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Comparison of Coccidioidin and Spherulin in Complement Fixation Tests for Coccidioidomycosis

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Coccidioidin, an extract from the saprophytic mycelial form of *Coccidioides immitis*, has been a very useful antigen preparation in serological tests for coccidioidomycosis. Its sensitivity has been very good for detecting most types of clinical disease, but tests with coccidioidin have been negative for 40% or more of patients with chronic pulmonary disease, the clinical entity which must be differentiated from other cavitary, nodular, or fibrotic pulmonary disease, e.g., tuberculosis and cancer. The specificity of coccidioidin has also been good although it results in positive tests for an average of 16% among patients with noncoccidioidal mycoses. Recently spherulin, an extract from the parasitic endosporulating spherule form of *C. immitis*, was reported to be more sensitive than coccidioidin in concurrent complement fixation tests with sera from selected cases. We have compared coccidioidin and spherulin in concurrent complement fixation tests with 614 sera submitted routinely for coccidioidal serology and with 159 selected sera from patients with noncoccidioidal mycoses. Among the former, spherulin was positive with 25% and coccidioidin with 23%, and correlation of titer scores was highly significant. Statistical analysis revealed no significant differences with respect to frequency of positive specimens, titer scores, or diagnosis for current coccidioidomycosis. The results with sera from noncoccidioidal mycoses revealed marked differences. Coccidioidin was positive with 20%, and spherulin was positive with 48%. The titer scores with spherulin were consistently and significantly higher, and there was no correlation for results with the two antigens. Thus, coccidioidin and spherulin were equally sensitive, but spherulin was considerably less specific.

The excellent work of Smith et al. (22, 24-26) demonstrated the value of complement fixation (CF) tests for coccidioidomycosis, using coccidioidin as the antigen. The test was considered a diagnostic indicator when there was conversion from negative to positive or when the titer increased fourfold or more with serial specimens during the course of current illness. Among cases of primary nondisseminating coccidioidomycosis, 68% were positive after 1 month of illness, and 84% were positive by the end of the 2nd month. In addition, Smith et al. (24, 26) found a consistent prognostic pattern. A rising titer indicated progressively increasing severity of infection. Among patients with nondisseminating pulmonary disease, no more than 5% had CF titers greater than 1:16. The frequencies of CF titers exceeding 1:16 for patients with disseminated (extrapulmonary) disease were 15% among those with a single demonstrable extrapulmonary (nonmeningeal) lesion, 49% among those with meningitis, and 83% among patients with extensive disseminated disease (nonmeningeal).

Although quantitative CF results have been very useful to clinicians as diagnostic and prognostic aids for most clinical types of coccidioidomycosis, there has been an acknowledged need for more sensitive and specific tests. Serological tests have been negative with approximately 40% of cases with chronic residual pulmonary disease and with 24% of spinal fluids from patients with meningitis (26). In addition, serological tests with coccidioidin on sera from patients with noncoccidioidal mycoses have been occasionally positive (3, 9-11, 23, 25). More recently, Scalarone et al. (20) reported that spherulin was more sensitive than coccidioidin in CF tests for coccidioidomycosis. Spherulin is a water-soluble preparation derived from in vitro-grown spherules, the parasitic form of *Coccidioides immitis*, whereas coccidioidin is de-

rived from the saprophytic mycelial form of this fungus. Tests with both antigens were performed on 100 sera. Twenty of these had yielded moderate to strong CF reactions with coccidioidin, and the concurrent tests with both antigens revealed no significant difference in results. The remaining 80 sera were selected for negative or weak reactions with coccidioidin. Spherulin was reactive with approximately one-third of specimens nonreactive with coccidioidin.

Since the greater sensitivity of spherulin had been detected only with sera selected for negative or weak reactions with coccidioidin, and since this selection had not been balanced by a similar selection of specimens yielding negative or weak reactions with spherulin, it seemed appropriate to repeat a comparison of spherulin and coccidioidin with all specimens submitted for coccidioidal serology. The results demonstrated no significant difference in sensitivity for either antigen. In addition, CF tests were done on specimens from patients with noncoccidioidal mycoses, and the results indicated that coccidioidin was significantly more specific than spherulin.

