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Reaction of An Aspergillus Polysaccharide with Cryptococcus Capsules and Various Acidic Polysaccharides.* (25086)

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Recently, a polygalactosamine (galactosaminan) has been isolated from cultures of *Aspergillus parasiticus* by electromigration(1,2). This polysaccharide contains only galactosamine, 35% of which is acetylated(2), and when a solution of the polysaccharide is exposed to an electric current in an electrophoresis or electrodialysis apparatus it moves toward the cathode, presumably due to the positive charge conferred by non-acetylated galactosamine. The capsular polysaccharide of *Cryptococcus neoformans* moves to the anode under similar conditions due to its content of glucuronic acid(3,4). That the first of these polysaccharides possesses positively charged amino groups led to the prediction that it might react with the carboxyl groups of the *C. neoformans* polysaccharide, or, for that matter other acidic polysaccharides. Such a reaction might result in precipitation if both polysaccharides were in solution when mixed or in a "capsular reaction"(5-7) if the acidic polysaccharide were in the form of a capsule surrounding a microorganism. Such reactions occurred and a preliminary study of the interaction is presented here.

Materials and methods. Samples of polygalactosamine (APS) from *Aspergillus parasiticus*(2) were furnished by Dr. Saul Roseman, Univ. of Michigan. Stock solutions were prepared by dissolving APS (1 mg/ml) in M/100 HCl or M/20 H_3PO_4. Dilutions of the stock were made in water, saline solution, or buffer. Capsular polysaccharides of pneumococcus types 2 and 3 (S2 and S3) were isolated from broth culture as described in(8). Commercial (USP) grade gum acacia was used without further purification. Samples of dextran were kindly furnished by Dr. Allene Jeanes. Cryptococcus polysaccharides were precipitated with ethanol in presence of acetic acid and sodium acetate(4,9). Samples of Type A polysaccharide (SA) used were further purified by electromigration in an electrodialysis chamber(4). Each of the 3 cells contained 100 ml of veronal buffer pH 8.6 or acetate buffer pH 5.0 (0.025M). In addition, the center cell contained SA dissolved in buffer at a concentration of 1 mg/ml. A current of 100 m.a. was applied for 5 to 6 hours and then the SA was deposited in a gelatinous layer on the anode membrane and was scraped off and dissolved in water for final ethanol precipitation. For precipitin tests, all polysaccharides were dissolved in 0.9% sodium chloride solution. Preliminary tests indicated that good precipitation of acidic polysaccharides could be obtained with as little as 50 μg APS/ml; therefore 0.5 ml APS (100 μg/ml) was added to each 10 x 75 mm test tube followed by 0.5 ml of acidic polysaccharide or dextran at various concentrations. Tests were read after

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TABLE I. APS Capsular Reaction and Agglutination of *C. neoformans* in Saline Solution.

Reaction	*C. neoformans* cells	APS (μg/ml) 50	25	12.5	6.2	3.1	1.5	.8
Capsular reaction*	CN- 6	CR	CR	(CR)	(CR)	(—)	—	—
	15	CR	CR	(CR)	(CR)	(—)	—	—
Agglutination†	6	+	+	+	+	±	±	—
	15	+	+	+	+	±	±	—

Strength and variability of capsular reaction is indicated in decreasing order by symbols CR, (CR), (—).

* Read after 30 min. at room temperature.

† Read after additional 18 hr in refrigerator.

2 hours at room temperature and stored at 0-4°C for 4 days at which time a final reading was made. For capsular reactions, cells of *C. neoformans,* Type A, strain 6, or Type B, strain 15 were grown 2-4 days in neopeptone broth with constant agitation(4). The cells were washed 3 times by centrifugation and suspended in water, saline or buffer (10^7 cells/ml). For the test, equal volumes of cell suspension and APS dissolved in appropriate vehicle were added to small test tubes. After 15-30 minutes at room temperature and again after 18-24 hours refrigeration a drop was removed from each tube, transferred to microscope slide and examined under cover glass at 440x.

