Isolation of Histoplasma capsulatum from Two Natural Sources in the Mohawk Valley; One the Probable Point Source of Two Cases of Histoplasmosis

ELIZABETH L. HAZEN; GEORGE N. LITTLE; and VERNA MORDAUNT

An unusual opportunity to study the mycology, serology, and epidemiology of histoplasmosis was put to excellent use. There is a reminder here of the ubiquity of this dangerous fungus.

Emmons [2, 3] in 1949 and again the following year found that Histoplasma capsulatum grows saprophytically in soil. These findings, which have been repeatedly confirmed not only by Emmons [4] but by other investigators,[5-13] have led to a greater understanding of the epidemiology of the disease. At the present time the consensus seems to be that the infection is acquired by the inhalation of dust containing the fungus spores.

The microorganism has most frequently been isolated from soils contaminated by chickens. Zeidberg, Ajello, and associates [8] first called attention to the association of chickens and H. capsulatum in soil, and they later reported [14] that the microorganism was found predominantly inside chicken houses and chicken yards. Emmons [15] in 1954 in a summary of results of a study of 1,751 specimens for pathogenic fungi reported an obvious association of H. capsulatum with the presence of chickens.

Seventy-three of 104 soil specimens from which he had isolated H. capsulatum were from inside or under chicken houses or in adjacent soil. Very recently Zeidberg, Ajello, and Webster [16] found that soils positive for H. capsulatum had an appreciably higher acidity and moisture-holding capacity than negative soils. They suggested that these factors may explain the greater number of positive findings in soils associated with chickens, since such soils are rich in organic matter and their high humus content tends to increase moisture-holding capacity.

Grayston, Loosli, and Alexander [6] in 1951 reported the isolation of H. capsulatum from a specimen of dried vegetable material and sawdust obtained from the floor of an old unused silo. Grayston and Furcolow [10] in their epidemiologic studies of 13 epidemics of histoplasmosis discovered the point source of all but two. These authors implicated wood as a vehicle of infection in four instances and emphasized that it may hold a position of importance in the growth of H. capsulatum in nature.

This report concerns primarily the study of specimens from two natural sources in the Mohawk Valley (N. Y.) with the purpose of establishing, if possible, the probable point source of histoplasmosis in two brothers (J.M. and L.M.) occupied as lumberjacks. The mycologic and serologic findings on specimens from these patients are also briefly discussed together with pertinent clinical data.

One (J.M.) became acutely ill with chest pains, temperature 103° F., and

nausea, and 12 days later was admitted to the Albany Veterans Administration Hospital with a pulmonary infection of unknown etiology. He was discharged three and one-half months later, apparently clinically recovered. X-ray examination of the chest revealed diffuse, coarse, nodular disease throughout both lung fields, with enlarged hilar areas.[1] The tuberculin and coccidioidin skin tests were negative and the histoplasmin skin test was strongly positive in a dilution of 1:100. Specimens of sputum and lymph nodes from a supraclavicular fat pad removed from J. M. were studied by cultural and animal inoculation for incitants of fungus disease. H. capsulatum was isolated from the lymph nodes by both technics. The microorganism was not recovered from the sputum.

Serial blood specimens were examined over an eight-month period by the quantitative complement-fixation test employing as antigens a heat-killed saline suspension of the yeastlike cells of H. capsulatum and B. dermatitidis. In the test six 50 per cent units of complement and fixation for 24 hours at 3°–6° C were used and an additional period of 30 minutes at 37° C for the hemolytic system. The antibody titers of these specimens with the Histoplasma antigen are shown graphically in Figure 1. There was a rapid rise in antibody concentration to a peak titer of 210 approximately three weeks after onset of illness and a gradual decline to a low of seven after about 37 weeks. No cross-reactions occurred with the Blastomyces yeastlike cell antigen and the serial blood specimens.

