laboratories of Sharp and Dohme in 1925 by the injection of defatted tubercle bacilli. The immunological properties of this serum have been studied by a number of investigators[1] including Heidelberger,[2] who found its antibody-content to be almost entirely antipolysaccharide. In our own tests the following substances were used as a possible source of antigenic material.

I. *Old Tuberculin* from 4 different companies.

II. *Protein-derivatives of Tuberculin.* (a) P. P. D.—Parke Davis and Company. This purified derivative of tuberculin was dissolved in buffer solution furnished by the company.

(b) T. P. T.—No. 16 (trichloracetic-acid precipitated) prepared at the Henry Phipps Institute of Philadelphia.†

III. *Polysaccharides* representing 4 samples derived from various lipids and soluble wax fractions from 3 strains of human tubercle bacilli and one strain of leprosy bacillus. These sugars had been kept in a desiccator since their preparation by Dr. R. J. Anderson.‡ They were readily soluble in physiological salt solution.

IV. *Crude Aqueous Extracts* from cultures of human tubercle bacilli which had been grown on modified Dorset's and glycerol agar. These extracts were prepared from weighed amounts of

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1 Thomas, R. M., and Duran-Reynals, F., *Yale J. Biol. and Med.*, 1940, **12**, 525.

2 Heidelberger, M., and Mengel, A. E. O., *J. Biol. Chem.*, 1937, **118**, 79.

† I am indebted to Dr. Florence B. Seibert for this preparation.

‡ I am indebted to Dr. R. J. Anderson of the Department of Chemistry of Yale University for this material.

dried bacilli, suspended in saline solution. The suspensions were then shaken for 20 minutes with glass beads and passed through a Seitz filter. The resulting clear filtrates were used for the tests.

V. *Phosphatide* prepared from human tubercle bacilli by Dr. R. J. Anderson.‡ Suspensions were made by grinding 10 mg of the phosphatide in 10 cc of distilled water. The resulting suspension was opalescent but showed no tendency to sediment at the end of 48 hours. When diluted 10^{-1} it was almost water clear. However, none of the immunological tests proved satisfactory with this colloidal suspension because of the fact that positive reactions were also obtained with normal horse serum.

Methods. (a) *Precipitin-reactions* were set up in small tubes, inside diameter 4 mm, using 0.3 cc of antigen and 0.1 cc of anti-H37 tubercle-bacillus serum. They were left in the incubator for 2 hours and then kept in the icebox until read at the end of 24 hours and again at 48 hours. All antigens were set up with normal horse-serum controls.

(b) *Collodion-particle Agglutination.*§ The principles underlying this technic have been laid down by Freund,[3] and Cannon and Marshall[4] and developed by Goodner.[5] Tests were set up in standard agglutination tubes, with 0.5 cc antigen, 0.1 cc collodion-particle suspension, 0.1 cc antiserum, and 0.3 cc saline. The suspension was added before the antigen and antibody were allowed to combine. The tubes were incubated at 37°C for 2 hours, centrifuged and then read. All tests were set up with normal horse-serum controls.

Results. The results of a typical experiment are shown in Table I in which dilutions of antigen have been tested against undiluted serum. As all of the 4 different brands of tuberculin reacted quite similarly, results with only a single brand are shown. This also holds true for the polysaccharides. It will be seen that the collodion-particle agglutinations were about 10 times more delicate than the precipitin-reaction in this series. In Table II antigens have been tested against dilutions of serum. As in Table I no positive readings were encountered with the tuberculoprotein derivatives and so they have not been included. Here, the collodion-particle agglutination is from 8 to 16 times more delicate than the precipitin reaction.

Summary. 1. With a single lot of antiserum which has been

§ Some of the collodion-particle suspension used in these tests was kindly supplied by Dr. Jules Freund of the New York City Health Department Laboratories.

[3] Freund, J., *Am. Rev. Tubere.*, 1925, **12**, 124.

[4] Cannon, P. R., and Marshall, C. E., *J. Immunol.*, 1940, **38**, 365.

[5] Goodner, K., *Science*, 1941, **94**, 241.

TABLE I.
Titration of Antigens Against Undiluted Anti-H137 Tubercle-bacillus Serum. Precipitin vs. Collodion-particle Agglutination.

Dilution of antigen	I Old tuberculin 1 cc = 1,000 mg		IIA P.P.D. 1 cc = 0.05 mg		IIB T.P.T. 1 cc = 1 mg		III Polysaccharide 1 cc = 1 mg		IV Crude aqueous extract of human tubercle bacilli 1 cc = 1 mg	
	Ptn.	C.P.A.	Ptn.	C.P.A.	Ptn.	C.P.A.	Ptn.	C.P.A.	Ptn.	C.P.A.
Original	—	0	—	—	—	—	++	+++	++	+++
10^{-1}	—	+++	—	—	—	—	++	+++	+	++
10^{-2}	++	+++	—	—	—	—	+	+++	—	++
10^{-3}	++	++	—	—	—	—	+	++	—	+
10^{-4}	+	++	—	—	—	—	—	+	—	—
10^{-5}	—	+	—	—	—	—	—	—	—	—
10^{-6}	—	—	—	—	—	—	—	—	—	—

C.P.A. = Collodion-particle agglutinations:
+++ = disc.
++ = coarse particles.
+ = fine particles.
— = negative.
0 = not done.
Ptn. = Precipitin-reactions:
++ = precipitation in 24 hr.
+ = " " 48 "

TABLE II.
Titration of Antibody (Anti-H37 Tubercle bacillus Serum) Against Various Antigens. Precipitin vs. Collodion-particle Agglutination.

Dilution of serum	I Old tuberculin 1 cc = 10 mg		III Polysaccharide 1 cc = 1 mg		IV Crude aqueous extract of human tubercle bacilli 1 cc = 1 mg	
	Ptn.	C.P.A.	Ptn.	C.P.A.	Ptn.	C.P.A.
Original	++	+++	++	+++	++	+++
1:2	++	+++	++	+++	++	+++
1:4	+	+++	+	+++	+	+++
1:8	+	++	+	++	—	++
1:16	+	++	+	++	—	++
1:32	—	++	—	++	—	+
1:64	—	++	—	++	—	—
1:128	—	+	—	+	—	—
1:256	—	+	—	—	—	—
1:512	—	—	—	—	—	—

Legend as in Table I.

artificially produced by injecting defatted tubercle bacilli into horses, a series of comparative immunological tests have been carried out. Results indicate that collodion-particle agglutination-tests are not only satisfactory for this type of immunological determination but are more delicate than precipitin-tests, particularly for the detection of antibody.