reproducible titers, and proved to be more sensitive than those done by the CPE method using monolayers in tubes with fluid medium. The virus dose employed also appeared to be less critical.

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Histoplasmosis: To Skin Test or Not to Skin Test? (32333)

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The histoplasmin skin test has played a vital role in the present knowledge of histoplasmosis. Subsequent development of serologic tests provided a valuable diagnostic aid. That repeated skin tests are unnecessary and can stimulate antibody titer increases was first reported from this laboratory(1-3). As recently reviewed(4), there have been conflicting reports concerning the antibody stimulating effect of a single skin test on serologic results. Previous studies suggested that a single skin test did not alter serologic results in the tests employed in this laboratory(1,2,5). The interpretation of the significance of the serologic data in 2 suspected cases of histoplasma pericarditis, to be described separately, suggested reevaluation of the effect of a single skin test on serologic data obtained in this laboratory.

Materials and methods. A total of 187 persons consisting of 130 male prisoners 21-35 years of age and 57 medical technology students 18-22 years of age was used in this study. A single intradermal histoplasmin skin test employing a 1:100 dilution of the commercially-available antigen (Parke, Davis) routinely used in this institution was performed in all 187, and 35 of the 57 students also were skin tested concurrently with 1:100 dilutions of blastomycin (Park, Davis) and coccidioidin (Cutter). The test group was bled immediately prior to skin testing, at 8 weekly intervals thereafter, and then every 2 weeks through the 3rd month. Serum was separated promptly, stored at —30°C, and then all 11 samples from each subject were tested simultaneously.

The 2 serologic tests routinely employed in this laboratory, the collodion agglutination and yeast phase complement-fixation tests, were used. The collodion agglutination test was performed as originally described(6) employing an optimal dilution of histoplasmin prepared from the G-13 strain of *Histoplasma capsulatum*. A modification of the yeast phase complement-fixation test originally described

TABLE I. Effect of Single Histoplasmin Skin Test on Serologic Results.

Histoplasmin skin test	No. showing significant titer increase by: Collodion agg.	Latex agg.	Yeast phase comp.-fix.	Histoplasmin comp.-fix.
Positive (90 subjects)	0	0	0	24 (26.7%)
Negative (97 subjects)	0	0	0	0
Total (187 subjects)	0	0	0	24 (12.8%)

(5,7) which has been employed in this laboratory for 17 years was used. Antigen was prepared by growing the G-13 strain in the yeast phase for 3 days at 37°C on Brain Heart Infusion (Difco) containing 1.2% agar. Growth was washed off with 3.0 ml of 0.85% saline per slant, and the resulting suspension was heated twice at 60°C for 1 hour on 2 separate days. The yeast cells were then washed 3 times with 0.85% saline, and diluted to the original concentration. Merthiolate (Lilly) was added to a final concentration of 1:10,000. Antigenic and anticomplementary values were then determined in a standard box titration using positive serum. In the test proper, 0.25 ml of each dilution of serum was mixed with 0.25 ml of the optimal dilution of antigen and refrigerated for 20 minutes. Two full units of complement in 0.5 ml were added and the tests were refrigerated for 15-18 hours and then warmed in a 37°C waterbath for 10 minutes prior to addition of 0.5 ml of sensitized 1% sheep erythrocytes. Antigen anticomplementary controls consisted of antigen in the presence of 2.0, 1.5, 1.0 and 0.5 units of complement. Tests were read exactly 10 minutes after lysis of the 2-unit control; 2+ fixation was taken as the end-point.

For comparative purposes, histoplasmin complement-fixation and latex agglutination tests were carried out on all sera. Procedures used in performing the former were identical to those described above for yeast phase tests, except that commercially-available (Parke, Davis) histoplasmin complement-fixation antigen was employed. The agglutination test used was a commercial modification (Colab) of the histoplasmin latex test developed in this laboratory(8,9) and was performed according to the directions provided with the antigen.

Results. As shown in Table I, 90 (48.1%) of 187 individuals exhibited a positive histoplasmin skin test (5 mm or more of induration at 48 hours). During the 12-week observation period no significant antibody changes were detected in any of the sera when measured by the yeast phase complement-fixation, collodion agglutination or latex agglutination tests. The sera of 2 skin-test positive individuals with negative base-lines developed 2+ agglutinations up to 1:20 dilutions by the 3rd week in the latex agglutination test. However, in this test(9) as in the collodion agglutination(10) it has been emphasized that 4+ agglutinations are required before significance of titer can be assessed in relation to clinically-suspected histoplasmosis.

In a similar manner none of 97 skin-test negative individuals developed significant increases in antibody titers as measured by the histoplasmin complement-fixation test. However, in contrast, 24 (26.7%) of 90 skin-test positive persons developed significant 4-fold or greater titer increases which were not evident 1 week after skin test, but were thereafter. As shown in Table II, by the 2nd week all 24 showed titers varying from 1:5 to 1:80, and peak titers of 1:10 to 1:160 were observed in all by the 3rd to 4th weeks. Antibody titers began to decline slowly beginning with the 5th to 6th weeks, but at the end of the 12-week observation period sera from only 4 of the 22 available for the entire study had become negative.