MATERIALS AND METHODS

Specimens. A total of 691 sera were received for serology for coccidioidomycosis. After excluding those which were anticomplementary or contained insufficient volume, CF results with both antigens were obtained for 614 sera. Similarly, CF tests were performed on 175 sera from patients with mycoses other than coccidioidomycosis. These were obtained from G. Baum, Veterans Administration Hospital, Cleveland, Ohio; M. Gordon, New York State Department of Health; L. Kaufman and S. Blumer, Center for Disease Control, Atlanta, Ga; J. Bennett, National Institutes of Health, Bethesda, Md.; and D. W. R. Mackenzie and C. M. Philpot, Mycology Reference Laboratory, London School of Hygiene and Tropical Medicine. The noncoccidioidal diseases from which these sera had been obtained are listed in Table 8.

Antigens. Coccidioidin was prepared and standardized according to methods published previously (5, 6). Spherulin was purchased from a commercial source (Berkeley Biologicals, Berkeley, Calif.). The recommended optimal dilution for the commercial spherulin was verified in a concurrent test with a reference spherulin supplied by G. M. Scalarone (University of California, Naval Supply Center, Naval Biomedical Research Laboratory, Oakland).

CF procedures. Since the objective was to compare results obtained with the two antigens, potential differences due to dilution of sera had to be avoided. Specimens were diluted (series of doubling dilutions) in sufficient volumes so that appropriate amounts could be transferred quantitatively from the set of dilutions to three additional sets of tubes. One was for the addition of coccidioidin, one was for spherulin, and the last set received diluent instead of antigen as a control for anticomplementary activity of the specimen. This protocol was modified further for CF tests with specimens from noncoccidioidal mycoses. After the three sets of serum dilutions had been prepared, the diluted specimens were numbered and rearranged according to a table of random numbers before the addition of antigen or diluent. Decoding of the scrambled sets was not done until all results had been recorded.

Two procedures were used according to the following protocol. Agar gel immunodiffusion and latex particle agglutination tests for coccidioidomycosis were done on all specimens received in the Veterans Administration Special Reference Laboratory for Coccidioidomycosis Serology (6, 8). CF with the 0.5-volume Kolmer procedure (24, 26) was performed by this laboratory on specimens yielding positive results in either test. Specimens which were negative with both the agar gel immunodiffusion and latex particle agglutination tests were transferred to the Mycology Research Laboratory, where CF was performed by the micro-adaptation of the LBCF procedure (30). Previous reports had demonstrated that comparable results were obtained with these methods when antigens were standardized appropriately for each (7, 12). Incubation for fixation of complement was done overnight at 5°C.

There have been a variety of approaches toward decision making with respect to recording the titer of CF tests. Smith et al. (24) considered the last tube (in a doubling dilution series) yielding complete fixation (4+) as the titer. Scalarone et al. (20) used 3+ or stronger as positive, and our practice with Smith's Kolmer procedure had been the same. The LBCF method used percent hemolysis, with 30% or less hemolysis as positive, and this could be converted to a score as indicated in Table 1 (30). Since our objective was to compare results obtained with two antigens, it seemed unreasonable to use these conventional titer systems when a difference of only 10% hemolysis or 1 unit in a score would be recorded as a twofold difference in titer (Table 2). Therefore, we have recorded results for each specimen as the total score obtained by adding the scores for each tube in dilution series. The examples presented in Table 2 illustrate that the total score was a more accurate comparison of the results obtained with each antigen than conventional titer, either by percent hemolysis or by the score in the last tube with fixation of complement. A score ≤ 2 was considered negative, since this represented less than 50% fixation of complement in a 1:2 dilution of serum.

Statistical analyses. Since the frequency distribu-

TABLE 1. *Conversion of percent hemolysis to a score*

% Hemolysis	Score
0–30	4
>30–50	3
>50–75	2
>75–90	1
>90–100	0

tion of quantitative results with each antigen did not follow a normal distribution curve, nonparametric statistics were used. Therefore, the serological results with each antigen were analyzed by the Spearman rank correlation coefficient and the Wilcoxon matched-pairs signed-rank test (21). The chi-square test was used to evaluate results with each antigen relative to a diagnosis of coccidioidomycosis for illness at the time the specimen was obtained.

RESULTS

The commercial spherulin and the reference spherulin were tested simultaneously against 12 sera to determine whether the former was comparable to the latter. The two antigens differed only slightly, and the differences were not significant (Table 3). Therefore, the commercial spherulin was considered equivalent to the reference spherulin, and the former was used throughout the remainder of the study.

