Serologic Studies in Histoplasmosis*

SAMUEL SASLAW, M.D., PH.D., CAPTAIN, M.C., A.U.S., AND CHARLOTTE C. CAMPBELL

Department of Bacteriology, Army Medical Department Research and Graduate School, Washington, D. C.

THE coincidence of pulmonary scarring or calcified nodules and histoplasmin skin sensitivity in tuberculin-negative persons was first described in detail by Christie and Peterson [1] and Palmer.[2] These and subsequent studies [3-16] have emphasized that lesions which in the past would have been interpreted as tuberculous in origin may be the result of an infection with *Histoplasma capsulatum* or some other related organism.

Widespread surveys correlating the roentgenographic appearance of pulmonary infiltrates with histoplasmin sensitivity, as well as recent descriptions of non-fatal cases [17, 18] have not only stimulated interest in the disease process known as histoplasmosis but have considerably altered the earlier concept that infection with *H. capsulatum* was rarely encountered and invariably fatal. The increasing cognizance of this alternate concept has resulted in a relatively larger incidence of detected infections. This, in turn, lends support to the postulate that histoplasmin sensitivity may be a direct result of previous infection with *H. capsulatum* and that a benign form of the disease is prevalent throughout a wide geographical area of the United States. The correctness of such a postulate can be strengthened by detecting a sufficient number of cases in the early acute phase of the disease. Unfortunately, the exact diagnosis of an infection so protean in nature as histoplasmosis, is not without its complexities and at present is largely dependent upon the isolation of the organism from infected tissues. This latter procedure, even in fulminating cases, is sometimes achieved only with great difficulty.

Several groups of investigators [19-27] have therefore turned to evaluating the use of serologic tests for the detection of humoral antibodies against *H. capsulatum*. Preliminary results of such studies in this laboratory [24, 27] as well as those conducted by Salvin [19] and by Furcolow and associates [21] have indicated that they may be of value as an aid in the diagnosis of histoplasmosis.

Studies in this laboratory have utilized two basically different tests and antigens. For the complement-fixation test a ground antigen derived from the yeast phase of *H. capsulatum* [23, 24] has been employed, since this is the form in which the organism is found in parasitized tissues. Conversely, the skin test antigen, histoplasmin, derived from the mycelial phase of the organism was incorporated into the collodion agglutination technic.[26-27] The nature of this antigen lends itself admirably to this latter procedure in that union with the microscopic collodion particles is readily achieved and positive agglutinations are macroscopically visible and distinct.

The data presented herein include the results obtained with sera from "normal" individuals, both skin test positive or negative, suspected or proven cases of histoplasmosis, and from persons representing a variety of illnesses.

* Presented at a Joint Session of the Epidemiology and Laboratory Sections of the American Public Health Association at the Seventy-seventh Annual Meeting in New York, N. Y., October 26, 1949.

TABLE 1

Repeatability of Quantitative Tests for Histoplasmosis Using Immune Rabbit Serum

Specimen Number	*Tests*	*Titers Frequency*							
		< 1:5	*1:5*	*1:10*	*1:20*	*1:40*	*1:80*	*1:160*	*1:320*
1	10 F*	9	1						
	10 A+	10							
2	10 F	2	4	4					
	10 A	10							
3	10 F						8	2	
	10 A					10			
4	9 F					8	1		
	9 A			3	6				
5	5 F								5
	5 A							5	
6	5 F				1	2	2		
	5 A				4	1			

F*—Complement-fixation
A+—Collodion Agglutination

METHODS

The complement-fixation technique employing ground yeast phase antigens in the presence of serial serum dilutions and the 50 per cent endpoint of hemolysis has been previously described[22-24] as has the collodion agglutination test.[25-27]

RESULTS

Repeatability of tests—In order to evaluate the reliability and reproducibility of the two tests a number of human as well as rabbit serum specimens were divided into a series of separate tubes and labeled with numbers in such a manner that only the referee knew the contents of each tube. Four persons then performed the dilutions of the serum specimens, selected at random. Because of this latter factor, a one-tube dilution variation in results was to be expected and was not considered out of the range of probability. Such studies demonstrated that over 98 per cent repeatability was obtained in both tests, utilizing either rabbit or human sera. Table 1 demonstrates the results obtained with a representative group of immune rabbit sera of varying degrees of reactivity. On the basis of repeated observations of this nature it was assumed that the techniques and materials employed were reliable and that the results obtained on test samples were not often affected by chance variations in the performance of either serologic test.