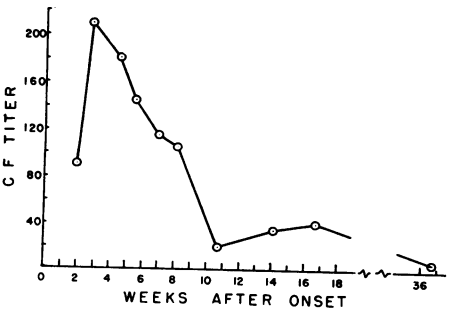

Figure 1—Complement Fixation Antibody Pattern of Sera from J. M. with Yeastlike Cell Antigen of H. capsulatum

The physicians,[1] upon questioning J. M. about his activities prior to his illness, learned that he and his brother (L.M.) had felled an old decayed tree at the edge of a woods two weeks prior to his illness and that they had been exposed to a considerable amount of dust at that time. It was found also that this patient lived on a farm 20 miles from the site of the decayed tree and that in his barn was a coop where chickens had formerly been housed; he stated, however, that the coop had not been cleaned for more than two years and that his brother (L.M.) had had no association with the farm.

The brother (L.M.) had not reported being ill, but a blood specimen was obtained from him for the quantitative complement-fixation test for histoplasmosis and a histoplasmin skin test was performed. The skin test was positive. A reaction, titer 85, occurred in the test with the blood serum and the yeastlike cell antigen of H. capsulatum and a cross-reaction, titer 40, with the yeastlike cell antigen of B. dermatitidis. With a second blood specimen collected four weeks later reactions, titers 40 and 25, occurred with the Histoplasma and the Blastomyces antigens, respectively. The marked fall in antibody titer, the positive histoplasmin skin test, and the exposure to the dust from the decayed tree at the same time as his brother (J.M.) on whom a definitive diagnosis of histoplasmosis had been established were considered strong presumptive evidence of an asymptomatic benign histoplasmosis. An x-ray of his chest at the time the first blood specimen was collected

The authors are associated with the Division of Laboratories and Research, New York State Department of Health, Albany, N. Y.

and another a month later revealed no findings of significance.[1]

It seems of interest to mention that a third brother (H.M.), who had no part in chopping down the tree, but who later hauled away the wood, showed no clinical or serologic evidence of histoplasmosis.

Materials and Methods

A large specimen of material from the rotted tree stump, consisting of dried, powdery wood chips, dried leaves, and a few remains of insects, was received in a Mason jar for examination for H. capsulatum. Two weeks later litter scraped from the floor of the chicken coop was submitted in a Mason jar and seven days after that a second specimen from this same source was received in a cardboard box. A portion of each of the three specimens was examined for the presence of H. capsulatum, following the technic of Emmons,[15] on the day received at the laboratory and a second portion of the material from the decayed tree was also examined one week later.

Each specimen was treated as follows: about 15 ml of the specimen was placed in a 100 ml sterile cylinder and sterile physiologic salt solution added to 10 times its volume. The suspension was shaken vigorously for about five seconds and then allowed to settle for 15 minutes. About 8 ml was pipetted from the top layer of the supernatant for microscopic examination for presence of macroconidia of Histoplasma and for intraperitoneal injection of mice. Four ml of the supernatant from the suspension of the material from the decayed tree stump was combined with 1 ml of an antibiotic solution in distilled water containing 2 mg of streptomycin and 5 mg of penicillin per ml. One ml of the mixture was injected intraperitoneally into each of four young white mice (Albany strain); on the second examination of this material four young Swiss mice were used. With the supernatants from the two suspensions of the litter from the chicken coop 6 ml of each was combined with 1.5 ml of the antibiotic solution and 1 ml of the mixture was injected, respectively, into each of six mice (Albany strain).

Each of the four groups of mice was observed for four weeks after which they were sacrificed and autopsied. The spleen and a portion of the liver were removed and macerated separately. The macerated spleens were spread over the surface of one slant each of brain heart infusion agar with blood and antibiotics[17] and Sabouraud's glucose agar. The cultures on the medium containing blood were incubated at 37° C and those on Sabouraud's glucose agar at room temperature; they were observed from four to six weeks before being discarded.

