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Complement Fixation Studies in Experimental Histoplasmosis.

S. B. SALVIN. (Introduced by Charles Armstrong.)
From the Division of Infectious Diseases, National Institute of Health, Bethesda, Md.

Chiefly because of the absence of a specific, potent antigen, no satisfactory complement-fixation test has been recorded for histoplasmosis. Van Pernis, Benson and Holinger[1] conducted complement-fixation tests on a human patient with histoplasmosis, but obtained negative results. The antigens they used were (a) the undiluted filtrate from a dextrose broth culture medium inoculated with *H. capsulatum* and (b) the acetone precipitate redissolved in saline.

However, antigens have been prepared and successfully used for complement-fixation tests in other mycotic diseases. For example, Martin[2,3] employed antigens derived from the yeastlike phases of *B. dermatitidis* and *Candida sp.* for the fixation of complement in the presence of homologous antisera, whereas Martin,[4] Fonseca[5] and Smith[6] derived their complement-fixation antigens from the mycelial phase in their studies on chromoblastomycosis, South American blastomycosis and coccidioidomycosis respectively.

Since the causative organism of histoplasmosis appears in the yeast-like form in infected animals and human beings, an antigen derived from this phase might be expected to produce the most satisfactory serologic tests for this disease. After the yeastlike form of *H. capsulatum* was successfully grown in a semi-fluid medium in large quantities,[7] serologic studies were initiated. The purposes of these investigations were (1) to determine the binding power and specificity of the yeastlike phase of *H. capsulatum* as a complement-fixation antigen, and (2) to obtain information on the time of appearance, the maximum titer and the persistence of complement-fixation antibodies in animals infected with histoplasmosis. In this paper the results of these investigations are presented.

Materials and Methods. a. *Complement-fixation antigens.* The yeastlike phase of *H. capsulatum* was grown at 37°C in semi-fluid medium[7] for 3 weeks, centrifuged, washed in Tyrode's solution at pH 7.0, and killed by 0.5% formalin. The cells were then centrifuged at 32-36°F, washed twice in Tyrode's solution, and stored at 2-5°C. The antigen employed in the complement-fixation tests consisted of whole washed formalin-killed cells of the yeastlike phase, and will henceforth be referred to as "YP antigen." Although all lots had approximately equal potency, one lot of YP antigen (derived from strain 6521) was used in the following experiments. The antigenic titer was 1:256 against rabbit immune serum and the anticomplementary titer was 1:4-1:8. This antigen exhibited similar binding power in the presence of antisera against the homologous strain and against five other strains of *H. capsulatum.*

Histoplasmin is the broth filtrate from the growth of the mycelium of *H. capsulatum* on a modified Long's medium.[8] The fungus was grown at room temperature in the dark, with samples removed periodically for assay. During approximately the first 3 weeks of growth, the filtrate had no binding power in the presence of homologous antiserum. However, for the next 20-30 days a marked rise in binding power of the filtrate appeared (Fig. 1). Thereafter the titer remained at a fairly con-

1 Van Pernis, P. A., Benson, M. E., and Holinger, P. H., *J. A. M. A.*, 1941, **117**, 436.

2 Martin, D. S., *J. Infect. Dis.*, 1935, **57**, 291.

3 Martin, D. S., *Am. J. Trop. Med.*, 1942, **22**, 295.

4 Martin, D. S., *Am. J. Trop. Med.*, 1938, **18**, 421.

5 da Fonseca, O., *An. brasil. de dermat. e sifil.*, 1939, **14**, 112.

6 Smith, C. E., personal communication.

7 Salvin, S. B., *J. Bact.*, 1947, **54**, 655.

8 Emmons, C. W., Olson, B. J., and Eldridge, W. W., *Pub. Health Rep.*, 1945, **60**, 1383.

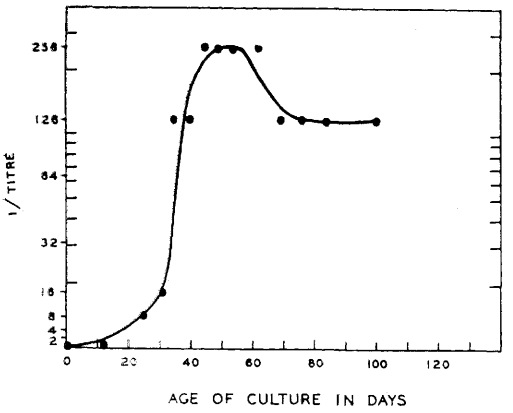

FIG. 1.

