THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

# A STUDY OF STRAINS OF *HISTOPLASMA CAPSULATUM*

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

JAMES EDWARD GREER

Norman, Oklahoma

1956

A STUDY OF STRAINS OF HISTOPLASMA CAPSULATUM

APPROVED BY

Howard L. Lamb

D. L. Crawl

Kirk L. Worley

H. Wender

J. B. Clark

Leon S. Young

DISSERTATION COMMITTEE

ACKNOWLEDGMENT

It is a pleasure to acknowledge the help and encouragement of Dr. Howard W. Larsh, Chairman, Department of Plant Sciences, University of Oklahoma, and Mycologist, Communicable Disease Center, Kansas City Field Station, University of Kansas Medical Center, Kansas City, Kansas, who directed this work. Gratitude is also expressed to Dr. Harrison L. Chance, Dr. J. Bennett Clark, and Dr. Norman Boke of the Department of Plant Sciences for reading the manuscript and offering valuable suggestions.

The author especially wishes to express his appreciation to the following for supplying him with cultures for this study, and with reprints of much of the literature that is not readily accessible: Michael L. Furcolow, M. D., Medical Director, U. S. Public Health Service, CDC, Kansas City Field Station, University of Kansas Medical Center, Kansas City, Kansas; John H. Robinson, D. V. M., Department of Microbiology, University of Louisville School of Medicine, Louisville, Kentucky; H. I. Lurie, B.S., M.B., Ch. B.,

Pathologist, South African Institute for Medical Research, Johannesburg; Chester W. Emmons, Ph. D., Principal Mycologist, National Institutes of Health, Bethesda, Maryland; Gerbert Rebell, B. S., Research Associate, Oklahoma Medical Research Foundation; and Mrs. Norma H. Broom, B. A., Bacteriologist, Department of Health Laboratories, Detroit, Michigan.

Finally, a debt of gratitude is acknowledged to Clarence S. Livingood, M. D., Physician-in-Charge, Division of Dermatology, Henry Ford Hospital, Detroit, Michigan, who granted permission, time, and facilities for the completion of this study, and to the Upjohn Company, Kalamazoo, Michigan, whose funds supported, in part, this work.

TABLE OF CONTENTS


| | Page |
|---|---|
| LIST OF TABLES ..... | vi |
| Chapter | |
| I. INTRODUCTION ..... | 1 |
| II. MATERIALS AND METHODS ..... | 9 |
| III. RESULTS ..... | 19 |
| IV. DISCUSSION ..... | 24 |
| V. SUMMARY ..... | 35 |
| BIBLIOGRAPHY ..... | 38 |

## LIST OF TABLES


| Table | | Page |
|---|---|---|
| 1. | Human Strains of *H. capsulatum* Obtained from Dr. Furcolow | 10 |
| 2. | Human Strains of *H. capsulatum* Obtained from Other Donors | 11 |
| 3. | Animal Strains of *H. capsulatum* Obtained for Study | 12 |
| 4. | Soil Strains of *H. capsulatum* Studied | 13 |
| 5. | Results of Viability Studies Using 357 Subcultures of *H. capsulatum* | 22 |

# A STUDY OF STRAINS OF *HISTOPLASMA CAPSULATUM*

## CHAPTER I

## INTRODUCTION

*Histoplasma capsulatum* is a diphasic, pathogenic fungus of the *Fungi Imperfecti*. It was first described by Samuel Taylor Darling (1-4), first recognized as a fungus by da Rocha-Lima (5), and first cultured by DeMonbreun (6). The organism exhibits thermal dimorphism. It has a small, unicellular, budding, yeastlike form when grown at 37° C. on moist, enriched media or in animal tissues. However, at room temperatures it is a white, filamentous mold with septate, branching hyphae from which the characteristic thick-walled tuberculate chlamydospores are produced. Either form may be converted to the other. Intermediate forms occur. The phenomenon of dimorphism has been recently reviewed by Scherr and Weaver (7). A cultural study of the life cycle of the organism has been made by Conant (8), Howell (9), and Umanzio (10-12), who described coremia formation as well as

the intermediate forms. H. capsulatum may be cultivated on a great variety of laboratory media, in tissue culture, and in the embryonated hen egg, the monkey, grassfrog, mouse, cavy, hamster, rat, rabbit, and gerbil.

The organism occurs in soil (13), water (14), air (15), and in diseased and apparently healthy animals (16,17) such as the dog, cat, house mouse, brown rat, roof rat, skunk, bear, opossum, racoon, and horse. H. capsulatum has also been reported in the ferret, cow, monkey, anteater, two-toed sloth, hen, and bat, but these reports are based on microscopic findings without cultural identification (18,19). Bat and "oil bird" droppings, chicken and pigeon manure have been associated with infections and epidemics, but neither the animals nor their excreta have been proved to be sources of the organism. In Panama, Darling searched for the fungus in water and in mosquitoes and other insects, cattle, monkeys, rats, mice, frogs, racoons, marsupials, and lizards. Similar surveys have been made in Surinam (19). Although Darling found organisms resembling the fungus in the inflamed colon of a rat, it has been cultured from a living source on the Isthmus in only one canine and four human infections, all fatal (20-22). Recently, the organism has been isolated from Panamanian soil (23). Methods of

isolation employed culturing from sections of infected tissue, animal inoculation with tissue, direct isolation from soil, and cultures and animal inoculations following the flotation methods with soil, which have been evaluated by Larsh, Hinton, and Furcolow (24).