Results with miscellaneous human sera—In order to ascertain the presence or

TABLE 2

Complement-fixation Reactions with Human Sera

Number of Persons	*Skin Reactions*	*Number of Sera Fixing Complement Serum Dilutions*				*Total*	*Per cent*
		1:5	*1:10*	*1:20*	*1:40*		
230	Positive	8	15	5	3	31	13.5
323	Negative	4	10	2	0	16	4.9
Total 553		12	25	7	3	47	8.5
303	?	6	14	3	2	25	8.2

absence of detectable humoral antibodies in so-called "normal" ambulatory persons, complement-fixation tests were performed on a total of 553 serum specimens obtained from a heterogeneous adult group representing residents of nearly every state in the union. Of this number, 230 were from skin-test-positive persons and 323 from individuals failing to react to histoplasmin. As noted in Table 2, 31 sera, or 13.5 per cent of the skin-test-positive group fixed complement in serum dilutions varying from 1:5 to 1:40. Relatively few reactions were obtained at the higher dilutions—viz: 5 or 2.2 per cent at 1:20 and 3 or 1.3 per cent at 1:40. Of the skin-test-negative group, 16 or 4.9 per cent of the 323 sera fixed complement in 1:5 to 1:20 dilutions. Thus, relatively more positive reactions were obtained in the skin-test-positive group. Also, as can be seen in Table 2, 25 or 8.2 per cent of 303 random sera originally submitted for other studies and on which skin sensitivity data were not available showed complement-fixing antibodies. This latter figure is remarkably close to that of 8.5 per cent attained in the combined total of both the skin-test-negative and -positive group above.

A similar study with the collodion agglutination technique revealed, as demonstrated in Table 3, that agglutination was obtained in 55 or 26.7 per cent of 206 skin-test-positive persons and in 65 or 21.4 per cent of 303 skin-test-negative individuals. All but 7 of these sera gave weak 1+ to 2+ reactions in low titer which we have not considered as significant (4+) in terms of active infection. The 7 sera giving 4+ reactions were from student nurses, of whom 5 were converters from Kansas, Ohio, Michigan, and Minnesota. The sixth was an Ohioan with questionable pulmonary calcification and the seventh a skin-test-negative, x-ray negative person from Kansas City. A total of 103 or 25.5 per cent of a miscellaneous group of 403 serum specimens yielded weak reactions. The 25.5 per cent is likewise remarkably close to the combined reactor rate of 23.6 per cent of the skin-test-positive and -negative group above.

The above results indicated that the presence of antibodies against either the yeast phase antigen or histoplasmin may occur in low dilutions in a small percentage of individuals exhibiting no evidence of the specific disease, and tends to emphasize the well known serologic fact that results on single specimens should not supersede the value of serial studies where a more critical evaluation of test results can be made in terms of the clinical picture.

Results with serum from proven cases—The ideal manner of evaluating the humoral antibody response to any organism is to obtain serial serum specimens during the course of the disease process. Aside from the difficulties normally encountered in the early diagnosis of

TABLE 3

Collodion Agglutination Reactions with Human Sera

Number of Persons	*Skin Reaction*	*No. of Sera Causing Weak Agglutinations (1+—2+) Serum Dilutions* 1:5	1:10	1:20	1:40	*Total*	*Per cent*
206	Positive	20	15	14	6	55	26.7
303	Negative	25	27	10	3	65	21.4
—		—	—	—	—	—	—
Total 509		45	42	24	9	120	23.6
403	?	51	31	17	4	103	25.5