Tests were performed to determine the range of difference in score attributable to experimental error. Duplicate tests for each of 36 specimens were done with coccidioidin and also with spherulin. The results (Table 4) demonstrated that a score difference of 4 was not exceeded for the duplicates of any specimen with either antigen. Therefore, when results with coccidioidin and spherulin on the same specimen differed by a score of 4 or less, it was reasonable to consider that these were equivalent. It is of interest that a score ≤4 is within a one-tube difference in the twofold dilution series (see Table 1), although a score difference of 4 might extend through two tubes of the series, e.g., three at a 1:2 dilution (score = 3) and three at a 1:4 dilution (score = 7).

Earlier reports had demonstrated that results obtained by the 0.5-volume Kolmer and by the micro-adaptation of the LBCF (microtiter) CF procedures were equivalent when antigens were standardized for each method (7, 12). In each of these reports, the tests were done in the same laboratory, but in the present study the tests were performed in separate laboratories. Therefore, 52 sera that had been reactive by the Kolmer method in the Reference Laboratory were tested by the microtiter method in the Mycology Research Laboratory (Fig. 1). Statistical analysis yielded a highly significant correlation coefficient, and therefore results obtained by these two methods on the same specimens were considered equivalent (Table 3).

Coccidioidin and spherulin were compared for sensitivity by simultaneous CF tests with 614 specimens submitted for serological testing for coccidioidomycosis. Of these specimens, 442 were negative with both antigens. The results with the 172 reactive specimens (at least one antigen with score ≥3) are presented in Fig. 2. The difference between scores with each anti-

TABLE 2. *Rationale for using score rather than titer for comparing CF results obtained with two different antigen preparations*

Antigen	CF result			CF titer	Total score
	Dilution	% Hemolysis	Score		
A	1:2	40	3		3
B	1:2	30	4	1:2	4
A	1:16	40	3	1:8	15
B	1:32	30	4	1:32	20

TABLE 3. *Statistical analyses of results when comparing commercial with reference spherulin and Kolmer with microtiter CF methods*

Comparison	n[a]	Spearman test[b]		
		r	95% CL	P
Spherulins	12	0.89	0.59–0.97	<0.001
Methods	52	0.88	0.80–0.93	<0.001

[a] *n*, Number of specimens.
[b] *r*, Spearman rank correlation coefficient; 95% CL, 95% confidence limits; *P*, probability.

TABLE 4. *Score differences for 36 specimens in duplicate tests with each antigen to determine the range attributable to experimental error*

Antigen used	Frequency of score difference					
	Equal	1	2	3	4	>4
Coccidioidin	24	7	2	1	2	0
Spherulin	18	7	5	4	2	0

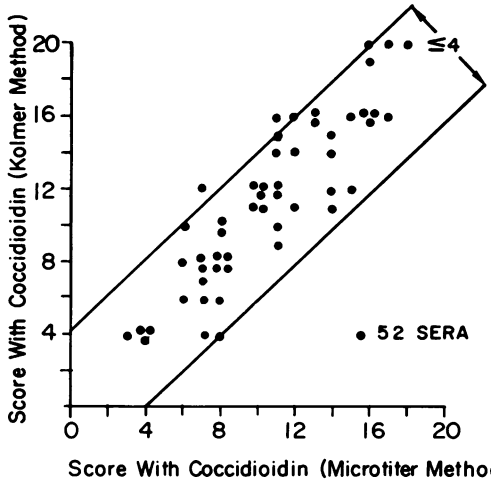

FIG. 1. *CF results obtained with Kolmer and microtiter procedures. Equivalent results (score difference ≤ 4) contained in outlined area.*

gen fell within the equivalent range (≤4) for 74% (128/172) of the reactive specimens. The score with spherulin was ≥5 than that with coccidioidin for 19 specimens, and the score with coccidioidin was ≥5 than that with spherulin for 25 specimens. Analysis of these results yielded a highly significant correlation coefficient (Table 5). Therefore, the null hypothesis that there was no association among these two sets of results would be rejected in favor of the decision that there was significant correlation, and one would conclude that there was no significant difference among results obtained with coccidioidin and spherulin in CF tests with specimens submitted for coccidioidal serology.