Portions of the material from the decayed tree stump and the first specimen of litter from the chicken coop were similarly studied after storage for six months at 3°–6° C to determine the effect of storage at this temperature upon the viability of the fungus spores.

Results

Microscopic—Structures thought to resemble macroconidia of H. capsulatum were found only in the material from the decayed tree stump examined on the day received and after one week's storage at 3°–6° C (Figure 2).

Mice—Eight mice, injected with the supernatants of the saline suspensions of material from the decayed tree, prepared on the day received and one week later, were found to have notably enlarged spleens. H. capsulatum was isolated between five and 30 days from all the cultures of the livers and spleens of three of four Albany mice receiving the first suspension and from the livers and spleens of all four young Swiss mice injected one week later with the super-

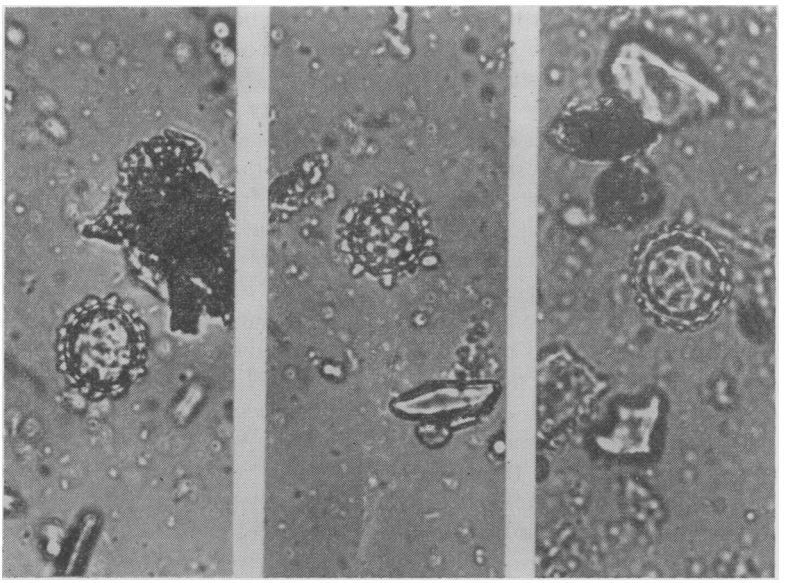

Figure 2—Macroconidia of H. capsulatum Found in Material from Decayed Tree Stump (magnification X600)

natant from the same material freshly prepared.

The six mice injected with the preparation from the first specimen of litter from the chicken coop also showed definitely enlarged spleens. H. capsulatum was recovered on the blood medium only between seven and 30 days from the spleen or both the liver and spleen of four of the six mice. The fungus was not recovered from mice injected with the supernatant of the second specimen of litter due to overgrowth of the culture media by fungal and bacterial contaminants.

H. capsulatum was isolated from the spleens, only, of three of six mice (Albany strain) injected with the supernatant of the suspension prepared from the material from the decayed tree after storage for six months at 3°–6° C. Three of the six mice died within four days after receiving the material. The microorganism was not recovered from the six mice injected with the supernatant of the suspension of litter from the chicken coop after storage for six months at 3°–6° C.

The identity of the strains isolated from the spleens and livers of the mice was established by demonstration of the characteristic tuberculate macroconidia (Figure 3).

Summary and Discussion

The study of specimens from two natural sources in the Mohawk Valley, N. Y., where two brothers with histoplasmosis lived and worked and apparently acquired the infection, is presented in detail. The mycologic and serologic findings on specimens from these patients, one having an acute pulmonary form of the disease, the other a mild or subclinical infection, are also briefly discussed together with pertinent clinical data.

H. capsulatum was isolated on three separate examinations of portions of material obtained from the remains of a decayed tree, believed to have been the point source of the infection, on the day received, after one week's storage at 3°–6° C, and finally after six months' storage at 3°–6° C. The fungus was

also isolated from litter from the chicken coop on J. M.'s farm on the day it was received, but not after six months' storage at 3°–6° C.

