16551

A Method for Demonstrating Antibodies in Rabbit Sera Against Histoplasmin by the Collodion Agglutination Technic.

SAMUEL SASLAW AND CHARLOTTE C. CAMPBELL. (Introduced by Maurice Landy.)
From the Department of Bacteriology, Army Medical Department Research and Graduate School, Army Medical Center, Washington, D. C.

Serologic tests for histoplasmosis may prove to be valuable adjuncts for both the diagnosis of the disease and for a more critical evaluation of the significance of the positive skin test reaction to histoplasmin. To date, the complement fixation tests reported from this laboratory[1] and others[2,3] have been the only *in vitro* immunologic procedures described. An improved method for the preparation and use of sensitized collodion particles for the detection of specific antibodies in blood sera has been reported recently by Cavelti.[4] The collodion agglutination test appeared to offer a potential technic for measuring antibodies against histoplasmin in positive skin reactors and in experimental sera derived from animals immunized with *Histoplasma capsulatum*. This report describes the results obtained in testing the blood sera of normal and immunized rabbits against suspensions of collodion particles sensitized with histoplasmin.

Methods. The collodion particles used in these studies were prepared by the method of Cavelti.[4] A suspension of particles diluted to match the turbidity of the McFarland scale No. 2 was sensitized by adding an equal volume of stock histoplasmin H-40* diluted 1:200 in physiologic saline. This optimal dilution of histoplasmin was determined by titration as shown in Table I. The histoplasmin and collodion mixture was shaken thoroughly and kept at room temperature for one hour to permit sensitization of the collodion particles. All serum specimens were inactivated at 60°C for 30 minutes and serial dilutions made in physiologic saline solution so that each tube contained 0.5 ml. To each serum dilution 0.5 ml of the suspension of sensitized particles was added. The tubes were shaken vigorously, incubated at room temperature for 2 hours and then centrifuged for 3 minutes at 1400 rpm in a No. 2 International horizontal centrifuge. The results were read by gently flicking the tubes in front of a black screen above which a microscope lamp served as a source of light. Agglutinations were considered 4+ when the diameter of the flakes was about 1 to 1½ mm or over with the decreasing gradations of 3+, 2+ and 1+. In the 1+ reaction the particles were very fine but still readily visible. A test was considered doubtful (±) if particles were just barely visible and negative when no particulate matter was observed after resuspension.

Young healthy rabbits were immunized with yeast phase strains of *H. capsulatum* and with the other agents used in this study according to the method described in a previous report.[1] The sera of rabbits immunized with 3 strains of *H. capsulatum* (G-2, G-5, G-6),† 2 strains of *Blastomyces dermatitidis*

[1] Saslaw, S., and Campbell, C. C., *J. Lab. and Clin. Med.*, 1948, **33**, 811.

[2] Salvin, S. B., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 342.

[3] Tenenberg, D. J., and Howell, A., *Pub. Health Rep.*, 1948, **63**, 163.

[4] Cavelti, P. A., *J. Immunol.*, 1947, **57**, 141.

* Issued by the Office of Field Studies, Tuberculosis Control Division, United States Public Health Service. Lot H-40 histoplasmin was prepared from the pooled broth filtrates of the mycelial phase of 3 strains of *Histoplasma capsulatum* and standardized by the method outlined by Howell.[5]

[5] Howell, A., *Pub. Health Rep.*, 1947, **62**, 631.

† The G-2, G-5 and G-6 yeast phase strains of *H. capsulatum* were reverted from the mycelial phase of strains 715, 952 and 650, respectively, from the collection of Dr. N. F. Conant at Duke University. The A-1 strain of *B. dermatitidis* and A-5 (Duke 930) and the strain of *B. braziliensis* (Duke 871B) are all human isolates received from Dr. Conant.

TABLE I.
Determination of the Optimal Dilution of Histoplasmin for Collodion Agglutinations Using Serum Prepared in Rabbits Against the G-2 Strain of *H. capsulatum*.

