EVALUATION OF HISTOPLASMIN AND YEAST PHASE ANTIGENS DERIVED FROM A SINGLE STRAIN OF HISTOPLASMA CAPSULATUM IN THE COMPLEMENT FIXATION TEST

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A complement fixation procedure for the serological diagnosis of histoplasmosis in a human patient was attempted first in 1941 by Van Pernis, Benson, and Hollinger. The results were negative with an antigen which consisted of the undiluted filtrate (histoplasmin) from a glucose broth medium inoculated with *Histoplasma capsulatum*. Since that time other types of antigens have been developed by various investigators. Salvin (1947) found that yeast phase cells of *H. capsulatum* reacted with sera from rabbits infected with the same fungus, but that histoplasmin failed to give reactions. This reactivity of yeast phase antigen with rabbit sera was confirmed by Saslaw and Campbell (1948*a*). Tenenberg and Howell (1948), however, found that histoplasmin was a satisfactory antigen for detecting complement-fixing antibodies in guinea pigs experimentally inoculated with *H. capsulatum*. Furcolow, Bunnell, and Tennenberg (1948), using Howell's antigen, detected 14 positive complement fixation reactors among 300 patients suspected of having histoplasmosis. On the basis of comparative tests with whole yeast phase cells, ground yeast phase cells, and histoplasmin, on sera from two proven human cases of histoplasmosis and hyperimmunized rabbits, Grayston (1952) favored the yeast phase antigen over histoplasmin. That histoplasmins may vary widely in their antigenic potencies was shown by Schubert *et al.* (1953) who compared the antigenic levels of histoplasmin produced by 10 different isolates of *H. capsulatum*. Relatively high titers were obtained with sera from culturally proven human cases of histoplasmosis with certain histoplasmins and weak or negative reactions with other histoplasmin preparations. It was concluded that pretesting of several lots of antigen must be undertaken to insure the selection of potent lots of histoplasmin for use in serological tests. Recently, Martin (1953) prepared a sonic disintegrated yeast phase antigen of *H. capsulatum* that elicited low titers in two sera from proven cases of histoplasmosis, but no comparisons were made with other types of antigens.

Although all the above antigens currently are being utilized in complement fixation tests for histoplasmosis, extensive comparative studies have not been carried out to determine the merits, if any, of one particular type of antigen over another. The present investigation was undertaken to compare the relative values of particulate yeast phase and sonic disintegrated yeast phase antigens with histoplasmin. All three types of antigens were derived from a single strain of *H. capsulatum* and used in complement fixation tests for histoplasmosis with sera from a series of human and animal sources.

MATERIALS AND METHODS

Antigen. H. capsulatum strain 105 from the culture collection of the Communicable Disease Center was selected for use throughout this study on the basis of its known ability to produce a relatively potent histoplasmin (Schubert *et al.*, 1953) and its readiness of conversion to the yeast phase.

Histoplasmin. Histoplasmin was obtained by growing strain 105 for 6 months on the modified (Tuberculin) medium of the Bureau of Animal Industry (Smith *et al.*, 1948). This lot of antigen was not anticomplementary when used at a dilution of 1:8.

Yeast phase. Whole yeast phase cells were harvested from cultures grown on Francis glucose cystine blood agar at 37 C for 4 days. The cells were washed twice with sterile 0.01 per cent merthiolated physiological saline and volumetrically adjusted to produce a 7 per cent stock suspension. In the tests this antigen was used at a dilution of 1:6 at which level it was not anticomplementary.

Sonic disintegrated yeast phase. A 20 per cent suspension of yeast phase organisms was treated by means of an ultrasonic apparatus (Ultrason-Televiso Corporation, Chicago, Illinois)[1] for one hour with ultrasonic waves generated through an oil bath at a frequency of 450,000 kilocycles per second by 2,000 volts, with an energy output of 10 watts per square centimeter of the crystal surface. The oil bath was kept at 30–35 C by circulating the oil through a refrigerating apparatus. The treated suspension then was centrifuged at 2,000 rpm for 15 minutes and the sediment discarded. The opalescent supernate was not anticomplementary when used at a dilution of 1:8.

Sera. Complement fixation tests were carried out on the following groups of animal sera:

(1) a. Hyperimmune sera from 8 rabbits, each hyperimmunized with a different strain of heat killed yeast phase cells of *H. capsulatum*. b. Control sera from 8 normal rabbits.

(2) Sera from rabbits experimentally infected by inhalation of an aerosol of tuberculate spores of *H. capsulatum* (through courtesy of Dr. M. L. Furcolow).