Over 400 human infections with this fungus have been recognized, and cases have been reported from every continent; in the United States, cases have been found in half of the states. Two cases in Oklahoma have been proved by cultures (25-27), and there probably have been forty others. One epidemic of pneumonitis at Camp Gruber, Oklahoma, in 1943, involved five men; another in March, 1944, involved 35 men. Subsequent studies (28-34) leave little doubt that all of these men were infected with H. capsulatum.

The disease resulting from infection with H. capsulatum is most commonly called histoplasmosis. It has also been designated as Darling's disease, cytomycosis of Darling, and reticuloendothelial cytomycosis. The disease is so protean in its manifestations that it cannot be said to have a typical syndrome, as claimed by earlier writers. The symptoms vary, depending on the location, extent, and duration of the infection. They are often referable to more than one organ or system. Histoplasmosis must, therefore,

be considered in the differential diagnosis of granulomatous, neoplastic, hematopoietic, and respiratory diseases. The symptoms and pathogenesis of histoplasmosis in animals is similar to that in humans (35,36). Infections have also been observed in apparently healthy animals (17).

The past decade has shown that the disease is not always of the progressive, rapidly disseminating, fatal type as was first thought. More commonly it is a benign infection with recovery. Evidence for this is to be found in the numerous roentgenographic studies of pulmonary infiltration and subsequent calcification (37-46). Furthermore, cases of generalized histoplasmosis are known to have recovered (47-49). There is also some indication (50) that the fungus may exist in the human system for periods up to ten years before the onset of symptoms.

A positive diagnosis of histoplasmosis can be made only by isolating H. capsulatum from the sputum (51,52), gastric lavage material or other body fluids (53-55), feces, liver or scalene biopsy (56), or necropsy material (57). In addition to the roentgenograms mentioned above, there are other diagnostic adjuncts. The fungus may be recognized in blood and tissues (58-61) examined microscopically. Complement fixation tests (62), collodion agglutination tests

(63-65), and histoplasmin skin testing may aid in evaluating the clinical findings.

The histoplasmin test was first performed by Van Pernis, et al. (66), using a filtrate from a broth culture of an organism (mycelial phase of H. capsulatum) which they isolated from a patient. Christie and Peterson (67) later named this extract histoplasmin. It has been made from filtrates of both yeastlike and mycelial phases (68), but there is little if any difference in their antigenicity. The histoplasmin skin test has somewhat the same significance as the tuberculin test. Histoplasmin is now commercially available, and surveys have been made on every continent and in almost every country in which the disease has been reported (44). There is a striking correlation between pulmonary calcification (in individuals not reacting to tuberculin or coccidioidin) and histoplasmin sensitivity, and the geographic areas involved are coincident (42,69). There are marked geographic differences in histoplasmin sensitivity (42,46,70-72). In Tennessee, the incidence of histoplasmin sensitivity and pulmonary calcification, as well as histoplasmosis is greater in areas adjacent to streams and lesser on high, dry ridges and lands distant from these streams (73). In Kansas, histoplasmin sensitivity was far

more common in humid than in dry regions, and greater in rural than in urban areas (74).

The mode of transmission of the disease is not known, but very likely the respiratory system is at least one portal of entry (34), and infections by the oral route have been accomplished experimentally (75). Although cases have occurred among persons of the same household (49,76), and in dogs of the same kennel (77,78), there is no indication that familial environment is a factor (79) or that the disease is readily transmitted from one individual to another (80).

The disease has occurred in patients from one month to 70 years of age. The number of cases found among children under ten years of age and in adult men suggests that soil is the chief vector; men may be exposed by occupation, and children by playing on the ground or on the floor where the organism has been brought in on the feet. Zeidberg (81) has suggested that the red-yellow podzols are the most favorable for the growth of the fungus, as he noted the high percentage of histoplasmin sensitivity in areas where the red-yellow podzols occur. It has also been suggested (74), that the average rainfall may be the determining factor, but others (82) have maintained that H. capsulatum is spread through the Mississippi Valley by flood waters

carrying the fungus in washed-out soil which is blown as dust. Mochi and Edwards (44) have noted the association of histoplasmosis with large rivers running through low country.

Of particular interest are the papers recently published in Peru (83-87). They report on patients suffering from a type of "cave sickness" known locally as "La Fiebre de Tingo Maria" and having clinical manifestations like those of histoplasmosis. The cultures of H. capsulatum obtained from these patients and the histoplasmin sensitivity studies prove conclusively that this disease is histoplasmosis. Especially significant are the descriptions of the chalice-like rock formations containing droppings from the numerous "oil birds" (Steatornis caripensis) frequenting this "cave of the owls."

A great many chemotherapeutic and antibiotic agents have been tested both clinically and in vitro against the fungus, but at present there is no successful treatment. Immunization, however, has been accomplished in mice (88-90), but the immunizing antigen is in the cell wall only, not the protoplasm (91). Although some patients have recovered following various treatments such as resection and the use of antifungal agents, others have recovered following supportive therapy alone (92).