Dil. of histoplasmin before mixture with equal quantity of collodion*	Positive serum dilutions 0.5 ml							Controls‡	
0.5 ml	1:5	1:10	1:20	1:40	1:80	1:160	1:320	Antigen	Serum
1:5	—	—	—	—	—	—	—	—	—
1:10	—	—	—	—	—	—	—	—	—
1:20	—	—	—	—	—	—	—	—	—
1:40	2+	—	—	—	—	—	—	—	—
1:100	4+	4+	3+	1+	—	—	—	—	—
1:200†	4+	4+	3+	3+	1+	±	—	—	—
1:400	3+	2+	1+	±	—	—	—	—	—
1:800	2+	1+	1+	±					
1:1000	±	±	±	±	—	—	—	—	—
1:2000	—	—	—	—	—	—	—	—	—
1:4000	—	—	—	—	—	—	—	—	—
1:8000	—	—	—	—	—	—	—	—	—

* Collodion diluted to equal turbidity of No. 2 McFarland nephelometer.
† 1:200 dilution selected as optimum dilution on basis of comparative titers.
‡ In antigen control sensitized particles were mixed with 0.5 ml of saline. In serum control positive serum (1:5 and 1:10) was mixed with unsensitized collodion. Normal rabbit serum was similarly titrated as above and was negative throughout.

TABLE II.
Results of Collodion Agglutination Tests with Rabbit Sera.

Sera	Animal No.	Serum dilutions—0.5 ml 1:5	1:10	1:20	1:40	1:80	1:160
G-2 (*H. capsulatum*)	7	3+	2+	1+	—	—	—
G-2 " "	8	4+	4+	2+	2+	—	—
G-5 " "	10	4+	4+	2+	2+	1+	—
G-5 " "	11	2+	2+	1+	±	—	—
G-6 " "	12	2+	2+	1+	±	—	—
A-1 (*B. dermatitidis*)	14	+	—	—	—	—	—
A-5 " "	15	+	+	—	—	—	—
B. braziliensis	16	±	—	—	—	—	—
S. schenckii	19	±	—	—	—	—	—
C. albicans	21	±	—	—	—	—	—
C. neoformans	26	—	—	—	—	—	—
*Normal rabbit sera (20)	31-50	—	—	—	—	—	—

* All of 20 normal rabbit serum specimens failed to show any agglutination. Duplicate control titrations using unsensitized collodion particles diluted with an equal quantity of saline showed no agglutinations with any of the above sera.

TABLE III.
Effect of Varying Incubation Temperature on Collodion Agglutination.

Sera	2 hr incubation at °C	Serum dilutions—0.5 ml 1:5	1:10	1:20	1:40	1:80
G-2	Room temp.	3+	2+	1+	—	—
Normal	" "	—	—	—		
G-2	37	1+	±	—	—	—
Normal	37	—	—	—	—	—
G-2	52	—	—	—	—	—
Normal	52	—	—	—	—	—

(A-1 and A-5)* and one each of *Blastomyces braziliensis*,* *Candida albicans*,‡ *Cryptococcus neoformans*‡ and *Sporotrichum schenckii*‡ were tested against collodion particles sensitized with histoplasmin. In addition, sera of normal stock rabbits were similarly investigated.

Results. The data as presented in Table II reveal that the sensitized collodion particles were uniformly agglutinated in the presence of sera from animals immunized with all 3 strains of *H. capsulatum* with serum titers ranging from 1:20 to 1:80. None of the other fungal antisera tested gave positive agglutinations except *B. dermatitidis* antisera, which reacted in low titer. Twenty specimens of sera from normal stock rabbits likewise failed to agglutinate the sensitized particles. In control studies collodion particles diluted with saline instead of histoplasmin showed no agglutinations in the presence of the same series of serum specimens.

Effects of varying incubation time and temperature. In order to ascertain the conditions for optimum incubation, 2 sets of experiments were conducted. In one study known positive serum and sensitized collodion mixtures were incubated at 37°C, 52°C, and at room temperature for 2 hours. As can be seen in Table III the agglutination reactions were stronger after incubation at room temperature than at 37° C while at 52° C no agglutination occurred. The normal serum control was negative throughout. In the second series of studies the tubes were placed in the refrigerator overnight after the routine 2 hour room temperature incubation and reading. The following day the tubes were recentrifuged in the usual manner and read. Unlike the results following primary incubation, many instances of non-specific agglutinations occurred with normal and heterologous sera while the specific sera gave higher "titers" (Table IV). Thus to obtain optimum and specific results the 2-hour incubation at room temperature was employed as the method of choice.

Summary. A method for utilizing sus-

‡ Isolated from patient material at the Army Medical Center.

TABLE IV.

Specificity of Agglutination Results after 2-Hour Incubation at Room Temperature as Compared to Overnight Incubation in Refrigerator Using Collodion Sensitized with Histoplasmin.