TABLE 4

Early Serologic Picture in Two Non-Fatal Cases

		Serum Dilution								
Case 1										
Date '48		*1:5*	*1:10*	*1:20*	*1:40*	*1:80*	*1:160*	*1:320*	*1:640*	*1:1280*
8/24	F *	0	0	0	0	0	0	0	0	0
	A †	4+	4+	4+	4+	4+	3+	±	—	—
9/5	F	0	0	0	0	0	0	0	10	85
	A	ND				ND				ND
9/25	F	0	0	0	0	0	0	0	50	100
	A	4+	4+	4+	3+	2+	±	—	—	—
10/29	F	0	0	0	0	50	100			
	A	4+	4+	3+	2+	1+	—			
12/21	F	0	10	20	45	100				
	A	—	—	—	—	—				
Case 2										
8/24	F	0	0	0	50	100	100	100	100	100
	A	3+	3+	1+	—	—	—	—	—	—
10/20	F	0	0	0	0	0	45	100	100	
	A	4+	4+	4+	2+	1+	—	—	—	
2/10/49	F	×	45	95	100	100				
	A	1+	±	—	—	—				

F * — Complement-fixation — Positive = 50% or less Hemolysis
A † — Collodion Agglutination

TABLE 5

Serologic Picture in Rabbit, Infected with H. capsulatum

Number 3637

		Serum Dilution					
Week After Infection		*1:5*	*1:10*	*1:20*	*1:40*	*1:80*	*1:160*
0	F *	—					
	A †	—					
1	F	—					
	A	—					
2	F	0	0	0	0		
	A	4+	3+	1+	—		
3	F	0	0	0	0	0	30
	A	4+	4+	4+	4+	2+	±
5	F	0	0	0	0	40	
	A	4+	4+	2+	1+	—	
7	F	0	0	0	10	50	
	A	3+	2+	1+	—	—	
9	F	0	0	50			
	A	2+	1+	±			
12	F	0	20	60			
	A	2+	1+	1+			
18	F	30	80				
	A	—	—				
22	F	40	100				
	A	—	—				
26	F	65	100				
	A	—	—				

F * — Complement-fixation — Positive = 50% or less Hemolysis
A † — Collodion Agglutination

histoplasmosis, dependence upon sera referred from widely distributed sources makes such serial studies difficult to obtain. The present study, of necessity, therefore includes a number of single serum specimens that were obtained sometimes as late as 2 to 3 years after the diagnosis had been made. However, by fortuitous circumstances, sera were obtained shortly after the onset of illness from 2 children exhibiting classical symptoms of histoplasmosis. Subsequent serial studies presented a working background for comparison with the antibody response in other patients. The early serologic findings in these boys has been previously reported [24, 27] and the detailed history of the cases will be presented elsewhere.[18] Briefly, these 5 and 8 yr. old cousins living on the same farm in central Ohio were admitted to the hospital within a few days of each other. Although acutely ill they recovered spontaneously and are now living and well over 1 year later. A review of their chest films depicts the marked early pulmonary involvement with subsequent resolution and after about 8 months suggests early calcification. The serologic response of these patients has been charted in Table 4. It was on the basis of the high titers obtained within the 2nd to 3rd week of illness that cultural studies for *H. capsulatum* were instigated and carried out successfully. The initial high titers declined comparatively rapidly and levels obtained after 4 to 8 months, as noted below, were not unlike those observed in chronic cases or in a small number of so-called "normal" individuals. This transient nature of antibody response aroused our interest considerably and subsequent studies [28] with rabbits have likewise demonstrated that the majority of experimentally infected animals showed the highest peak of antibody titer 2 to 4 weeks after infection, and that within 5 to 8 months the titers had dropped off to baseline levels. Table 5 demonstrates the response in a representative animal.

Thus, it was not unexpected when certain serum specimens from known proved cases failed to demonstrate appreciable antibody titers. Sera from 16 cases, including the 2 above mentioned, were investigated by both tests for antibody content, and the results are shown in Table 6. The diversity of age, clinical status, and time of serum withdrawal present some difficulties in evaluation. Eleven of these patients are living while 5 died.