The current thinking is that H. capsulatum maintains itself as a saprophyte in nature, chiefly in soil rich in organic matter, particularly bird droppings (34,83). By contact with such soil or dust (15,93), man and animals have become infected.

This study was undertaken to determine whether the strains of H. capsulatum from animals, soil, and man have recognizable differences such as exist in the genera Microsporum, Mycobacterium, and Brucella, and by the use of various diagnostic procedures to characterize the fungus further as an aid in its identification and its differentiation from other fungi.

# CHAPTER II

## MATERIALS AND METHODS

Sixty-two strains of Histoplasma capsulatum were collected and studied. The human strains collected for study are listed in Table 1 and Table 2 together with such notes as could be obtained. The cultures have been designated by names and/or numbers. The names are often those of patients, but occasionally they denote an area from which the culture came or the person who made the isolation. The animal strains studied are listed in Table 3. All of the soil strains studied were obtained from Dr. Furcolow and are listed in Table 4.

### Morphological Characteristics

Stock cultures of the strains studied were maintained on Bacto-Sabouraud Dextrose Agar in test tubes and six ounce prescription bottles, with either screw caps or cotton plugs. In addition, the mycelial phase was grown on Mycophil Agar, Bacto-E.M.B. Agar, and Bacto-Endo Agar.

TABLE 1

Human Strains of H. capsulatum Obtained from Dr. Furcolow

| Designation | Source | Date of Isolation |
|---|---|---|
| Benner - 2464 | intestinal ulcer | October 31, 1950 |
| Marks | blood clot | September 26, 1951 |
| Ellis | | 1951 (St. Louis, Mo.) |
| Junk (Pt. who was | ill at farm where Iowa soil #4 isolated)- | 1951 |
| Chick | blood clot | November 27, 1950 |
| Dawson | gastric lavage | November 25, 1945 |
| Creator | pleural fluid | December 26, 1951 |
| Mitchell - H-629 | sputum | October 27, 1947 |
| Mitchell - 2363 | sputum | June, 1950 |
| Lupher - 929 | gastric lavage | April, 1948 |
| White - 2132 | sputum | November 29, 1949 |
| Reeves - 970 | sputum | April 22, 1948 |
| Tachjean - A-136 | necropsy | June, 1947 |
| Spitzli - 809 | tonsils | January 29, 1948 |
| Parnell - P5 | (Isolated by Dr. Peterson) | 1944 |
| Canham - 3223 | blood clot | January 12, 1953 |
| Dueffell - 3220 | sputum | February 16, 1953 |
| P 10 - C 936 | (Isolated by Dr. Portuondo) | 1944 (St. Louis, Mo.) |
| Wolkey - A-145 | necropsy | October 13, 1947 |
| Guest - A-140 | necropsy | December 22, 1947 |
| Anderson - 2012 | | Prior to 1950 |

TABLE 2

Human Strains of H. capsulatum Obtained from Other Donors

| Designation | Source | Date of Isolation | Donor |
|---|---|---|---|
| 6507 | hilar lymph nodes | 1941 | Emmons |
| Kudik | sputum | 1956 | Broom |
| 9A2 | ? | prior to 1952 | Rebell |
| #4<br>#55<br>#56 | (South African Institute for Medical Research, Johannesburg) | prior to 1953 | Lurie |
| Sallee | ? | 1952 | Robinson |
| ATCC 8136 | (American Type Culture Collection - from Children's Hospital, Cincinnati) | November 23, 1939 | Benedek |

Lactophenol cotton blue was used to make slide preparations for microscopic examinations. The yeast phase of these organisms was obtained by using Castaneda bottles (94) containing Bacto-Brain Heart Infusion Agar with 10% human citrated blood, with and without Bacto-Brain Heart Infusion. Adequate moisture is essential, and the water of condensation in the screw capped bottles was sufficient for the solid-and-liquid technique of Marwin (95). The morphology of the different strains was also observed on the various media mentioned in connection with the biochemical tests.

TABLE 3

Animal Strains of H. capsulatum Obtained for Study

| Designation | Source | Date of Isolation | Donor |
|---|---|---|---|
| E 6562 | skunk | ? (94) | Emmons |
| Craghead | dog | June 28, 1951 | Furcolow |
| Thompson Dog #2 | dog | March 6, 1953 | Furcolow |
| Racoon 3222 | racoon | January 28, 1953 | Furcolow |
| Racoon 3299 | racoon | April 27, 1953 | Furcolow |
| Thompson Cat #10 | cat | February 27, 1953 | Furcolow |
| Dog 2917 | dog | February 7, 1953 | Furcolow |
| Dog 1023 | dog (Nashville, Tenn.) | June 14, 1953 | Furcolow |
| E 6617 | skunk (Ga.) | ? (94) | Emmons |
| E 6568 | rat (Ga.) | ? (94) | Emmons |
| E 6569 | rat (Ga.) | ? (94) | Emmons |
| Cat #4 | cat | 1953 | Furcolow |
| Thompson Dog #4 | dog | 1953 | Furcolow |
| E 6515 | dog | ? | Emmons |