Of the two natural sources containing the infectious spores, the dust from the decayed tree would appear to be the likely source of the infection, since both men were exposed at the time of felling the tree about two weeks prior to their illness. The chicken coop on the farm of J.M. had not been cleaned by him for more than two years and, hence, he had not been exposed to dust from this source; furthermore, his brother (L.M.) had had no association with the farm.

The isolation of this fungus from material from an old decayed tree stump recalls the findings of Grayston and Furcolow.[10] These investigators in 1953 reported the isolation in Illinois of H. capsulatum from dried, powdery wood chips and flakes intermixed with sandy soil scooped out of the bottom of a hollow tree trunk considered to be the probable source of histoplasmosis in two young male cousins. Prior to their illness the boys had played for a short time in the hollow tree trunk.

These studies reveal a new area of endemicity of histoplasmosis in New York State. Grayston and Furcolow[10] reported the isolation of H. capsulatum in 1953 from the dirt basement of a church in Plattsburgh, N. Y., in the Champlain Valley, near the site of an epidemic of acute miliary pneumonitis of unknown etiology described by Nauen and Korns.[18]

The St. Lawrence and Lake Champlain Valleys were incriminated as endemic areas by White and Hill[19] in 1950 following a study of 114 persons with pulmonary calcifications. They found that 106 of the 114 persons were from these valleys and that 79 of 84 of those tested for Histoplasma-sensitivity yielded positive reactions.

The unusual opportunity to study the mycologic, serologic, and epidemiologic aspects of a small epidemic of histoplasmosis, as well as to follow the clinical features of the disease, is rarely afforded the laboratory. For this opportunity

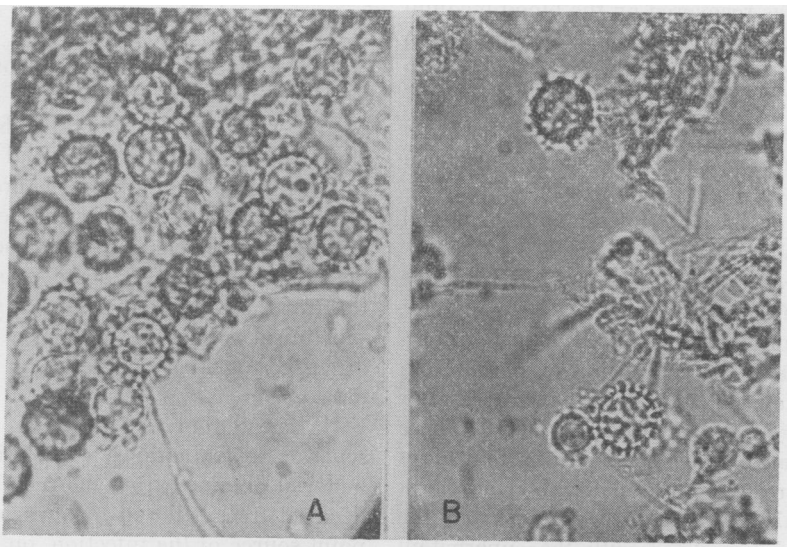

Figure 3—H. capsulatum. A. Macroconidia Produced by One of the Isolates from Material from the Decayed Tree Stump. B. Macroconidia Produced by One of the Isolates from Litter from Chicken Coop (magnification X600)

the authors are greatly indebted to Drs. Cullen, Scholdager, and Beecher of the Albany Veterans Administration Hospital.