Development of binding power of histoplasmin from strain 6510 of *Histoplasma capsulatum* against homologous antiserum.

stant level, although there was a slow but distinct increase in the anticomplementary action as the culture aged, especially after the first 90 days of growth. For the experiments in which histoplasmin was used, a 60-day-old filtrate of strain 6510 was employed as the antigen.

b. *Complement-fixation test.* The method was that employed by Bengston.[9] Complement was titered in the presence of antigen each day tests were conducted and antigens were tested whenever new lots were employed. Four units of antigen, 2 of complement, and 1½ of amboceptor were used throughout the studies.

c. *Strains of fungi.* The fungi were from the collection in this laboratory. The yeastlike phase on which most of the studies were conducted was from a strain (No. 6521) from a naturally-infected dog.[10] The histoplasmin employed was produced by a culture of strain 6510.[11] Blastomycin was obtained from a broth culture of strain 6014 of *B. dermatitidis* isolated by Conant; and the coccidioidin, partly from a broth culture of strain 6210 of *C. immitis* isolated by Emmons, and partly supplied by C. E. Smith.

d. *Animals.* The experimental animals were adult albino rabbits, which were bled and their sera tested for complement-fixation antibodies in all cases with the appropriate antigens before inoculation. Among 228 animals tested, only 2 rabbits had sera that fixed complement in the presence of histoplasmin, and none in the presence of YP antigen. What caused these two rabbit sera to fix complement in serum dilutions of 1:8 and 1:16 was not learned, since no gross abnormalities were discernible. However, the two were discarded.

Experiments and Results. a. *Production of complement-fixation antibodies.* Fifteen rabbits were inoculated intravenously with a suspension of the live yeastlike phase of strain 6521 of *H. capsulatum.* They were bled from the ear veins periodically, and their serum assayed for complement-fixing antibodies with YP antigen.

For about 10 days after inoculation, no antibodies were demonstrable (Fig. 2). The antibody titer then began to increase sharply until by about the 30th day the antibody titer reached 1:512 for most of the animals. All rabbits developed antiserum that fixed complement in the presence of YP antigen. A rapid decrease in titer occurred for the 20 ensuing days, after which the titer remained more or less constant until the experiment was terminated 170 days after inoculation.*

When these same antisera were tested with histoplasmin as the antigen, much lower antibody titers were obtained (Fig. 2), the highest being 1:128 in comparison with the 1:512 with YP antigen. In addition, 7 of the 15 animals showed no positive titers at any time, in contrast to the positive titers demonstrated in all the animals with the YP antigen. With histoplasmin as the complement-fixation antigen, the maximum titer was attained during the third week after inoculation, after which the antibody titer decreased progressively until the eighth week, when no antibodies could be demonstrated.

b. *Reactions of H. capsulatum antigens with other fungal antisera.* (a) *Coccidioidomycosis.* YP antigen and histoplasmin were

9 Bengston, I. A., *Pub. Health Rep.*, 1944, **59**, 402.

10 Olson, B. J., Bell, J. A., and Emmons, C. W., *Am. J. Pub. Health*, 1947, **37**, 441.

11 McLeod, J. H., Emmons, C. W., Ross, S., and Burke, F. G., *J. Pediat.*, 1946, **28**, 275.

* Not all the rabbits were bled on each "bleeding day."

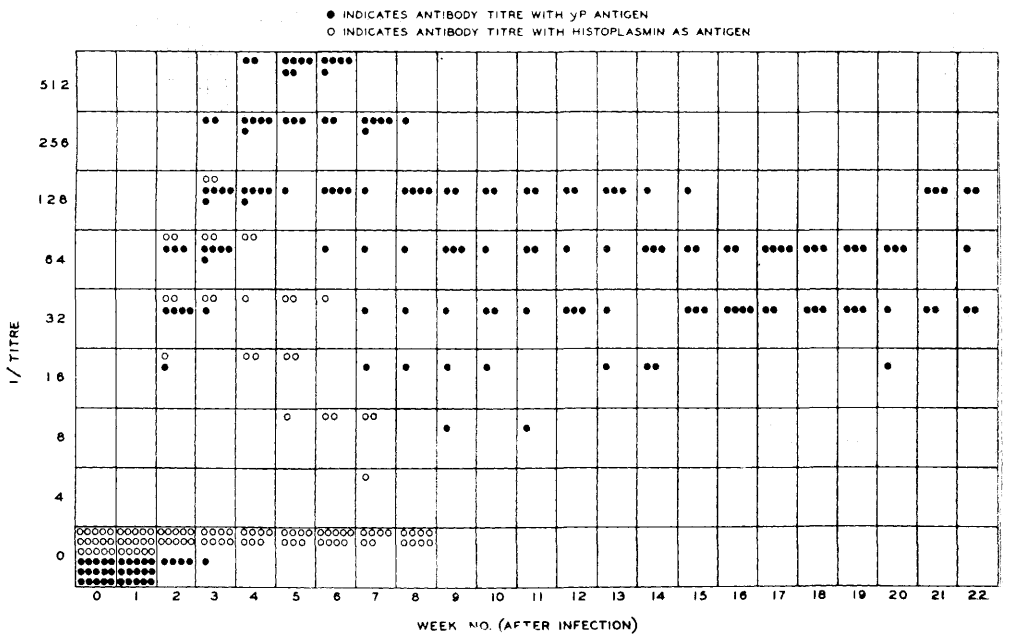

FIG. 2.

Development of antibody titer of experimentally infected rabbits in the presence of (a) YP antigen and (b) histoplasmin.