## Physiological Characteristics

Tests for proteolytic activity were made by growing all the strains of H. capsulatum on Bacto-Loeffler Blood Serum Agar and Bacto-Brain Heart Infusion Agar plus 10%

TABLE 4

Soil Strains of *H. capsulatum* Studied

| Designation | Source | Date |
|---|---|---|
| Camp Crowder | East side of barn near silo, McDonald Co., Mo. | November 11, 1952 |
| Lawson-Soil III | ? | 1953 |
| Camp Gruber | Inside storm cellar, Sequoyah Co., Okla. (31) | June, 1951 |
| Little | Chicken house in Platte Co., Mo. (32) | April 28, 1952 |
| Grayston | Inside of silo Lake Co., Ind. (95-97) | July, 1951 |
| Iowa Soil #4 | Near Junk farmhouse Jackson Co., Iowa | 1951 |
| Davis | Texarkana Cave, Little Rock, Arkansas | December, 1951 |
| Madison Soil #1 | Swamp, Dane Co., Wisc. | May, 1952 |
| Clemens Soil #2 | Near rose bushes, Woodson Co., Kansas | June 13, 1952 |
| Boone Co. Soil #23, #92, #6, #12, #2, #73, #40 | Boone Co., Mo. | February 16, 1953 |
| McNatt Soil #7, #10 | ? | 1953 |

human blood or plasma.

The inorganic nitrogen medium used consisted of 15 g agar, 1 g di-hydrogen ammonium orthophosphate, 10 g glucose, 0.2 g potassium chloride, and 0.2 g magnesium sulphate per liter of distilled water, with brom-cresol purple added as an indicator. Since the tubes had cotton plugs, it was necessary to incubate them in baskets supported over water in sealed glass museum jars to prevent dehydration and provide adequate humidity.

Bacto-Simmons Citrate Agar in screw capped tubes was inoculated in quadruplicate sets with all strains. A fifth tube containing Bacto-Simmons Citrate Agar plus 2% glucose was also inoculated with each strain.

Catalase production was tested by flooding both mycelial and yeast phase cultures with hydrogen peroxide. Cultures of staphylococci and non-hemolytic streptococci were used, respectively, as positive and negative controls.

For the oxidase test, 1% aqueous solutions of both dimethyl-p-phenylenediamine hydrochloride and tetramethyl-p-phenylenediamine dihydrochloride were used to flood yeast phase cultures of all the strains. Stock cultures of Neisseria were used as control organisms.

The phenomenon of fluorescence was studied by

observing all cultures under ultraviolet light. Two lamps were used, one emitting rays predominantly at 2537 A and the other at 3660 A. Wherever possible, cultures were examined without intervening glass. However, the glassware used, when containing cultures of Pseudomonas aeruginosa or hairs infected with Microsporum audouini, permitted fluorescence. In addition to the 62 strains of H. capsulatum, the following stock cultures, all approximately four months old and growing on Bacto-Sabouraud Dextrose Agar, were tested at both wave lengths: Microsporum audouini, M. canis, M. gypseum, Trichophyton schoenleini, T. gallinae, T. concentricum, T. ferrugineum, T. violaceum, T. rubrum, T. mentagrophytes, T. megnini, Epidermophyton floccosum, Piedra hortai, Blastomyces dermatitidis, B. brasiliensis, Monosporium apiospermum and Allescheria boydii, Hormodendrum pedrosoi, Candida albicans, Cryptococcus neoforman, Nocardia madurae, Phialophora verrucosa, and Sporotrichum schencki.

Coagulase production was tested by adding 0.5 ml of plasma to 0.2 ml of one day old suspensions of yeast and mycelial phases of H. capsulatum in Bacto-Brain Heart Infusion in Wassermann tubes. Incubation at 37°C was for three hours during which time observations were made for clot formation. Two human strains (Kudik and Rebell 9A2),

two soil strains (Boone Co. 92 and 40), and two animal strains (Emmons 6515 and 6562) were tested with rabbit (Bacto-Coagulase Plasma) and human (Diagnostic Plasma, Warner-Chilcott) plasma. Negative controls consisted of tubes containing only broth and plasma, whereas positive controls contained a broth culture of a coagulase producing staphylococcus and plasma.

Urease production was initially observed with 15 strains (8 human, 7 soil) of H. capsulatum grown on Bacto-Urea Agar. To facilitate medium preparation by eliminating Seitz filtration, the method of Tidwell, Heather, and Merkle (100) was used to prepare an autoclave-sterilized urea medium which included 1.5% agar. Tubes of this medium were inoculated with all strains. Finally, Bacto-Urea Broth sterilized by Seitz filtration was used to test all strains. Comparison tests were then made with two strains of Blastomyces dermatitidis, one strain from the University of Texas Medical Branch, Department of Dermatology and Syphilology, and the other from the University of Michigan, Department of Dermatology. Stock cultures of Proteus mirabilis and paracolons were used for positive controls. Negative controls consisted of tubes of a medium having all the ingredients of Bacto-Urea Agar except urea.