As noted above, 35 individuals were skin tested simultaneously with histoplasmin, blastomycin and coccidioidin and 11, 0 and 2 gave positive skin tests, respectively. Four of the 11 with positive histoplasmin skin tests developed significant histoplasmin complement-fixation titer increases. The 2 individuals with positive coccidioidin skin tests had positive and negative histoplasmin skin tests, respectively, and neither of the 2 showed

TABLE II. Distribution of Histoplasmin Complement-Fixation Antibody Titers at Intervals After Positive Skin Test.

Weeks post-skin test	No. of subjects	Reciprocal of HCF titer						
		0	5	10	20	40	80	160
0	24	24	—	—	—	—	—	—
1	24	24	—	—	—	—	—	—
2	24	—	3*	8	10	2	1	—
3	24	—	1	8	12	2	—	1
4	24	—	—	10	12	1	1	—
6	22	—	5	10	5	2	—	—
8	22	1	3	10	7	1	—	—
10	22	1	7	8	5	1	—	—
12	22	4	4	8	5	1	—	—

* No. of subjects with titer of 1:5 at 2 wk.

significant titer increase in any of the 4 tests. Thus, in 35 persons receiving all 3 skin tests, no augmentation of serologic response was observed.

Analysis of serologic response in relation to size of skin-test reaction is included in Table III. As can be seen in persons showing induration measuring 5 to 10 mm, only 2 of 28 or 7.1% developed significant titer increases. In contrast, in those exhibiting reactions over 10 mm in diameter, which varied from 11 to 30 mm, 22 of 62 (35.5%) showed titer increases.

Sufficient sera were available from 18 persons who had developed significant titer changes in the histoplasmin complement-fixation test for comparative studies. Thus, baseline and 3-4 week post-skin test sera were submitted to 2 other laboratories, A and B,* with no prior knowledge of the purpose of the request. Both laboratories reported significant titer increase with the histoplasmin complement-fixation tests in 17 of 18 and 18 of 18, respectively. With yeast phase antigen complement-fixation, each laboratory observed significant titer increase with the sera of 2 people, but there was no correlation since these represented 4 different individuals. In addition, serial specimens from 28 persons negative in all 4 tests in this laboratory were submitted to laboratory A, and showed no titer changes in both yeast phase and histoplasmin complement-fixation tests.

TABLE III. Relationship of Size of Histoplasmin Skin-Test Reaction to Titer Change in Histoplasmin Complement-Fixation Test.

Induration (mm)	No. of subjects	Titer increases	
		No.	%
5	1	0	0.0
7	3	1	33.3
8	3	0	0.0
9	5	0	0.0
10	16	1	6.3
Total 5-10	28	2	7.1
11	5	2	40.0
12	19	6	31.6
13	15	4	26.7
14	11	3	27.3
15	7	5	71.4
16	2	1	50.0
18	1	0	0.0
20	1	0	0.0
30	1	1	100.0
Total 11-30	62	22	35.5
Total 5-30	90	24	26.7

Discussion. Every serologic test must be interpreted in terms of the clinical picture. Factors other than the disease which could alter serologic data should be recognized. Differences in results obtained after a single histoplasmin skin test have varied from laboratory to laboratory as recently reviewed (4). It is apparent that variables such as skin-test antigen, type of serologic test and antigens employed therein, and size of group studied could account for differences in results. It is therefore incumbent on each laboratory performing such tests to evaluate periodically these tests in terms of response to the skin-test antigen employed. Over the past 17 years in this laboratory, periodic checks on small groups have demonstrated that the collodion agglutination and yeast phase complement-fixation tests were not af-

* A—Arthur H. Bauer, Division of Laboratories, Ohio Department of Health.

B—Charlotte Campbell, Dept. of Microbiology, Harvard Univ. School of Public Health.

fected by a single skin test. The present study with a larger group has confirmed this observation. In the commercially-available latex agglutination test, a modification of the procedure originally described from this laboratory, 4+ agglutination at 1:8 or higher is considered significant. The sera of 2 persons in this study showed "increases in titer" but none of the agglutination reactions exceeded 2+ in intensity. It is of interest in a report(11) that latex agglutination antibodies were stimulated by a single test in 8 of 32 individuals, that 2+ agglutination was used as the criterion of a positive test.

The relation of size of induration to serologic conversion was not considered significant in a small series of 12 individuals where the test was not read as positive unless 10 mm or more of induration was observed(4). In most institutions 5 mm is considered a positive test. Thus, in comparing size of induration to serologic response in the histoplasmin complement-fixation test, reactions over 10 mm resulted in a significantly greater incidence of skin-test induced antibodies.

The protean manifestations of histoplasmosis have been emphasized from this institution(12,13). The skin test is a valuable tool when properly employed. *Repeated* skin tests, particularly in the skin-test positive person, offer no new information and may lead to false positive serologic tests. Constant monitoring of the effect of a *single* test on the serologic tests employed in each laboratory, and appreciation of this relationship or lack of relationship can be invaluable in the overall assessment of the patient with suspected histoplasmosis.

Summary. A single skin test with commercially-available histoplasmin did not stimulate significant antibody response in the sera of 187 persons when measured by the yeast phase complement-fixation, collodion agglutination or histoplasmin latex agglutination tests. Similarly, no effect was noted with the histoplasmin complement-fixation test in the sera of 97 skin-test negative individuals; in contrast 26.7% of skin-test positive persons developed significant titer increases.

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