In the former group, excluding cases 1 and 2 discussed above, a single serum specimen obtained from No. 3 on the 4th month of illness fixed complement at 1:80 while the collodion agglutination test gave 4+ reactions and a titer of 1:40. This patient has also made a complete recovery.

Patient No. 4 who has had several bouts of vague undiagnosed illness for the past 2 years, which may or may not have been histoplasmosis, developed antibodies in his most recent episode and *H. capsulatum* was isolated from his larynx. During the 5th, 7th, and 8th months of his present illness, complement-fixation antibodies were present only in low titer (1:10). However, collodion agglutination reactions were positive at 1:80–1:160 during this period. We have insufficient data at this date to explain why the latter test showed such an appreciably higher titer. Serum from patients with evidence of chronic disease (Nos. 5–9), obtained from 8 months to several years after clinical onset, showed complement-fixation antibodies in low titer (1:5 to 1:40). These titers were comparable to those observed in the small percentage of so-called "normal" individuals discussed above. No 4+ collodion agglutinations were obtained in any of these sera. Case No. 10 represents the results obtained with the serum of an infant with disseminated histoplasmosis in which recovery is not expected. A single serum

TABLE 6

Serologic Reaction According to Approximate Month of Disease

Case No.	*Test*	*Age*	*Status*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*12*	*18*	*24*	*24+*	*Comments*
1	F *	C	L	1:1280	1:320	1:80		1:40								R
	A †			1:160	1:80	1:80		—								
2	F	C	L	1:40		1:160				1:10						R
	A			1:20		1:80				—						
3	F	A	L				1:80									R
	A						1:40									
4	F	A	L					1:10		1:10	1:10					Laryngeal
	A							1:80		1:80	1:160					R — possible
5	F	A	L									1:10	1.5	1:5		Chronic
	A											—	—	—		
6	F	A	L								1:10					"
	A										—					
7	F	A	L									1:5				"
	A											—				
8	F	A	L											1:20	1:20	"
	A													—	—	
9	F	A	L											1:40	1:20	"
	A													—	—	
10	F	I	L				—									Acute disseminated
	A						—									R — unlikely
11	F	I	L					—			—		—			R — old serum
	A							—			—		—			
12	F	A	D			1:40	D									Pharyngeal disseminated
	A					1:40										
13	F	O	D				D									Autopsy Blood
	A						—									
14	F	O	D				—	D								Laryngeal
	A						1:20									
15	F	O	D									1:10	1:10	D		
	A											—	—	—		
16	F	A	D									—				7 weekly samples negative—Disseminated
	A											—				

F * — Complement-fixation
A † — Collodion Agglutination

C — Child
A — Adult

I — Infant
O — Old

L — Living
D — Dead

R — Recovered

specimen, obtained approximately in the 4th month of illness, showed no antibodies. Case No. 11, an infant who recovered from the disease, showed no antibodies at 4, 8, and 12 months. If an antibody response occurred it may have been present earlier in the disease or the fact that these particular serum specimens were about 2 years old may have accounted for the lack of detectable antibodies.

The 5 fatal cases were all adults, 3 of whom were aged persons. Serum from case No. 12, in which pharyngeal histoplasmosis with dissemination resulted in rapid termination 4 months after onset, showed a 1:40 titer in both tests during the 3rd month. Knowledge of antibody response earlier in the disease, in this case, would have been of considerable interest. Case No. 13, who also died with the disseminated form in the 4th month, showed no antibodies in blood obtained post-mortem. Case No. 14, with pharyngeal histoplasmosis, showed no complement-fixation antibodies in the 4th month or 1 month prior to death, but did have a 1:20 titer in the collodion agglutination test. Thus, like the other 2 laryngeal or pharyngeal forms above (No. 4 and No. 12) collodion agglutination reactions were consistently positive. Case No. 15 represented a chronically ill old man who showed complement-fixing antibodies in low titer (1:10) at 12 and 18 months, but none at the time of death 2 years after onset. Collodion agglutinations were negative throughout.