The viability or survival of H. capsulatum in culture media was tested by subculturing onto Bacto-Sabouraud Dextrose Agar. The cultures had been maintained on Bacto-Sabouraud Dextrose Agar, Mycophil Agar, and fresh-potato dextrose agar. When it was observed in this study that cultures, some still moist in screw capped tubes and others in cotton plugged tubes and thoroughly desiccated, were viable after periods greater than one year, records were kept of the age of these cultures, the condition of the agar (state of desiccation), and viability as evidenced by subcultures. In the case of dried cultures, spores and mycelia were scraped from the surface and transferred, sometimes with flakes of dried agar. Subcultures from soft, moist agar cultures were made by transferring mycelia in small plugs of agar cut from the surface growth. All transfers were made using a stiff wire hammered to form a spatula at the end. The wire was heated to redness and immediately plunged into the agar of the recipient tube, thus cooling the inoculating spatula and coating it with agar to prevent dissemination of spores at the same time. The coated wire was then introduced into the culture, charged with inoculum, and returned to the recipient tube. The charged spatula was then sterilized by use of an electric coil (Bacti-Burner) to prevent

contamination of the working area. Subcultures showing no growth at the end of one month were discarded and recorded as negative. Those showing growth were checked for characteristic colonial morphology, pigmentation, or microscopic appearance to exclude the possibility of contamination.

# CHAPTER III

# RESULTS

## Morphological Characteristics

No difference has been detected in the gross morphology of the human, soil, or animal strains, on any of the media used, in either the mycelial or the yeast phase. Nor has any difference been noted microscopically. The characteristic tuberculate chlamydospores have been produced by all strains, but the time of their development was neither regular nor predictable. When grown on Bacto-Endo Agar, there were no differential characteristics although the color (basic fuchsin) was absorbed by the mycelium. On Bacto-Levine E.M.B. Agar the mycelium was white, but there was some suggestion of color as the fungus grew down into the agar. In the mycelial phase, H. capsulatum resembled Blastomyces dermatitidis. Pleomorphism, so common in fungi, was not detected in old cultures. There was no peculiarity noted in the yeast phase of these strains. No difference

was detected between old and recent isolates.

## Physiological Characteristics

No proteolytic activity by any of the strains was observed on blood or plasma agar or Bacto-Loeffler Blood Serum Medium. The initial growth appeared yeastlike and was often orchid colored on the Loeffler slants, but attempts at getting satisfactory yeast phase cultures on this medium were unsuccessful. There was no lysis of red blood cells.

All the strains were able to utilize the inorganic nitrogen of the di-hydrogen ammonium orthophosphate agar. Growth was slow and scant. The characteristic pigment was formed, however, and the indicator was changed from purple to yellow.

Not any of the strains was able to grow on Bacto-Simmons Citrate Agar. Luxuriant growth occurred, however, and the brom-thymol blue was changed from grass green to yellow in the tubes with glucose added to the medium.

Both yeast phase and mycelial cultures of all strains gave positive catalase tests as evidenced by the evolution of bubbles when the fungi were in contact with hydrogen peroxide. The staphylococci were, of course, also positive, and the nonhemolytic streptococci were negative.

The oxidase test was negative with the yeast phase cultures of all strains but positive with the Neisseria cultures with either reagent.

Fluorescence was not observed in any of the 62 strains of H. capsulatum or in any of the pathogenic fungi tested at either wave length.

Coagulase tests using both yeast phase and mycelial cultures of the human, soil, and animal strains were negative with both rabbit and human plasma. Control tests were satisfactory.

Urease was produced by all strains of H. capsulatum, for all urea media were changed from neutral to alkaline. The control medium was unchanged, and the control organisms gave characteristic reactions. The two strains of Blastomyces dermatitidis produced prompt alkaline reactions in the Bacto-Urea Agar but not in the Bacto-Urea Broth.

The viability of H. capsulatum in culture media was observed over a five year period. The results with 357 subcultures are shown in Table 5. These data show that dried spores may be viable for periods up to three years, and that moist cultures may be viable for five years. There was no indication that any strain survives longer than the others. Both human and animal strains were among the five

TABLE 5

Results of Viability Studies Using 357 Subcultures of *H. capsulatum*

| Age in months | Hard and Desiccated Agar | | Soft and Moist Agar | | TOTAL |
|---|---|---|---|---|---|
| | Positive | Negative | Positive | Negative | |
| 5 | | | 16 | 1 | 17 |
| 13 | 8 | 5 | | | 13 |
| 14 | 7 | 3 | | | 10 |
| 16 | 1 | 4 | | | 5 |
| 19 | 1 | 69 | 1 | 7 | 78 |
| 20 | | 11 | | 3 | 14 |
| 24 | | | 1 | 1 | 2 |
| 27 | 1 | 1 | 14 | 4 | 20 |
| 30 | 2 | 7 | 10 | | 19 |
| 34 | | | 4 | 22 | 26 |
| 35 | | | 1 | 22 | 23 |
| 36 | 5 | 28 | | 8 | 41 |
| 37 | 3 | 7 | | | 10 |
| 38 | 2 | 3 | | | 5 |
| 50 | | 8 | | | 8 |
| 61 | | 20 | | | 20 |
| 63 | | 10 | 6 | 15 | 31 |
| 66 | | 11 | | 4 | 15 |
| Totals | 30 | 187 | 53 | 87 | 357 |

year old positive cultures, but no soil strain had been maintained for that long. For the most part, all cultures were kept on shelves or in cabinets away from direct light.