Results. When mixtures of *C. neoformans* cells and APS were examined under the microscope a pronounced clarification of the capsular outline was seen in contrast to control cells in water or saline where no capsule was visible. When electrolyte was present, this resembled the "capsular reaction" obtained with specific antibody(4-7); however, if the reactants were in distilled water, a marked contraction of the capsule occurred in response to APS. In this case, the capsule had an irregularly crenated margin and was contracted so that it was sometimes hard to differentiate from the cell wall. In either water or saline, easily discernible reactions were obtained with 50 μg APS/ml although at this concentration not all capsules reacted to the same degree. Results of titration in serial 2-fold dilutions of APS in saline solution are presented in Table I. Some reaction was obtained with as little as 3.1 μg/ml after 30 minutes incubation at room temperature; however at this concentration reactions were weak and variable and many capsules did not react at all. Additional storage of mixtures in the refrigerator for 18 hours resulted in some intensification of the capsular reaction and in agglutination of cells. At higher APS concentrations, the agglutinated cells were firmly adherent to the bottom and sides of the test tube.

Before setting up the capsular reaction, cryptococcal cells were washed several times by centrifugation. The advisability of this procedure is demonstrated in Table II where it is shown that addition of an excess of dissolved polysaccharide (SA) to cryptococcal Type A cell suspensions before exposure to APS resulted in inhibition of capsular reaction. This was apparently due to competition between the soluble SA and the intact capsule for APS, and demonstrates identity of SA with the capsular substance. It should be noted that in this experiment the final APS concentration was held constant at 50 μg/ml. Use of a smaller amount would conceivably result in more striking inhibition. It can also be seen from Table II that the capsular reaction and its inhibition by excess SA occur over a fairly wide range of pH; more extensive studies on the effect of pH are in progress.

When APS was mixed with certain polysaccharides, precipitation resulted. Typical re-

TABLE II. Inhibition of APS*—Cryptococcus Capsular Reaction by Excess SA at Various pH Values.

Buffer (M/20)	SA conc. (μg/ml) 0	1000	100	10
HCl-KCl pH 2	CR	—	(CR)	CR
Citrate pH 4	"	—	—	"
Phosphate pH 6	"	—	—	"
Veronal pH 8	"	—	—	"

CR = capsular reaction. (CR) = weak capsular reaction.

* APS conc. = 50 μg/ml.

TABLE III. Precipitation of Various Polysaccharides by APS.

Polysaccharide	Polysaccharide conc. (μg/ml) 1000	500	250	125	62	31
Cryptococcus SA	C	C	CP	P	P	(P)
Dextran	—	—	—	—	—	—
Pneumococcus S2	P	P	P	P	P	(P)
" S3	C	CP	P	P	(C)	—
Gum acacia	P	P	P	P	C	(C)

C = cloudy, CP = cloudy + precipitation, P = precipitation, ( ) = weaker reaction.

sults are presented in Table III. Precipitation occurred with all acidic polysaccharides tested, including Cryptococcus SA, Pneumococcus Types 2 and 3, and gum acacia. Although not shown in the Table, Cryptococcus SB and SC also precipitated with APS. Dextran, the only non-acidic polysaccharide tested, failed to react.

Discussion. The combination of APS, the polygalactosamine produced by *A. parasiticus*, with microbial capsules and dissolved polysaccharides introduces another interesting form of microbial interaction.

It is not yet known whether the combination of APS with microbial cells would inhibit their growth; however, this would seem to be a possible application of practical value. Combination of APS with the microbial cell surface might also serve to enhance phagocytosis of the cell. The reaction bears a superficial resemblance to the antigen-antibody reaction and may be useful as a model in studying certain aspects of the antigen-antibody combination. It also recalls the non-specific combination of proteins with microbial capsules(7) except that the APS reaction seems less dependent on pH. The APS reaction might also be of value in studying such intriguing interactions as that reported between 2 mating types of yeast by Brock(10). The APS reaction serves to emphasize that antigen-antibody-like combinations can result from the reaction of an "antigen" with a compound that bears only a superficial resemblance to an antibody; such interactions occurring among various polysaccharides, proteins and complexes within the human body may conceivably give rise to certain diseases of unknown or obscure etiology about which it is currently popular to speculate on the etiologic role of an antigen-antibody reaction.

Summary. When polygalactosamine (APS) produced by *Aspergillus parasiticus* was mixed with cells of *Cryptococcus neoformans* it produced a "capsular reaction" and agglutination similar to that caused by specific antibody. The positively charged APS molecule also caused precipitation of various negatively charged (acidic) polysaccharides including gum acacia and capsular polysaccharides of *C. neoformans* and pneumococcus. Dextran was not precipitated by APS.

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