Serum at weekly intervals obtained from a fatal disseminated case (No. 16) showed no demonstrable antibodies with either test during the last 7 weeks of life.

Results in suspected cases—At present the serologic picture is being followed in 2 children who present symptoms highly compatible with early acute histoplasmosis and in whom recovery seems likely.

Table 7 shows the antibody titers noted thus far in these 2 children. Serum obtained from "A" shortly after the onset of illness fixed complement at 1:40 and gave a positive collodion agglutination through 1:160. These results suggest active infection. After 3 months the complement-fixation titer was 1:20 and the collodion 1:80. In case B the exceptionally high complement-fixation titer of 1:320 and the 1:80 collodion results likewise seem to confirm the clinical impression of histoplasmosis. The early serologic results

TABLE 7

Early Serologic Results in Two Children with Clinical Picture Suggestive of Histoplasmosis

Case	Month of Disease	Test	Serum Dilutions 1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
A	1	F*	0	0	0	5	100				
		A†	4+	4+	4+	4+	4+	1+	—		
	2	F	0	0	0	30	100				
		A	4+	4+	4+	3+	1+	—			
	3	F	30	35	50	80					
		A	4+	4+	4+	4+	2+	—			
B	1	F	0	0	0	0	0	0	0	0	60
		A	4+	4+	4+	4+	3+	1+	—	—	—
	2	F	0	0	0	0	0	0	0	0	80
		A	4+	4+	4+	3+	1+	—			

F* — Complement-fixation — Positive = 50% or less Hemolysis
A† — Collodion Agglutination

obtained thus far are similar to those discussed in the proved cases 1 and 2 above. Efforts to recover this organism have thus far been unsuccessful, but in view of the difficulty encountered in other infections such as brucellosis and certain forms of tuberculosis one may have to rely on other laboratory data to support the clinical diagnosis. It is precisely in this type of case that serologic tests could be most useful as a diagnostic aid.

DISCUSSION AND SUMMARY

The studies described herein as well as similar ones by Salvin[19] and by Furcolow, Bunnell, and Tenenberg,[21] tend to substantiate the observations from this laboratory on the potential value of serologic studies in the laboratory diagnosis of histoplasmosis.[24, 27] Because of previously reported cross-reactions between *H. capsulatum* and other antigens[13, 22-27, 29, 30] and low antibody levels sporadically appearing in certain individuals with no known history of the disease, it is felt that the quantitative methods applied throughout these studies are of importance.

Quantitative methods readily differentiate low-titered sera from those showing a marked antibody content and offer a means of comparing homologous and heterologous titers when related antigens such as *Blastomyces dermatitidis* are simultaneously titrated. Both of these aspects are important in the elimination of nonspecific reactions and in the eventual evaluation of serologic studies in this disease. Skin sensitivity does not necessarily imply the coincidence of circulating humoral antibodies. Thus, the occurrence of positive serologic findings in only a small percentage of skin-test-positive individuals is not improbable. By alterations of technique, the sensitivity of the employed tests might be increased to such an extent that a higher correlation between skin reactivity and humoral antibodies could be obtained. However, this could also lead to decreased specificity. Since it has been our purpose to devise a test which would assist in the diagnosis of the active disease process, the procedures developed have been directed toward detecting antibody levels of greater intensity than those observed in "normal individuals," or in persons merely reacting positively to histoplasmin. We again emphasize the importance of serial specimens for more accurately evaluating the relationship of humoral antibodies to the disease process, since in both human and animal studies the transient nature of the antibody response has been marked. Failure to augment early clinical suspicion of histoplasmosis with serial serologic studies has undoubtedly greatly increased the number of cases forever relegated to the "fever of undetermined origin" category. On the other hand, positive serologic findings early in the disease may result in a more intensive and appropriate search for the causative organism, and thereby increase the number of definite diagnoses. This does not imply that antibodies will always be found early in the disease for in the rapidly fulminating forms our tests have shown titers to be minimal or entirely lacking. More cases will have to be studied before one can fully evaluate this and many other as yet untouched aspects of the disease. However, on the basis of our very limited observations, it is our feeling that the absence of antibodies in high titer early in the disease indicates a poor prognosis, while a self-limiting course may be expected in cases showing an appreciable antibody response.