# CHAPTER IV

## DISCUSSION

The tests and media used have purposely been the standard and recognized procedures of diagnostic medical microbiological laboratories. Thus any diagnostically useful tests discovered by these studies could be applied in such laboratories without requiring additional media or reagents.

The cultures of the 62 strains of Histoplasma capsulatum used in this study were clinically and geographically representative, and covered a period of 17 years. Of the strains observed, 31 were human isolates, 18 were from soil, 6 from dogs, and two each from cats, racoons, rats, and skunks. Occasionally, multiple isolates were obtained from the same source. These were considered as the same strain. Among the human isolates were strains from intestinal ulcers, hilar lymph nodes, blood clots, gastric washings, pleural fluid, sputum, and tonsils. Both old and recent

isolates were included. The oldest culture was that from the American Type Culture Collection and isolated in 1939. The most recent was isolated in 1956. Human isolates have been obtained from both fatal and non-fatal cases. Three of the cultures were isolated from cases in South Africa and the remainder from the United States.

## Morphological Characteristics

Moore (100,101) studied two strains of H. capsulatum which he called Posadasia capsulata and P. pyriformis. He considered these to be different species, the former being smaller in size and in amount of growth. Their biochemical tests were identical. This classification was not confirmed by others and has been abandoned. Howell (9,102,103) studied the same strains. The P. capsulata strain was from a case reported by Dodd and Tompkins (104), which was the first case to be diagnosed ante mortem. The P. pyriformis was from a case reported by Hansmann and Schenken (105). Howell found the effects of temperature and pH to be similar for these two strains, but a marked difference in virulence was noted in five strains compared by intracerebral inoculation of mice (106). On the other hand, Rowley and Huber (107) studied four strains (2 human, 1 skunk, 1 cat) which showed

no significant difference in pathogenicity. Not any of these strains required biotin in media containing casein hydrolysate, calcium pentothenate, glucose, and agar (108). Calcium pantothenate was required for one strain, but apparently not for the other three. Strain differences in streptomycin (109,110) and actidione (24) activity have also been noted. Antibiotic resistance alone, however, does not justify species distinction or even variety rank. Emmons (111) has stated that "despite minor differences, one species, Histoplasma capsulatum, causes both human and animal histoplasmosis."

## Physiological Characteristics

The various media containing blood, plasma, serum, egg white and egg yolk were used in obtaining and observing the yeast phase cultures in this study. The two strains tested by Moore (101) failed to liquefy gelatin. DeMonbreun (6) reported only slight liquefaction of gelatin stab cultures after six week incubation.

That all strains of H. capsulatum tested could utilize di-hydrogen ammonium orthophosphate as a sole source of nitrogen was shown by growth on this medium and on citrate agar when glucose was added. The use of sealed jars was

found necessary in preliminary experiments in which no growth occurred with cultures known to be capable of growth. The high humidity requirements of the fungus have been previously demonstrated (112).

Not any of the strains was able to utilize sodium citrate as a sole source of carbon. That nothing else was lacking and that the other ingredients were not inhibitory was shown by the luxuriant growth obtained when 2% glucose was added. DeMonbreun (6), Moore (101), Scheff (113), and others have reported the failure of this fungus to produce acid or gas on a variety of carbohydrates. McLaurin et al. (114) studied only human strains but these were all citrate negative.

It is not surprising that all strains are catalase positive since this is characteristic of many filamentous fungi. The catalase activity is of special significance, however, because of its variation in another pathogen which is chiefly pulmonary, the tubercle bacillus. Since catalase negative tubercle bacilli have drug resistance but decreased pathogenicity, it is of interest to note that none of these strains of H. capsulatum show that variation although some strains have been isolated following drug therapy. The fungus is an obligate aerobe. Some workers have believed

it to be microaerophilic and recommended the use of sealed tubes and carbon dioxide for culture. Screw capped or paraffin sealed tubes and the use of candle jars have served, in the writer's experience, only to prevent evaporation.

Two test solutions were used for the oxidase tests because the tetramethyl form is considered less toxic and viable subcultures can more readily be made. The dimethyl form, however, is more economical and has an added advantage in that it does not discolor the medium.

There have been claims that cultures of fungi fluoresce and that this characteristic may be used as an aid in identification (115). Because of these reports, H. capsulatum as well as other fungi were examined with a Wood's light and a germicidal lamp. No evidence has been found in these observations to support these claims. Furthermore, Robinson et al. (116) have shown that the fluorescent material in tinea capitis was in the hair, not the fungi. It is possible that the earlier reports of fluorescence were positive because of differences in the filters used. All the cultures used in this study were subcultures devoid of clinical material. It may be that the earlier reports of positive fluorescence resulted from the use of primary isolates containing some clinical material. Substrates were probably

different also, although this is said to be without influence.

Coagulase production is regarded as the best single criterion for pathogenicity of staphylococci, and probably serves to prevent invading organisms from dying as a result of phagocytosis. Therefore, this characteristic was studied since there are no previous reports concerning it. The plasma used were citrated and subject to false positive reactions with certain enterococci, but since H. capsulatum does not utilize citrate, false positives were not expected nor encountered.

