Separation of Specific Antigens of *Histoplasma capsulatum* by Ion-Exchange Chromatography.* (26037)

CURTIS H. GREENE, LOUIS S. DELALLA AND V. N. TOMPKINS
(Introduced by J. F. Kent)
Division of Laboratories and Research, New York State Department of Health, Albany

Histoplasmin, the filtrate derived from prolonged growth of the mycelial phase of *Histoplasma capsulatum* in liquid synthetic medium, has been employed for many years as the antigen in complement fixation, precipitin, and collodion agglutination tests for histoplasmosis(1,2). Recently Heiner reported its use in the Ouchterlony technic(3), which appeared to be a more sensitive and specific test for the disease. He detected six different antigens in crude mycelial filtrates, but only one, isolated by continuous-flow electrophoresis and designated as "h" antigen, was specific for active histoplasmosis(4).

Since use of purified "h" antigen would appear to provide more significant information to the clinician than does crude histoplasmin in the gel-precipitin test now in routine use in this laboratory, methods were explored for simple, reliable separation of this fraction from the crude filtrate. The purpose of this paper is to describe the initial separation of "h" antigen from other antigens by means of diethylaminoethyl-cellulose (DEAE) chromatography of partially purified histoplasmin.

Materials and methods. Preparation of crude antigen. The histoplasmin used in this study was prepared from cultures of *H. capsulatum* No. 4894, obtained from the late Dr. Rhoda Benham of Columbia University. Liquid asparagine medium, 1500 ml, was dispensed in 3000-ml Erlenmeyer flasks and inoculated with several small pieces of mycelium from Sabouraud agar cultures in Petroff flasks. After incubation of the cultures at room temperature (25°C) in the dark for approximately 6 months, the mycelial mats were broken up and sunk by violent agitation of the flasks. The medium was separated from the mycelial mats by filtration through paper, and sterilized by filtration through a Hormann filter. 'Merthiolate' in a final concentration of 1:10,000 was added to the crude filtrate as preservative. In the agar gel-precipitin technic, the crude preparation was shown to contain, in terms of antiserum from a patient with culturally proven histoplasmosis, at least 2 antigens. These were precipitated from solution by half saturation with ammonium sulfate or by addition of 2 volumes of acetone.

Chromatography. DEAE-cellulose was prepared by the procedure of Peterson and Sober(5). A 1.2 x 45 cm column containing 3 g of adsorbent was equilibrated with 0.01 M pH 8.0 phosphate buffer as suggested by Fahey *et al.*(6). The sample of partially purified histoplasmin was dialyzed for 24 hours against the same buffer in a revolving dialyzer. Three ml of the clear, but highly colored, solution containing approximately 150 mg of protein were applied to the column and washed down with several 5 ml portions of buffer. Gradient elution with increasing molarity from 0.01 M to 0.30 M phosphate and decreasing pH from 8.0 to 4.4 was accomplished by introducing 0.30 M NaH_2PO_4 through a 250 ml constant-volume mixing chamber containing the starting buffer. When the limit of the gradient was reached, salt concentration was further increased by introducing a 5% NaCl solution through the mixing chamber. Collection of the effluent in 3 ml fractions at a flow rate of 9 ml/hr was begun immediately after introduction of the sample. Optical densities of the fractions at 280 mμ were determined in a Beckman DU spectrophotometer and pH determinations were made in a Cambridge electron ray pH meter; fractionation was at 4°C.

Immunologic study. Large volumes of effluent were pooled and dried by lyophilization, reconstituted in water, and dialyzed against 0.85% NaCl. The concentrated pools were examined for antigenic activity by double

* The authors are greatly indebted to Drs. Ray K. Brown and Morris A. Gordon for invaluable assistance.

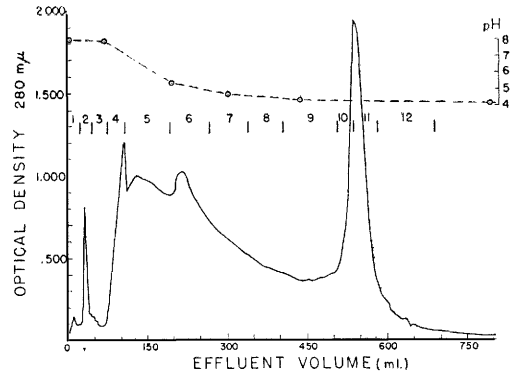

FIG. 1. Chromatogram of partially purified histoplasmin on DEAE-cellulose. Solid line represents optical density of column effluent at 280 mμ. Broken line and scale at right denote pH changes. Vertical lines demarcate pools of effluent.

diffusion in agar, using for antibody the serum from a person wth proven histoplasmosis.

Results. Fig. 1 shows the chromatogram obtained by plotting optical densities at 280 mμ against effluent volume. The pooled fractions are indicated in the figure by vertical lines, while the horizontal broken line and the scale at the right show pH changes. Pools 1 through 4 failed to exhibit antigenic activity when examined by the Ouchterlony technic. Pools 5, 7, and 8 each developed a single line while pool 6 and the crude antigen developed

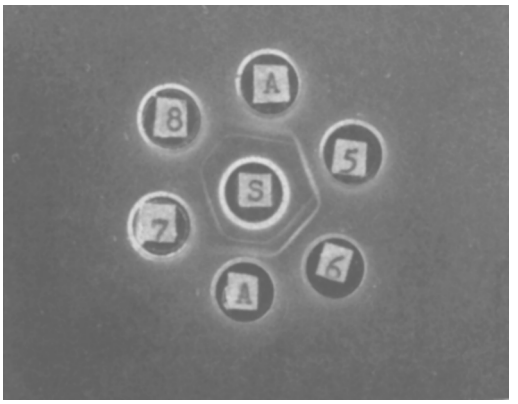

FIG. 2. Ouchterlony plate showing separation of "h" and "m" antigens. S: antiserum from patient with proven histoplasmosis; A: crude antigen; 5, 6, 7, and 8: pools of effluent from chromatographic column.

† We are greatly indebted to Dr. D. C. Heiner, who kindly identified the 2 lines developed with this antiserum as being due to the "h" and "m" antibodies.

2 lines corresponding to the "h" and "m" antigens of Heiner (Fig. 2).† One of these made identity with the line from pool 5 ("m" antigen), while the other made identity with those from pools 7 and 8 (the "h" antigen). No antigens were detectable in the remaining pools (9 through 12) by this very sensitive test.

Discussion. As demonstrated above, the "h" and "m" antigens of *H. capsulatum* can be separated from each other by column chromatography on DEAE-cellulose. In addition, a considerable degree of purification has been accomplished by removal of some 75-80% of inert material. Neither the precise degree of purity of these preparations nor the extent of contamination, if any, with other antigens has been determined. The crude mycelial phase histoplasmin used in this work, however, has produced no bands other than "h" and "m" when tested aganst several hundred sera from patients with culturally proven histoplasmosis or with strong presumptive evidence of the disease. It appears to be devoid of the other antigens reported by Heiner(4). The isolated fractions produced only one band when tested against several sera from culturally proven cases of histoplasmosis, or from those with strong presumptive evidence of the disease. Further purification and characterization of these preparations, as well as results of their use in immunologic tests, will be reported later.

Summary. "h" and "m" antigens apparently specific for detection of histoplasmosis have been separated from crude histoplasmin and purified by means of column chromatography on DEAE-cellulose. A gradient elution scheme with decreasing pH and increasing molarity of phosphate was used.

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Received July 14, 1960. P.S.E.B.M., 1960, v105.