After further experience and many, many more observations, it is possible that serologic studies may contribute not only to a better diagnosis but also to a more adequate evaluation of the prognosis. High incidence of skin-sensitivity to histoplasmin and an increasing number of reported non-fatal cases indicate

that histoplasmosis is a relatively more common disease than previously supposed, but the final solution of this fascinating problem lies in synthesizing the efforts of various investigators with diverse avenues of approach, including those of Emmons [31, 32] and many others. The serologic aspects as conducted in this and other laboratories may contribute to the armamentarium of the physician. The preliminary observations made thus far that the diagnosis of histoplasmosis can be accelerated by serologic methods is encouraging.

REFERENCES

1. Christie, A., and Peterson, J. C. Pulmonary Calcification in Negative Reactors to Tuberculin. *A.J.P.H.* 35:1131 (Nov.), 1945.
2. Palmer, C. E. Nontuberculous Pulmonary Calcification and Sensitivity to Histoplasmin. *Pub. Health Rep.* 60:513 (May 11), 1945.
3. Palmer, C. E. Geographic Differences in Sensitivity to Histoplasmin among Student Nurses. *Pub. Health Rep.* 61:475 (Apr. 5), 1946.
4. Christie, A., and Peterson, J. C. Histoplasmin Sensitivity. *J. Pediat.* 29:417 (Oct.), 1946.
5. Van Pernis, P. A., Benson, M. E., and Holinger, P. H. Specific Cutaneous Reactions with Histoplasmosis. *J.A.M.A.* 117:436 (Aug. 9), 1941.
6. Furcolow, M. L., High, R. H., and Allen, M. F. Some Epidemiological Aspects of Sensitivity to Histoplasmin and Tuberculin. *Pub. Health Rep.* 61:1132 (Aug. 2), 1946.
7. High, R. H., Zwerling, H. B., and Furcolow, M. L. Disseminated Pulmonary Calcification. A Report of 113 Cases. *Pub. Health Rep.* 62:20, (Jan. 3), 1947.
8. Sontag, L. W., and Allen, J. E. Lung Calcifications and Histoplasmin-Tuberculin Skin Sensitivity. *J. Pediat.* 30:657 (June), 1947.
9. Prior, J. A., and Allen, M. F. Geographic Distribution of Histoplasmin and Tuberculin Reactors among Ohio State University Freshmen and Student Nurses Training in Columbus, Ohio, Hospitals. *Pub. Health Rep.* 62:1608 (Nov. 7), 1947.
10. Hudson, E. H., Moore, F. R., and Phillips, E. A. Comparative Sensitivity to Histoplasmin and Tuberculin in Ohio University Students. *J. Lancet* 68:142 (Jan. 24), 1948.
11. Drug, E. S., and Glen, H. R. Comparative Studies in Tuberculosis and Histoplasmosis among 2,000 Students Entering Pennsylvania State College, September 1947. *J. Lancet* 68:206 (Feb. 7), 1948.
12. Dickie, H. A., and Clark, E. A. Histoplasmin and Tuberculin Sensitivity in Relation to Pulmonary Calcifications among University of Wisconsin Students. *Ann. Int. Med.* 28:1087 (June), 1948.
13. Christie, A., and Peterson, J. C. Pulmonary Calcification and Sensitivity to Histoplasmin, Tuberculin, and Haplesporangin. *J.A.M.A.* 131:658 (June 22), 1946.
14. Peterson, J. C., and Christie, A. Histoplasmosis and Pulmonary Calcifications. *Am. Rev. Tuberc.* 57:361 (Sept.), 1948.