Urease was produced by the 17 strains studied by McLaurin et al. (114), but these were all human strains and the method of testing was not given. Since the Bacto-Urea Agar is more nutrient but less well buffered medium, it gives positive reactions with some organisms such as paracolons that produce negative results in Bacto-Urea Broth. This was not true of the medium prepared by autoclaving a modified urea broth plus agar. The method of Tidwell et al. (117) for the preparation of urea media has been found to be satisfactory and to give reliable results with Proteus and paracolons. B. dermatitidis and H. capsulatum resemble each other closely in the mycelial phase, and only by the

production of tuberculated chlamydospores by the latter can they be distinguished microscopically. Since this may require quite some time and result in delay in identification and subsequent diagnosis and treatment, the urease test offers a rapid means of distinguishing the two organisms. Obviously, Christensen's urea agar is of no value in this test for both organisms produce urease, but urea broth (or a similarly buffered agar) can differentiate the two because only H. capsulatum gives a sufficiently strong reaction to turn the medium alkaline.

At the start of these studies, the only reports in the literature on the viability of H. capsulatum were those of Beamer et al. (118). They reported that organisms resisted drying and refrigeration for four months. Three other reports were published later. Carmichael (119) kept his cultures in a deep freeze at -20° C and found them viable after nine months. Ritter (120) studied the viability of H. capsulatum in sterilized tap water from the Kansas River. She found that survival of the mycelial phase increased as temperatures increased from 4° C to room temperatures (20-25° C) but survival decreased above 25° C until at 45° C cultures survived but three days. Nielsen and Evans (121) had cultures of H. capsulatum which were viable after two years

incubation.

The cultures of H. capsulatum under observation in this report were subjected to some changes in temperature, but the temperature range for the entire five year period is estimated not to have exceeded 10-40° C at any time. This might possibly have affected spore germination, but if spores did germinate it is not likely that the nutrients were sufficient to produce a new generation of spores to increase viability of the cultures. The objection might also be raised that some of these cultures were slowly growing and thereby periodically producing younger cultures rather than persisting in a dormant state. This does not seem possible in the desiccated cultures, and the possibility is discounted in the moist cultures since the inoculation was made to cover the entire surface. The nutrients in the medium must have been almost, if not entirely, exhausted. The high humidity requirements already mentioned further preclude the possibility of growth in the desiccated cultures.

Admittedly, the conditions under which these observations were made do not parallel soil conditions. Obviously, there were no competitive organisms. The presence of glucose in these media probably increased longevity and

resistance to desiccation. Iljin (122) reported experiments to support his hypothesis that, in green plants at least, certain colloidal substances and sugars exert a protective action against desiccation, and non-electrolytes exercise greater protection than electrolytes, which, in high concentration, produce injury and decrease tolerance. Also, gradual drying resulted in increased viability as did gradual rehydration. All of these conditions were such, in the cultures of H. capsulatum, as to favor viability.

It is true that the recorded viability in months, i.e., from date of inoculation until date of subculture, may exceed the age or longevity of the elements in the culture. The reason for this is that chlamydospores may not be formed for several weeks after the inoculation of a new culture. However, several weeks or a month may be subtracted from the recorded time and the period still exceed five years for the oldest viable cultures. This is three years longer than any comparable reports in the literature.

These data show that dried spores may be viable for periods up to three years, and that moist cultures may be viable for five years. Such facts are of significance in connection with the etiology and mode of transmission of histoplasmosis. These studies indicate that H. capsulatum

is a hardy fungus capable of surviving for long periods of time even under adverse conditions. As stated in Chapter I, the disease is most prevalent in valley regions and its distribution seems dependent upon moisture content of the soil. The data recorded here showing the persistence and viability of the organism for periods of many years lends credence to the theory that the organism may maintain itself as a saprophyte or remain dormant in soil for long periods of time and thus serve as a source of infection.

It is apparent from these studies that all of these strains belong to a single type. Other species of the genus Histoplasma are H. farciminosum and H. duboisii. The former is a misnomer which rightly should be farcinimosum since it is the causative agent of epizootic lymphangitis of horses, or equine farcy. The corruption of spelling has been perpetuated until the name is now not only used but recommended unanimously by the Committee on Medical and Veterinary Mycopathology at the Fifth International Congress of Microbiology. Microscopically, this organism is indistinguishable in tissues from H. capsulatum and in culture the two appear quite similar. The chief difference is in the host relationship, since no case is known of an epizootic becoming epidemic. Also, it has been recently reported (123)

that horses with farcy were histoplasmin negative. H. duboisii (124,125) is also a closely related fungus and possibly a morphological variant because one of its forms is apparently identical with H. capsulatum although the other is noticeably different. It has not been possible to obtain cultures of H. duboisii although two requests for cultures have been made.

# CHAPTER V

## SUMMARY

A study has been made of 62 strains of Histoplasma capsulatum, a dimorphic fungus causing disease in man and animals and having a saprophytic existence in soil. The strains were considered to be representative clinically and geographically. One object of the study was to determine if strains from animals, man, and soil have recognizable differences such as exist in the genera Microsporum, Mycobacterium, and Brucella. Another was to characterize the fungus further as an aid to classification and differentiation from other fungi.

