Effect of Histoplasmin Skin Testing on Serologic Results.* (20220)

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The booster effect of repeated positive histoplasmin skin reactions on the collodion agglutination test for histoplasmosis has been reported previously(1). The purpose of this study was to compare the effect of skin testing upon the histoplasma complement-fixation tests using both yeast phase and histoplasmin antigens, upon the collodion agglutination test, and upon the blastomyces and coccidiodes complement-fixation tests.

Methods. Twenty-five healthy volunteer medical students received 6 weekly intradermal injections with 0.1 ml of lot H-42 histoplasmin (1:100).† Blood for serum antibody studies was obtained prior to each skin test and at 7,26,30 and 40-44 weeks after the first skin test, whenever possible or indicated. Seven additional volunteers were bled prior to and weekly for 5 weeks after a single positive skin test. Histoplasmin lots H-15‡ H-17† and H-37† were used in parallel in the collodion agglutination test(2,3). Lot H-15 histoplasmin, lot 47-54§ coccidioidin and ground yeast phase histoplasma and blastomyces antigens were employed in 50% end-point complement-fixation tests(4,5).

Results. Fourteen of the 25 volunteers were skin-test positive. Of this number 12 developed antibodies detectable by one or more of the serologic tests for histoplasmosis after 2 or more skin tests. None of the 11 skin-test negative reactors developed demonstrable antibodies with any of the serologic procedures after 6 weekly skin tests. Similarly, none of 7 showed any humoral antibodies for 5 weeks after a single positive skin test.

Collodion agglutination. Eight of 14 (57.1%) skin-test positive persons developed 4+ agglutinations in peak titers varying from 1:10 to 1:40. As summarized in Table I, the sera of 4 (Nos. 5,10,12,13) became positive after 2 skin tests, the other 4 (Nos. 1, 3, 6, 14) after 3 skin tests, respectively. At the 5th and 6th weeks the serum of one (No. 12) became negative. Positive agglutinations were observed in only 2 instances (Nos. 5 and 14) 21 weeks after the last skin test, but these were negative when checked at 40 weeks. Thus the positive collodion agglutination reactions occurring after repeated positive skin tests were relatively transient in nature, and all were negative 35 weeks after the last skin test. This is in accord with previous studies (1).

Histoplasmin complement-fixation. When histoplasmin was used as antigen, 12 of 14 (85.7%) skin-test positive persons developed complement-fixing antibodies in titers varying from 1:5 to 1:40. Four were positive after the 2nd skin test, 8 after 3, 11 after 5, and the 12th person at 26 weeks (See Table I). At this latter time antibodies were still present in the sera of 8 of the 9 previously positive, while at 30 weeks, 4 of 5 specimens, previously positive at 26 weeks, fixed complement. At 40 to 44 weeks, 3 (Nos. 2, 7, 10) still remained positive. For comparative purposes 8 serum specimens from Nos. 2,7,10 and 11 taken at 26 to 44 weeks were sent to the Communicable Disease Center at Chamblee, Georgia‖ where histoplasmin is used as the antigen in complement-fixation. Their results confirmed our findings in all instances. In contrast to the transient nature of positive reactions in the agglutination test or in yeast-phase antigen complement-fixation (*vide infra*), the duration of positive reactions was much longer with histoplasmin as antigen in

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‖ Test performed by Dr. Joseph Schubert.

TABLE I. Effect of Repeated Positive Histoplasmin Skin Tests on Serologic Responses.†

Subject	Test	Time (wk) ‡	1*	2*	3*	4*	5*	6	26	30	40-44
1.	CA		0	0	10	20	10	10	0		0
	YF		0	5	5	5	5	10	0		0
	H		0	5	10	10	10	10	10		0
	B		0	0	0	5	5	5	0		0
2.	YF		0	20	10	20	20	20	0	0	0
	H		0	10	10	20	10	10	10	5	5
	B		0	10	10	20	20	20	0	0	0
3.	CA		0	0	10	10	10	10		0	0
	YF		0	0	0	0	5	5			0
	H		0	0	0	0	5	10			0
5.	CA		0	20	40	40	40	40	10		0
	YF		0	0	0	0	0	10	0		0
	H		0	0	10	10	10	20	5		0
6.	CA		0	0	5	10	10	5	0		
	YF		0	10	10	5	10	20	0		
	H		0	0	5	0	0	0	0		
	B		0	10	10	5	5	10	0		
7.	YF		0	0	5	10	20	20	0	0	0
	H		0	0	5	20	20	10	10	10	5
	B		0	5	5	20	20	20	0	0	0
8.	YF		0	0	0	0	5	5	0		
	H		0	0	0	0	5	5	0		
	B		0	0	0	0	5	10	0		
10.	CA		0	20	40	40	40	40	0	0	0
	YF		0	10	10	20	20	20	0	0	0
	H		0	5	40	40	20	40	10	10	10
	B		0	5	10	10	10	20	0	0	0
11.	YF		5	5	5	10	10	10	0	0	0
	H		0	5	5	10	10	10	5	5	0
	B		10	10	10	20	20	20	0	0	0
12.	CA		0	40	40	10	0	0	0	0	
	YF		0	ND	ND	5	ND	ND	0	0	
	H		0	ND	ND	5	ND	ND	5	0	
13.	CA		0	10	10	10	20	10	0		0
	H		0	0	0	0	0	0	5		0
14.	CA		0	0	20	40	40	40	10		0
	YF		0	10	10	40	40	40	0		0
	H		0	0	5	10	10	10	0		0
	B		0	5	20	40	40	40	0		0

* Positive histoplasmin skin test.