Inspection of Fig. 2 seemed to show that scores with coccidioidin were consistently greater than those with spherulin for specimens with higher CF titers, and this was apparent most frequently with specimen scores >12. Therefore, the Wilcoxon matched-pairs signed-rank test was used to compare results with all scores, and also for those scores ≤12 and those >12. It was apparent (Table 5) that the frequency by which coccidioidin scores exceeded spherulin scores was significant only for sera with scores >12, even when only sera with a score difference >4 were considered. When all sera were evaluated regardless of score, the frequency of score differences was not significant for either antigen (Table 5 and Fig. 3).

The results with both antigens are presented in Table 6 in terms of numbers of positive and negative specimens of those submitted for coccidioidal serology. Among the 172 specimens which were reactive, 12% were positive only with coccidioidin, and 19% were positive only with spherulin. Of the 33 specimens that were spherulin positive and coccidioidin negative, the total score with spherulin was 3 to 4 with 13 and ≥5 for the remainder. Similarly, coccidioi-

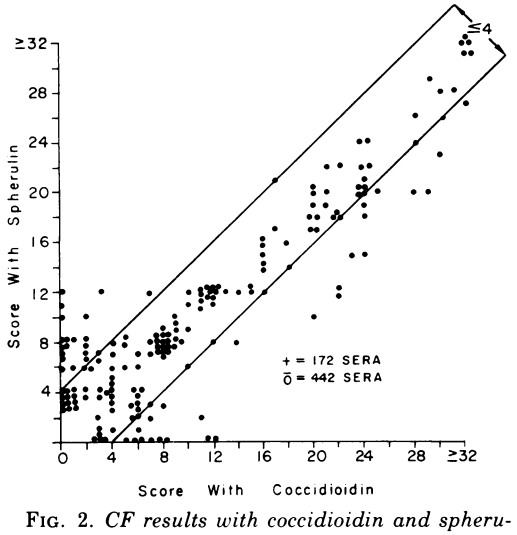

FIG. 2. *CF results with coccidioidin and spherulin on reactive sera submitted for coccidioidomycosis serology. Equivalent results (score difference ≤ 4) contained in outlined area.*

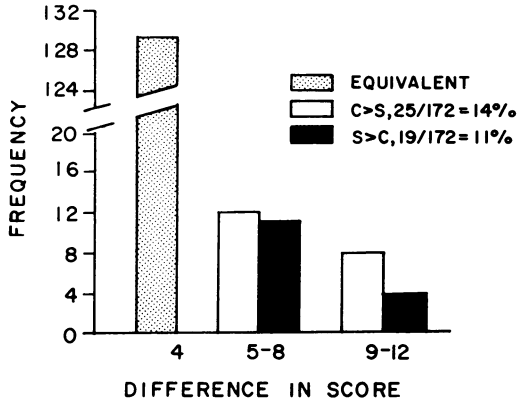

FIG. 3. *Frequency of differences in scores obtained with coccidioidin (C) and spherulin (S) in CF tests with sera submitted for coccidioidomycosis.*

TABLE 5. *Statistical analyses of results with sera submitted for coccidioidal serology*

Sera	Coccidioidin score	Statistical tests[a]		
		n^b	Spearman P	Wilcoxon P
All	0-38	172	<0.001	NS[c]
	≤12	118	<0.001	NS
	>12	54	<0.001	<0.001
Score	0-38	43	<0.03	NS
Difference	≤12	31	<0.001	NS
>4	>12	12	<0.001	0.002

[a] Spearman rank correlation coefficient and Wilcoxon matched-pairs signed-rank test; P, probability.

[b] n, Number of specimens.

[c] NS, Not significant, $P > 0.05$.

TABLE 6. *Comparison of CF results with coccidioidin (C) and spherulin (S), and of clinical diagnosis with sera submitted for coccidioidal serology*

CF			No. with diagnosis[a]	
C	S	n^b	Positive	Negative
+	+	119	97	21
+	−	20	6	10
−	+	33	4	24
−	−	442	11	392

[a] Diagnosis for coccidioidomycosis during current illness; 565/614 (92%) were available.

[b] n, Number of specimens.

din was positive and spherulin was negative with 20 specimens, and the total score with coccidioidin was 3 to 4 with 7 and ≥5 with 13. These differences were not significant (chi-square = 0.287, $P > 0.05$). When the CF results with each antigen were evaluated with respect to a current diagnosis of coccidioidomycosis, statistical analysis indicated that there was a significant difference among the overall results, but further analysis demonstrated (Tables 6 and 7) that this applied only to whether a specimen was serologically positive or negative (with either antigen) compared with the diagnosis of coccidioidomycosis; i.e., there were two different populations, one being CF positive with a diagnosis of coccidioidomycosis, and the other being both serologically and diagnostically negative. The remaining comparisons for CF results with each antigen with respect to a diagnosis of coccidioidomycosis were without significance.