(3) Sera from dogs naturally infected with *H. capsulatum* (through courtesy of Dr. D. A. Rowley).

(4) Sera from culturally proven cases of histoplasmosis.

(5) Sera from persons presumably infected by *H. capsulatum* as indicated by pulmonary lesions, clinical symptoms, and conversion from a negative to a positive histoplasmin skin test.

(6) Sera from patients with evidence of disease other than histoplasmosis and from normal individuals.

Complement fixation procedure. The complement fixation procedure used was an adaptation of the 50 per cent end point technique, employing barbital buffered physiological saline throughout. Antigens and serial dilutions of inactivated sera were tested in 0.25 ml amounts with 0.5 ml of complement containing 5 fifty per cent units. Fixation was allowed to proceed for 15–18 hours at 4–6 C. The tubes were then placed at room temperature until the sensitized cells (a 2 per cent suspension of sheep erythrocytes sensitized for 10

[1] The name of the company is used as a means of identifying the product under discussion and does not represent endorsement by the Public Health Service.

TABLE 1

Complement fixation titers obtained on sera from eight hyperimmunized and six experimentally infected rabbits

Hyperimmunized Sera	Titers* Obtained with Antigens from *Histoplasma capsulatum* in the Form of:		
	Histoplasmin	Whole yeast phase	Sonic disintegrated yeast phase
28†	16	64	32
105	32	128	128
A228	32	32	16
A233	32	64	32
419	128	128	64
420	128	32	64
421	64	512	256
481	512	512	256
Experimentally Infected Sera			
1	16	4	16
2	16	4	16
3	32	4	32
4	16	8	32
5	16	4	16
6	8	4	16

* Titers expressed as reciprocals of the dilutions.

† Number indicates strain of *H. capsulatum* used in immunization.

TABLE 2

Complement fixation titers obtained on human sera from culturally proven cases of histoplasmosis

Sera	Antigens from *Histoplasma capsulatum* in the Form of:		
	Histoplasmin	Whole yeast phase	Sonic disintegrated yeast phase
1. a. 11-30-53	4,096	2	8
b. 12-17-53	16,384	0	2
2.	1,024	0	0
3.	256	4	16
4. a. 12-29-49	32	8	16
b. 2-8-50	64	16	32
c. 4-11-50	2	0	0
5.	512	0	0
6.	128	0	0
7.	128	0	0
8.	64	4	32
9.	128	32	16
10. a. 3-25-53	1,024	0	0
b. 4-8-53	512	0	0
11.	256	8	16

TABLE 3

*Complement fixation titers obtained on human sera from presumptive cases of histoplasmosis**

Sera	Antigens from *Histoplasma capsulatum* in the Form of:		
	Histoplasmin	Whole yeast phase	Sonic disintegrated yeast phase
1	8	0	8
2	16	2	4
3	4	4	0
4	128	0	32
5	32	0	0
6	64	0	0
7	256	0	0
8	64	0	0
9	64	32	16
10	8	0	0
11	4	0	0
12	32	2	0
13	8	0	2
14	8	0	0
15	32	0	0

* Sera from individuals presumably infected by *H. capsulatum* as indicated by the presence of pulmonary lesions, clinical symptoms, and conversion from a negative to a positive histoplasmin skin test.

minutes in the proportion of 1:1 with 4 units of hemolysin in 0.25 ml) were prepared. The sensitized cells were added in 0.5 ml amounts and the tests read after a secondary incubation period of 30 minutes in a 37 C water bath. The titers were recorded as the highest dilution of serum which gave a 3 plus or 4 plus reaction (30 per cent hemolysis or less).

The complement binding power of the antigen was tested with 1.25, 2.5, and 5 fifty per cent units of complement. The antigen was considered to be anticomplementary when a 3 plus or 4 plus reaction was obtained with 2.5 fifty per cent units.

EXPERIMENTAL RESULTS

The results of the tests carried out on the different groups of sera are presented in tables 1 to 3.

DISCUSSION

The present status of serological tests as diagnostic tools in the study of histoplasmosis is confusing due to conflicting findings reported by various laboratories. Some of the observed discrepancies arise from the fact that the investigators have used different types of complement-fixing antigens such as histoplasmins from various sources, whole yeast phase antigens, and ground yeast phase antigens. Other types of serological tests employed include the collodion agglutination technique by Saslaw and Campbell (1948*b*) and the precipitin test as developed by Salvin and Furcolow (1954). The method of histoplasmin production (growth for periods of three to six months in a liquid medium) is, by its very nature, conducive to large variations in the antigenic potencies of various preparations. In addition, it has been shown in this laboratory that isolates of *H. capsulatum* vary greatly in their capacity to produce complement-fixing histoplasmin as measured by the results obtained in complement fixation tests with human sera. There can be no doubt that histoplasmin is a very complex material, probably containing a great variety of substances which may have different antigenic characteristics.