15. Furcolow, M. L., Mantz, H. L., and Lewis, L. The Roentgenographic Appearance of Persistent Pulmonary Infiltrates Associated with Sensitivity to Histoplasmin. *Pub. Health Rep.* 62:1711 (Dec. 5), 1947.
16. Beadenkope, William G., Loosli, Clayton, G., Lack, Herbert, Rice, Frederick A., and Slattery, Robert V. Tuberculin, Coccidioidin, and Histoplasmin Sensitivity in Relation to Pulmonary Calcifications. *Pub. Health Rep.* 64:17 (Jan. 7), 1949.
17. Bunnell, I. L., and Furcolow, M. L. A Report on Ten Proved Cases of Histoplasmosis. *Pub. Health Rep.* 63:299 (Mar. 5), 1948.
18. Friedman, V., Saslaw, S., and Wheeler, W. E. *Am. Dis. Child.* in press.
19. Salvin, S. B. Complement Fixation Studies in Experimental Histoplasmosis. *Proc. Soc. Exper. Biol. & Med.* 66:342 (Mar.), 1947.
20. Tenenberg, D. J., and Howell, A. A Complement Fixation Test for Histoplasmosis. I. Technic and Preliminary Results in Animal Sera. *Pub. Health Rep.* 63:163 (Feb. 6), 1948.
21. Furcolow, M. L., Bunnell, I. L., and Tenenberg, D. J. A Complement Fixation Test for Histoplasmosis. II. Preliminary Results with Human Sera. *Pub. Health Rep.* 63:169 (Feb. 6), 1948.
22. Saslaw, S., and Campbell, C. C. The Use of Yeast Phase Antigens in a Complement Fixation Test for Histoplasmosis. I. Preliminary Results with Rabbit Sera. *J. Lab. & Clin. Med.* 33: No. 7, 811 (July), 1948.
23. Campbell, C. C., and Saslaw, S. The Use of Yeast Phase Antigens in a Complement Fixation Test for Histoplasmosis. II. Results with Ground Antigens. *J. Lab. & Clin. Med.* 33, 10:1207 (Oct.), 1948.
24. Campbell, C. C., and Saslaw, S. Use of Yeast Phase Antigens in a Complement Fixation Test for Histoplasmosis. III. Preliminary Results with Human Sera. *Pub. Health Rep.* 64: No. 18, 651 (May 6), 1949.
25. Saslaw, S., and Campbell, C. C. A Method for Demonstrating Antibodies in Rabbit Sera Against Histoplasmin by the Collodion Agglutination Technic. *Proc. Soc. Exper. Biol. & Med.* 68:559, 1948.
26. Saslaw, S., and Campbell, C. C. A Comparison Between Histoplasmin and Blastomycin by the Collodion Agglutination Technique. *Pub. Health Rep.* 64:290 (Mar. 4), 1949.
27. Saslaw, S., and Campbell, C. C. A Collodion Agglutination Test for Histoplasmosis. *Pub. Health Rep.* 64:424 (Apr. 1), 1949.
28. Saslaw, S., and Campbell, C. C. Unpublished data.
29. Emmons, C. W., Olson, B. J., and Eldridge, W. W. Studies of the Role Fungi in Pulmonary Disease. I. Cross Reactions of Histoplasmin. *Pub. Health Rep.* 60:1383 (Nov. 23), 1945.
30. Howell, A. Studies of Fungus Antigens. I. Quantitative Studies of Cross Reactions Between Histoplasmin and Blastomycin in Guinea Pigs. *Pub. Health Rep.* 62:631 (May 2), 1947.
31. Emmons, C. W., Bell, J. A., and Olson, B. J. Naturally Occurring Histoplasmosis in *Mus musculus* and *Rattus norvegicus*. *Pub. Health Rep.* 62:1642 (Nov. 14), 1947.
32. Emmons, C. W. Isolation of *Histoplasma capsulatum* from Soil. *Pub. Health Rep.* 64:892 (July 15), 1949.