Sera	Rabbit No.	Serum dilutions											
		Room temperature						Refrigerator					
		1:5	1:10	1:20	1:40	1:80	1:160	1:5	1:10	1:20	1:40	1:80	1:160
G-2 (*H. capsulatum*)	7	3+	3+	1+	−	−	−	4+	4+	3+	1+	1+	±
G-5 ,,	10	4+	4+	2+	2+	1+	−	4+	4+	4+	3+	2+	±
G-6 ,,	12	2+	2+	1+	±	−	−	3+	3+	3+	1+	±	−
B. braziliensis	16	±	−	−	−	−	−	3+	2+	±	±	−	−
A-5 (*B. dermatitidis*)	15	−	+	−	−	−	−	3+	1+	1+	±	−	−
C. albicans	21	−	−	−	−	−	−	1+	1+	±	−	−	−
C. neoformans	26	−	−	−	−	−	−	1+	1+	−	−	−	−
Normal	31	−	−	−	−	−	−	±	±	−	−	−	−
,,	32	−	−	−	−	−	−	2+	−	−	−	−	−

pensions of collodion particles sensitized with histoplasmin for the determination of antibodies against histoplasmin is described. The sensitized suspensions uniformly showed the presence of antibodies against histoplasmin in rabbits which had been immunized with 3 different strains of *H. capsulatum*. Antisera against *B. dermatitidis* reacted in low dilution, while that of *B. braziliensis, S. schenckii, C. neoformans* and *C. albicans* as well as normal rabbit sera failed to agglutinate the histoplasmin-coated particles. These results suggest the use of this technic with human sera. The findings of such studies are now being analyzed in this laboratory.

16552

Intestinal Absorption of Vitamin A from Aqueous and Oily Menstruum.*

HANS POPPER AND BRUNO W. VOLK.

From the Hektoen Institute for Medical Research and the Departments of Pathology of Cook County Hospital and Northwestern University Medical School, Chicago, Ill.

Oral intake of vitamin A in aqueous dispersions produces a significantly higher rise of the blood vitamin A level than in oily menstruum.[1,2,3] This was considered evidence of improved intestinal absorption, especially since after intake of the aqueous preparation, the fecal excretion of vitamin A appeared reduced[2] and the storage in the liver increased.[2,4] The use of aqueous preparations appeared especially superior in improving the response to vitamin A in patients with liver[5] or celiac[6] disease. This suggested the direct observations of the intestinal absorption of vitamin A in aqueous and oily solution in rats by fluorescence microscopy.[7]

Material and Method. Thirty-three albino rats, all weighing between 140 and 170 g and previously on a stock diet, were sacrificed at different intervals following feeding of various doses of vitamin A as unsaponifiable fraction of fish liver oil in corn oil, or dispersed in water with a sorbitan monolaurate derivative. Both preparations† contained 25,000 U.S.P. units of vitamin A per cc and were made from the same vitamin A concentrate. Pieces of duodenum, upper jejunum, lower jejunum and ileum were fixed in 10% formalin. Frozen sections were prepared and studied microscopically for vitamin A fluorescence.[8] Total amount of vitamin A fluorescence and its site were recorded. Vitamin A fluorescence is seen in the intestinal wall of the rat only after feeding high doses of vitamin A.[7]

Results. Independent of variations of the amount of vitamin A fed and of the time interval between administration and sacrifice (Table I), the amount of vitamin A in the intestinal wall was larger after an equal dose of vitamin A in aqueous than in oily menstruum. The difference was especially evident with reduction of the amount of vita-

* Supported by a grant from Endo Products, Inc., Richmond Hill, N. Y.

1 Kramer, B., Sobel, A. E., and Gottfried, S. P., *Am. J. Dis. Child.*, 1947, **73**, 543.

2 Lewis, J. M., Bodansky, O., Birmingham, J., and Cohlan, S. Q., *J. Pediat.*, 1947, **31**, 496.

3 Popper, H., Steigmann, F., and Dyniewicz, H. A., *J. Lab. and Clin. Med.*, 1947, **32**, 1403.

4 Sobel, A. E., Sherman, M., Lichtblau, O., Snow, S., and Kramer, B., *J. Nutrition*, 1948, **35**, 225.

5 Popper, H., Steigmann, F., and Dyniewicz, H. A., *Gastroenterology*, in press.

6 May, C. D., and Lower, C. U., *J. Clin Invest.*, 1948, **27**, 226.

7 Popper, H., and Volk, B. W., *Arch. Path.*, 1944, **38**, 71.

† Supplied by Dr. Samuel M. Gordon of Endo Products Inc.

8 Popper, H., *Phys. Rev.*, 1944, **24**, 205.

9 Frazer, A. C., *Phys. Rev.*, 1946, **26**, 103.