The CF tests with sera from cases of noncoccidioidal mycoses revealed that spherulin was considerably less specific than coccidioidin (Fig. 4 and Table 8). Coccidioidin was positive with 20% (35/175) and spherulin with 48% (84/175) of these specimens. In addition, the score with coccidioidin was >4 than that with spherulin for only 3%, but the spherulin score was greater for 51% of the 92 reactive specimens (Fig. 5). It was apparent that there was a marked difference between these two antigens when results with sera for coccidioidal serology (Fig. 3) were compared with those for noncoccidioidal serology (Fig. 5). Statistical analyses demonstrated a complete reversal for specimens from noncoccidioidal mycoses (Table 9) compared with those submitted for coccidioidal serology (Table 5). There was no correlation among the CF scores, and there was a significant difference by the Wilcoxon matched-pairs test for these two antigens with sera from noncoccidioidal mycoses.

TABLE 7. *Statistical analysis (chi-square test) of CF results with coccidioidin and spherulin compared to current diagnosis for coccidioidomycosis*

Comparison[a]	No. with diagnosis		P^b
	+	−	
C or S+	107	55	<0.001
C and S−	11	392	
C+	103	31	NS[c]
S+	101	45	
C−	15	416	NS
S−	17	402	
C+, S−	6	10	NS
C−, S+	4	24	

[a] C, Coccidioidin; S, spherulin.
[b] P, Probability by chi-square test.
[c] NS, Not significant, $P > 0.05$.

DISCUSSION

The value of an antigen as a serodiagnostic aid depends on its sensitivity and specificity for

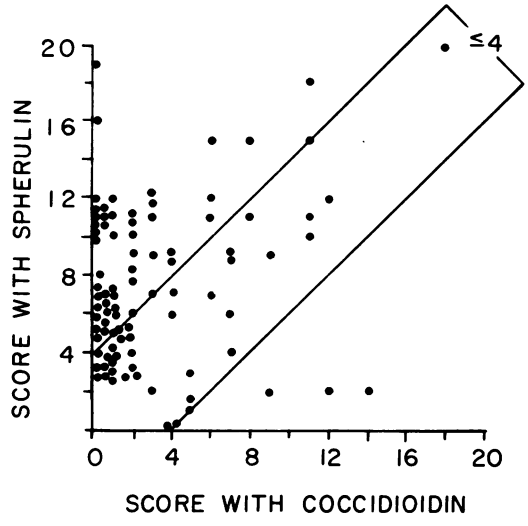

FIG. 4. *CF results with coccidioidin and spherulin on reactive sera from patients with noncoccidioidal mycoses.*

TABLE 8. *Specificity of CF results with coccidioidin (C) and spherulin (S), with sera from noncoccidioidal mycoses*

Etiology	No. of sera with reactions				
	No.	C+ S+	C+ S−	C− S+	C− S−
Molds					
Allescheria boydii	2	1	0	0	1
Aspergillus species	3	1	0	2	0
Blastomyces dermatitidis	4	0	0	0	4
Histoplasma capsulatum	116	20	8	40	48
Phialophora pedrosoi	1	0	0	0	1
Yeasts					
Candida albicans	9	2	0	6	1
Candida parapsilosis	1	1	0	0	0
Cryptococcus neoformans	32	1	0	6	25
Torulopsis glabrata	1	0	0	1	0
Actinomycetes					
Micromonospora faeni	3	0	0	2	1
Streptomyces somaliensis	1	1	0	0	0
Thermoactinomyces vulgaris	2	0	0	0	2

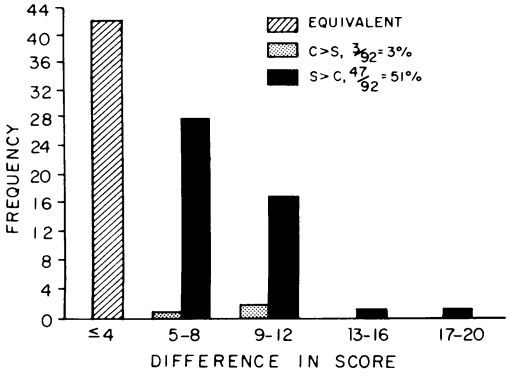

FIG. 5. *Frequency of differences in scores obtained with coccidioidin (C) and spherulin (S) in CF tests with sera from patients with noncoccidioidal mycoses.*