Note: The decrease in the number of readings per week is due to the deaths of an increasing number of animals.

studied in complement-fixation tests with antisera from 8 rabbits experimentally infected with *C. immitis* and from 10 human patients with localized or disseminated coccidioidomycosis.† All the rabbits infected with *C. immitis* developed potent complement-fixation antibodies in the presence of the homologous antigen. However, at no time did YP antigen fix complement in the presence of coccidioidomycosis antibodies, although histoplasmin did, both with the rabbit and human antisera.

(b) *Blastomycosis.* Eight rabbits, inoculated intravenously with the living yeastlike phase of *B. dermatitidis,* were bled periodically and their antisera analyzed for the presence of complement-fixation antibodies with blastomycin as antigen. Antibodies were demonstrated about 20 days after infection, and remained until the ninth week. Throughout this period of demonstrable antibody with the homologous antigen, YP antigen produced no complement-fixation with the blastomycosis antisera at any time. However, histoplasmin showed reactions with these heterologous antisera at the low titers of 1:8 and less.

(c) *Moniliasis.* Four rabbits were immunized by daily intravenous injections of formalin-killed cells of the budding phase of *Candida albicans.* The resulting antisera, active with the homologous antigen, were analyzed for complement-fixing properties in the presence of histoplasmin and YP antigen. Histoplasmin produced complement-fixation in antiserum dilutions as high as 1:8, whereas YP antigen showed no positive reactions whatsoever.

Discussion. Antigens from the yeastlike phase of *H. capsulatum* show promise as agents for determining the presence of complement-fixation antibodies, since these antigens have specificity, good binding power, and low anticomplementary titers with experimentally produced rabbit antisera. When histoplasmin was used as antigen, the titers were lower, of shorter duration, and less specific than with the YP antigen. Also of

† Thanks are expressed to Dr. C. E. Smith for providing these antisera.

interest was the persistence of a positive titer in the presence of YP antigen in the sera of rabbits with histoplasmosis after gross external symptoms of the disease had disappeared and blood cultures were no longer positive.

During the course of these studies on laboratory animals, two human patients appeared from whom *H. capsulatum* was isolated.‡ Their sera fixed complement in the presence of YP antigen in dilutions of 1:32 and 1:128. More than 30 "normal" persons from whom *H. capsulatum* was not isolated had no complement-fixing antibodies in the presence of YP antigen.

Summary. An antigen from the yeastlike phase of *Histoplasma capsulatum* fixed complement in the presence of antisera from experimentally infected rabbits from the 2nd to the 23rd weeks following infection. In contrast to histoplasmin, this antigen did not react with several other fungal antisera.

‡ Thanks are expressed to Drs. F. C. Kelly and J. Wells for making these sera available.

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Sulfactin. Bacterial Spectrum, Toxicity and Therapeutic Studies.*

HARRY E. MORTON. (Introduced by A. N. Richards.)

From the Department of Bacteriology, School of Medicine, University of Pennsylvania, Philadelphia, Pa.

A new antibiotic, sulfactin, has been described by Junowicz-Kocholaty, Kocholaty, and Kelner.[1] A description of the actinomyces, the method for growing the organism, and the purification and chemical properties of the antibiotic substance were given by the above authors. It is the purpose of this publication to present results of studies on the bacterial spectrum and the toxicity and therapeutic action in mice.

Experimental. The sterile crude culture filtrate of Actinomyces, R-30, was employed for determining the bacterial spectrum. In general, the *in vitro* antibacterial tests were carried out at the time similar tests were being made with actinorubin and lavendulin employing Bacto-nutrient broth adjusted to pH 7.3 and by the technic described previously by Kelner and Morton.[2] Since sulfactin is more inhibitory for gram-positive than for gram-negative organisms, it was standardized against *Staphylococcus aureus,* P210. The smallest amount of sulfactin per ml of Bacto-nutrient broth, pH 7.3 which prevented growth of *S. aureus,* under the conditions of the test, was designated as one dilution unit.

The crude culture filtrate of Actinomyces, R30, was titrated against *S. aureus,* P210, in Bacto-nutrient broth, pH 7.3, and in the same medium to which had been added 0.5% NaCl. In each medium the activity titrated to the same tube, representing 6,400 dilution units. Thus the antibacterial action of sulfactin is not appreciably reduced in the presence of 0.5% NaCl. The presence of 10% sterile, defibrinated, normal horse blood in the Bacto-nutrient broth with salt necessitated 10 times more sulfactin to inhibit *S. aureus.*

There was received on June 20, 1946, from Dr. Junowicz-Kocholaty about 60 mg of recrystallized sulfactin which had been dried over $CaCl_2$. By the agar streak technic of assay it was estimated that 0.0254 μg of this material equalled one unit. The material was put in solution as follows: To 10.43 mg of sulfactin was added one ml of chloroform and warmed to 45°C until dissolved. One ml of

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1 Junowicz-Kocholaty, R., Kocholaty, W., and Kelner, A., *J. Biol. Chem.,* 1947, **168**, 765.

2 Kelner, A., and Morton, H. E., *J. Bact.,* 1947, **53**, 695.