Other factors which may have contributed to differences in serological results include the tendency to employ hyperimmune rabbit sera as the agents to titrate antigenic potency and the failure to distinguish between antibody patterns developed in hyperimmunization and those resulting from infection. In addition, it has been known for many years that antibodies from various species of animals react differently in the complement fixation test which employs guinea pig complement, depending upon whether or not protein or carbohydrate antigens from bacterial sources are used as antigens. Martin (1953) showed that anti-Blastomyces hyperimmune rabbit serum fixes guinea pig complement with a nonprotein surface material from *Blastomyces dermatitidis*, whereas fixation with the same antigen does not occur with sera from infected humans. On the other hand, sera from both hyperimmune rabbits and infected humans fix guinea pig complement with an antigen liberated from the fungus cells by sonic vibration.

The experiments presented in this paper, although preliminary in nature, emphasize some of the points discussed above. The sera from eight hyperimmune rabbits (table 1) fixed complement almost equally well with each of the three antigens tested, giving mean titers of 1–64, 1–120, and 1–71 with the histoplasmin, yeast phase, and ultrasonic antigens, respectively, the yeast phase

antigen giving slightly higher titers. Mean titers of 3.0 and 6.6 were obtained with histoplasmin on sonic disintegrated yeast phase antigen, respectively, in tests with sera from 9 normal rabbits. The whole yeast phase antigen gave no reaction with these sera at the 1:4 dilution. In direct contrast, the results obtained with serum specimens from 11 culturally positive (table 2) and 15 suspected cases (table 3) of human histoplasmosis showed wide differences in complement fixation with mean titers of approximately 1–79, 1–2, and 1–3 for the histoplasmin, yeast phase, and ultrasonic antigens.

The limited number of sera from experimentally infected rabbits gave results more closely resembling those of the naturally infected humans with relatively high titers to the histoplasmin and negative or low titers to the yeast phase antigens (table 1). In contrast to the results obtained in human sera, the titers to the ultrasonic antigen were comparable to those obtained with the histoplasmin antigen, emphasizing again the possibility that antibodies from various animals may respond differently to similar test antigens.

Tests with all three antigens on sera from 9 normal humans and two patients suffering with tuberculosis and amoebiasis, respectively, all gave negative reactions with the following exceptions. With histoplasmin, titers of 1:4 were obtained with one normal human serum and with each of the sera from the tuberculosis and the amoebiasis cases. A titer of 1:8 was obtained with one normal serum with the whole yeast phase antigen. Only two of the normal human sera reacted at the 1:4 dilution with the sonic disintegrated yeast phase antigen.

The sera from six naturally infected dogs showed an entirely different pattern in that the reactions to the histoplasmin were all negative, but responses to both yeast phase and ultrasonic antigens could be demonstrated. Two dog sera gave titers of 1:8, and one gave a titer of 1:16 with the whole yeast phase antigen. With the sonic disintegrated yeast phase antigen, three dog sera gave titers of 1:8, one gave a titer of 1:16, whereas the titer of the other serum was 1:64.

The experiments reported above were all carried out with a single isolate of *H. capsulatum* that was known to produce a potent histoplasmin antigen for the complement fixation test. It is possible that different results might be obtained with histoplasmins prepared from other isolates of this fungus. It is emphasized, however, that titrations of antigens with hyperimmune rabbit sera cannot be used as criteria for establishing the potency of a substance intended for use as a diagnostic antigen in naturally infected humans.

SUMMARY

The antigenic capacity of three different antigens derived from the same strain of *Histoplasma capsulatum*—histoplasmin, whole yeast phase cells, and the supernate from sonic disintegrated yeast phase cells—was tested against various types of sera.

In tests with sera from hyperimmunized rabbits, all three antigens were of relatively equal activity, but with sera from infected rabbits, the whole yeast phase antigen was inferior to the other two antigens.

Histoplasmin was completely unreactive when tested with sera from infected dogs. However, in tests with human sera obtained either from culturally proven cases of histoplasmosis or from presumptive cases, histoplasmin was more reactive than the whole yeast phase or the sonic disintegrated yeast phase antigens used in this study.

It is suggested that *H. capsulatum* antigens for use in complement fixation tests with human sera should not be preselected on the basis of tests with nonhuman sera.

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