TABLE 9. *Statistical analyses of results with sera from patients with noncoccidioidal mycoses*

Sera	Coccidioidin score	n[a]	Statistical tests[b]	
			Spearman P	Wilcoxon P
All	0-20	92	NS[c]	<0.001
	≤12	90	NS	<0.001
	>12	2	ND[d]	ND
Score Difference >4	0-20	50	NS	<0.001
	≤12	49	NS	<0.001
	>12	1	ND	ND

[a] n, Number of specimens.
[b] Spearman rank correlation coefficient and Wilcoxon matched-pairs signed-rank test; P, probability.
[c] NS, Not significant, $P > 0.05$.
[d] ND, Not done, $n < 6$.

detecting infection caused by the organism from which the antigen was prepared. When the etiological agent occurs in both a saprophytic form and a parasitic form, one anticipates that extracts from the latter will contain a spectrum of antigens more representative than an extract from the saprophytic form with respect to induced antibody formation in the host. This occurs in coccidioidomycosis, where the fungus *Coccidioides immitis* exists as a mycelial form in nature and as an endosporulating spherule in an infected host. Therefore, one would anticipate that an extract from the spherule form (spherulin) would be more sensitive than a preparation from the mycelial form (coccidioidin) for detecting an antibody response in coccidioidomycosis. The question of specificity differs. Whereas sensitivity would depend on what antigens and how many different antigens are present in the extract, specificity would be determined primarily by the qualitative antigenic content. A preparation could be highly specific but lack sensitivity because too few antigens were present, or highly sensitive but lack specificity because it contained antigens partially or completely identical to those found among other agents to which the host had been exposed. The ideal preparation would be both highly sensitive and specific.

Coccidioidin has been used extensively as a serological reagent for coccidioidomycosis for almost 30 years. Smith et al. (24, 26, 27), using coccidioidin in a tube precipitin test and CF tests with all specimens, found that the time during the course of illness at which specimens were obtained and the type of clinical disease were important factors determining the frequency of positive results with each test. Among serologically reactive patients with primary nondisseminating coccidioidomycosis, 91% were positive by the 2nd week of illness with the tube precipitin test, but this frequency decreased rapidly to 10% positive by the 4th month. Among the same patients, the frequency of reactors with the CF test was only 29% at 2 weeks, but increased gradually to 84% by the 2nd month of illness and reached 89% by the 6th month. The frequencies of positive serology among patients with disseminated coccidioidomycosis were 63% with the tube precipitin test and 99% with the CF test, reflecting the later time during the course of illness at which specimens were obtained and the more extensive disease that was present (26). In contrast, tube precipitin tests were positive for only 6% and CF tests for 62% of patients with pulmonary cavitary lesions, and these frequencies were considerably less among patients with other types of chronic pulmonary disease (22). Thus, the sensitivity of coccidioidin has been very good, but there is much room for improvement.

The specificity of coccidioidin has been equally good, but this preparation has been reported to yield positive CF tests with serum from patients with other mycoses, especially histoplasmosis (1-3, 11, 16-19, 25). The cited reports included 325 cases and thousands of specimens. With the exception of one report, the frequency of reactions to coccidioidin ranged from none to 27%, with an average of 16% for those in which cross-reactions to coccidioidin were found. The single exception was that by Kaufman and Clark (11), in which they reported 13/16 cases of histoplasmosis and blastomycosis reacting with coccidioidin in the CF test. The objective of this study, however, was

to assess the value of the agar gel immunodiffusion test with coccidioidin for resolving the etiology among sera reactive with several fungal antigens in the CF test; therefore, these sera had been selected for the study because they demonstrated multiple reactivity (personal communication).