† Only serologic tests developing positivity are listed.

‡ 0 wk all 0.

CA, collodion agglutination; YF, yeast phase histoplasma complement fixation; H, histoplasmin complement fixation; B, blastomyces yeast phase complement fixation; ND, not done.

complement-fixation tests.

Histoplasma yeast phase complement-fixation. Eleven of the 14 (78.6%) skin test positive persons demonstrated complement-fixing antibodies in titers ranging from 1:5 to 1:40 between the 2nd and 6th weeks after the initial skin test, *i.e.* one week after the 2nd to 5th weekly skin test. None of these persons sera was positive when samples were obtained at 26 weeks. Thus, with yeast phase antigen a relatively transient rise in antibodies usually followed skin testing and no positive sera were detected 21 weeks after the last skin test (See Table I).

Blastomyces yeast phase complement-fixation. Cross-reacting titers were observed in the sera of 8 of 14, or 57.1%. These appeared at similar time intervals as with homologous antigens and frequently in equal or occasionally even higher titer. However, as with yeast phase histoplasma antigens, all sera were negative when checked at 26 weeks, or 21 weeks after the last skin test (Table I).

Coccidioides complement-fixation. None of the sera reacted with coccidiodin antigen following histoplasmin skin testing.

Discussion. With the increased use of histoplasmin as a skin testing agent one must consider its potential effect on homologous and heterologous serologic tests. It was, therefore, not surprising that antibodies were demonstrated frequently following 2 or more positive skin tests. When the same type of antigen, histoplasmin, was used in complement-fixation as was used in skin testing, the antibody titer demonstrated more permanence than when yeast phase antigen was used. Since no serologic alterations were observed in skin test negative individuals, one could assume that in the positive skin reactors a previously attained immune response was stimulated by skin testing. The fact that such reactions were found in healthy individuals would suggest that a similar or an even greater response could be obtained during the active or convalescent stages of histoplasmosis. In our experience, peak titers with yeast phase complement-fixation tests in both animal and human infections have fallen by 5-8 months and low or negative results are seen after 12 to 18 months(6,7). Other studies in which histoplasmin was employed as antigen have shown positive tests as late as 3-4 years after the onset of the disease when the patient had been clinically well for 1-2 years(8). However, a review of these latter cases reveals numerous repeated positive skin tests. Positive agglutination reactions did not persist as long as did complement-fixing antibodies although histoplasmin was used as antigen

in both tests. However, the latter test is more sensitive when antibodies are low in titer and the collodion agglutination test is frequently negative after the 3rd month or so of active disease(7,9). Cross-reacting tests with blastomyces antigen were not unexpected, and have been described previously(2,4,5). Results from these laboratories demonstrated that at different stages of histoplasma infections heterologous blastomyces antibody titers often equaled and sometimes surpassed, the homologous histoplasma titers(10).

It is felt that repeated skin tests would alter and confuse the utilization of serologic data in the diagnosis of histoplasmosis.

Conclusions. Repeated positive histoplasmin skin tests in normal medical students caused false positive reactions in the collodion agglutination test (57.1%) and in the complement-fixation test with histoplasma yeast phase antigen (78.6%) or with histoplasmin as antigen (85.7%). The response was relatively transient in the former 2 tests, and negative titers were usually restored by 21 weeks after the last skin test. However, positive results were noted as late as 21, 25 and 39 weeks in complement-fixation tests employing histoplasmin as antigen. A transient response was observed in complement-fixation studies with cross-reacting yeast phase blastomyces antigens (57.1%), but no sera reacted in the same test with coccidiodin antigen. A single positive skin test and repeated negative skin tests did not alter serologic results. Thus, repeated histoplasmin skin testing of skin-test positive persons may invalidate complement-fixation data for long periods with histoplasmin as antigen, and for much shorter periods with yeast phase histoplasma and blastomyces complement-fixation or histoplasmin collodion agglutination tests.

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Effects of Large Doses of Desoxycorticosterone Acetate, Cortisone Acetate and ACTH in Intact Rhesus Monkeys.* (20221)

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In the course of a study designed primarily to investigate the effects of relatively large doses of desoxycorticosterone acetate, cortisone acetate and ACTH on the morphology of the adrenal cortex of the rhesus monkey(1), additional observations were recorded relating to the physiological effects of these hormones in the intact animal. These are reported in the present paper.

Materials and methods. The 14 monkeys (9 males and 5 females) used in this study were quartered in individual cages located in an air-conditioned room maintained between 78° and 80°F. Food(2) and water were supplied *ad libitum* except for a 12-hour fast preceding each bleeding. The monkeys were bled at three- to four-day intervals prior to and

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