REFERENCES

1. Cullen, J. H.; Hazen, E.; and Scholdager, R. Two Cases of Histoplasmosis Acquired in Felling a Decayed Tree in the Mohawk Valley. New York State J. Med. (In press.)
2. Emmons, C. W. Isolation of Histoplasma capsulatum from Soil. Pub. Health Rep. 64:892–896, 1949.
3. Emmons, C. W. Histoplasmosis: Animal Reservoirs and Other Sources in Nature of the Pathogenic Fungus, Histoplasma. A.J.P.H. 40:436–440, 1950.
4. ————. The Isolation From Soil of Fungi Which Cause Disease in Man. Trans. New York Acad. Sc. 14: 51–54, 1951.
5. Ajello, L., and Zeidberg, L. D. Isolation of Histoplasma capsulatum and Allescheria boydii From Soil. Science 113:662–663, 1951.
6. Grayston, J. T.; Loosli, C. G.; and Alexander, E. R. The Isolation of Histoplasma capsulatum in an Unused Silo. Ibid. 114:323–324, 1951.
7. Loosli, C. G.; Grayston, J. T.; Alexander, E. R.; and Tanzi, F. Epidemiological Studies of Pulmonary Histoplasmosis in a Farm Family. Am. J. Hyg. 55:392–401, 1952.
8. Zeidberg, L. D.; Ajello, L.; Dillon, A.; and Runyon, L. C. Isolation of Histoplasma capsulatum From Soil. A.J.P.H. 42:930–935, 1952.
9. Furcolow, M. L., and Larsh, H. W. Direct Isolation of Histoplasma capsulatum From Soil: Probable Etiological Relationship to Camp Gruber Pneumonitis (19584). Proc. Soc. Exper. Biol. & Med. 80:246–248, 1952.
10. Grayston, J. T., and Furcolow, M. L. The Occurrence of Histoplasmosis in Epidemics. Epidemiological Studies. A.J.P.H. 43:665–676, 1953.
11. Gordon, M. A.; Ajello, L.; Georg, L. K.; and Zeidberg, L. D. Microsporum gypseum and Histoplasma capsulatum Spores in Soil and Water. Science 116:208, 1952.
12. Ajello, L. Occurrence of Histoplasma capsulatum and Other Human Pathogenic Molds in Panamanian Soils. Am. J. Trop. Med. & Hyg. 3:897–904, 1954.
13. Kier, J. H.; Campbell, C. C.; Ajello, L.; and Sutliff, W. D. Acute Bronchopneumonic Histoplasmosis Following Exposure to Infected Garden Soil. J.A.M.A. 155:1230–1232, 1954.
14. Zeidberg, L. D., and Ajello, L. Environmental Factors Influencing the Occurrence of Histoplasma capsulatum and Microsporum gypseum in Soil. J. Bact. 68:156–159, 1954.
15. Emmons, C. W. The Significance of Saprophytism in the Epidemiology of the Mycoses. Trans. New York Acad. Sc. 17:157–166, 1954.
16. Zeidberg, L. D.; Ajello, L.; and Webster, R. H. Physical and Chemical Factors in Relation to Histoplasma capsulatum in Soil. Science 122:33–34, 1955.
17. Howell, A. The Efficiency of Methods for the Isolation of Histoplasma capsulatum. Pub. Health Rep. 63:173–178, 1948.
18. Nauen, R., and Korns, R. F. A Localized Epidemic of Acute Miliary Pneumonitis Associated with the Handling of Pigeon Manure. Paper read at the Annual Meeting of the American Public Health Association (Oct.), 1944.
19. White, F. C., and Hill, H. E. Disseminated Pulmonary Calcification. Am. Rev. Tuberc. 62:1–16, 1950.

National Science Foundation Senior Postdoctoral Award Program

September 4, 1956, is the final date for applications for a second group of senior postdoctoral fellowships to be awarded by the National Science Foundation during the current calendar year. Fellowships will be awarded in mathematical, physical, medical, biological, engineering, and other sciences, including anthropology, psychology (other than clinical), geography, certain interdisciplinary fields, and areas of convergence between the natural and social sciences. In addition, candidates must have at least five years' experience beyond the science doctorate or its equivalent, have demonstrated ability and special aptitude for advanced training and productive scholarship in the sciences, and be a United States citizen. Names of successful fellowship candidates will be announced on October 16, 1956.

Applications and further details from the Division of Scientific Personnel and Education, National Science Foundation, Washington 25, D. C.