Even though coccidioidin has demonstrated good sensitivity and specificity in serological tests for coccidioidomycosis, a better reagent would be desirable. Levine, Stevens, et al. (13–15, 28, 29) had demonstrated convincingly that spherulin was more sensitive and equally specific in comparison with coccidioidin as a skin-testing reagent. Scalarone et al. (20) compared coccidioidin and spherulin in the CF test, and reported that spherulin was more sensitive than coccidioidin among low-titered sera, since it was reactive with approximately one-third of specimens nonreactive with coccidioidin. Moderate- to high-titered sera yielded equivalent results with both antigen preparations. This comparison was incomplete, in our opinion, because 80 of the 100 sera tested had been selected for negative or weak reactions with coccidioidin, leaving open the question of whether sera that were negative or weakly reactive with spherulin might be more reactive with coccidioidin. Our results with consecutive serum specimens confirmed that spherulin was reactive with 33 sera that were negative with coccidioidin, but we found also that 20 specimens were coccidioidin positive and spherulin negative and that all these differences occurred among sera with low titers (Table 6 and Fig. 2). In addition, 38% (6/16) of specimens that were coccidioidin positive-spherulin negative, but only 14% (4/28) that were spherulin positive-coccidioidin negative, were from patients diagnosed as having coccidioidomycosis at the time the specimen was obtained (Table 6).

It is quite possible that many of the cases that were positive with only one antigen may have had coccidioidomycosis in the past and had retained residual CF antibody. If so, then spherulin would indeed be more sensitive than coccidioidin, as reported by Scalarone et al. (20), but less reliable with respect to current illness. Although these differences with coccidioidin and spherulin are apparent in our results, it must be noted that none are statistically significant (Table 7) with respect to a diagnosis of coccidioidomycosis, and by this objective analysis we must conclude that they are equivalent in sensitivity.

It is possible, however, that these two extracts from different growth forms of *C. immitis* may differ qualitatively or quantitatively in content of antigens. Evidence supporting this is found in the results obtained with sera from patients with mycoses other than coccidioidomycosis. Here the differences are most impressive with 20% (35/175) of sera reactive with coccidioidin compared with 48% (84/175) positive with spherulin. The cross-reactivity with coccidioidin approximates the average of 16% reported by other investigators (vide ante). Furthermore, the results with these two antigens differ not only in the number of reactive sera from noncoccidioidal mycoses, but also in the scores (i.e., titers) obtained. This is most apparent when comparing Fig. 3 and 5, where there is very little difference in paired scores with sera submitted for coccidioidal serology but marked differences in paired scores among specimens from cases of other mycoses. In contrast to the homologous results, the differences for the specimens from heterologous mycoses are highly significant (Table 9). Nevertheless, the question of whether these two antigen preparations differ qualitatively and/or quantitatively cannot be answered by information currently available; this question is now being investigated.

The possibility of dual infections must be considered also. It was because of this possibility that the sera from noncoccidioidal mycoses were obtained from areas east of the Mississippi River, i.e., well outside of the endemic area for coccidioidomycosis. Although this question cannot be answered definitively, it seems highly unlikely that almost one-half of this population would have satisfied the two conditions required for infection by *C. immitis*: presence in the endemic area (or exposure to fomites), and actual infection while there.

The more plausible explanation for the difference in specificity would be that infection with *C. immitis* induces antibody to one or more antigens found also in other fungi. It is quite common for serum from humans and animals with coccidioidomycosis to yield positive CF tests with antigens from *Histoplasma capsulatum* and *Blastomyces dermatitidis*, even to higher titers than with the homologous antigen (2–4, 11, 16, 18, 23). This would imply that the parasitic form of *C. immitis*, the spherules and endospores, elaborated the common antigen(s). Therefore, it should not be surprising that an extract (spherulin) from this parasitic form is markedly cross-reactive. The evidence that coccidioidin also reacts with sera from noncoccidioidal mycoses, but with lesser frequency, would indicate that this extract from the saprophytic form also contains some of the common antigen(s).

We must conclude, therefore, that coccidioidin and spherulin are equivalent in sensitivity for detecting currently active coccidioidomycosis, but that coccidioidin is considerably more specific than spherulin. Since spherulin did detect a few cases of coccidioidomycosis that were serologically negative with coccidioidin, some consideration must be given to the use of both preparations for maximum coverage. Spherulin alone, however, would be inadvisable because of the high frequency of false positive reactions and the possibility of erroneous diagnoses. The reactivity of both coccidioidin and spherulin with specimens from patients with noncoccidioidal mycoses and actinomycoses is particularly disturbing, since so many reports have indicated that coccidioidin is relatively specific. From these reports and our current results, it appears that the nonspecificity of coccidioidin is of the order of 16 to 20%. Additional studies to evaluate this question more completely are indicated.

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