The tests and media used have purposely been the standard and recognized procedures of diagnostic medical microbiological laboratories in that any useful tests discovered by these studies could be applied in such laboratories without requiring additional media or reagents.

The morphological characteristics of both mycelial

and yeast phases were observed on a variety of media and microscopic studies were made, but there were no significant differences in animal, soil or human strains. No proteolytic activity of any strain on blood, plasma, or coagulated serum was found. Red blood cells in the agar were not lysed. All strains could utilize di-hydrogen ammonium orthophosphate as a nitrogen source, but none could utilize sodium citrate as a sole carbon source. Glucose and other carbohydrates are satisfactory carbon sources. All strains were found to be catalase positive but oxidase negative. The coagulase tests on both human and rabbit plasma using two human, two animal, and two soil strains were negative. No fluorescence was observed in the cultures of any of the 62 strains at either 2537 A or 3660 A. In addition, 23 cultures of other pathogenic fungi gave no fluorescence although there are numerous reports in the literature to the contrary.

All strains of H. capsulatum were found to produce urease in sufficient amounts to give a positive test with urea broth, which is well buffered. In contrast, Blastomyces dermatitidis, which at times is morphologically indistinguishable, gave positive results with the less buffered urea agar but negative results with the broth. This provides a means of distinguishing the two fungi.

The viability or survival of H. capsulatum in culture media was observed to exceed three years for desiccated cultures and five years for moist cultures. These data exceed the viability reports in the literature on any of the pathogenic fungi. The significance of this in relation to the etiology and transmission of histoplasmosis is discussed.

## BIBLIOGRAPHY


1. Darling, S. T. 1906 A protozoon general infection producing pseudotubercles in the lungs and focal necrosis in the liver, spleen and lymph nodes. J. Am. Med. Assoc., 46, 1283-1285.

2. Darling, S. T. 1907 Notes on histoplasmosis. A fatal disorder met with in Tropical America. Maryland Med. J., 50, 125-129.

3. Darling, S. T. 1908 Histoplasmosis: A fatal infectious disease resembling kala-azar found among the natives of Tropical America. Arch. Int. Med., 2, 107-123.

4. Darling, S. T. 1909 The morphology of the parasite (Histoplasma capsulatum) and the lesions of histoplasmosis. A fatal disease of Tropical America. J. Exptl. Med., 11, 515-531.

5. da Rocha-Lima, H. 1912 Histoplasmosis und epizotische lymphangitis. Arch. f. Schiffs. u. Tropen-Hyg., 16, 79-85.

6. DeMonbreun, W. A. 1934 The cultivation and cultural characteristics of Darling's Histoplasma capsulatum. Am. J. Trop. Med., 14, 93-116.

7. Scherr, G. H., and Weaver, R. H. 1953 The dimorphism phenomenon in yeasts. Bacteriol. Rev., 17, 51-92.

8. Conant, N. F. 1941 A cultural study of the life-cycle of Histoplasma capsulatum Darling 1906. J. Bacteriol., 41, 563-580.

9. Howell, A., Jr. 1939 Studies on Histoplasma capsulatum and similar form species. I. Morphology and development. Mycologia, 31, 191-216.

10. Umanzio, C. B. 1950 Morphological and cultural characteristics of Histoplasma capsulatum Darling 1906, with brief notes relating to Darling's histoplasmosis. Doctoral Thesis. Washington University, St. Louis.

11. Umanzio, C. B. 1951 Systemic infections. V. Further observations on Histoplasma capsulatum with brief notes on Darling's histoplasmosis. J. Osteopathy, 58, 16-23.

12. Umanzio, C. B. 1952 Morphological and cultural characteristics of Histoplasma capsulatum Darling 1906 with brief notes relating to Darling's histoplasmosis. Bacteriol. Proc., M37, 89-90.

13. Emmons, C. W. 1949 The isolation of Histoplasma capsulatum from soil. Publ. Hlth. Repts., 64, 892-896.

14. Gordon, M. A., Ajello, L., Georg, L. K., and Zeidberg, L. D. 1952 Microsporum gypseum and Histoplasma capsulatum spores in soil and water. Science, 116, 208.

15. Ibach, M. J., Larsh, H. W., and Furcolow, M. L. 1954 Isolation of Histoplasma capsulatum from the air. Science, 119, 71.

16. Emmons, C. W. 1950 Histoplasmosis: animal reservoirs and other sources in nature of the pathogenic fungus, Histoplasma. Am. J. Public Health, 40, 436-440.

17. Emmons, C. W. 1955 Histoplasmosis: Proved occurrence of inapparent infections in dogs, cats, and other animals. Am. J. Hyg., 61, 40-44.

18. Collier, W. A., and Winckel, W. E. F. 1952 Beiträge zur geographischen Pathologie von Suriname. 2. Vögel als Träger von histoplasma-artigen Mikroorganismen. Zeitschr. f. Hyg., 135, 338-340.

19. Collier, W. A., and Winckel, W. E. F. 1952 Beiträge zur geographischen Pathologie von Suriname. 6. Histoplasmose bei Säugetieren in Suriname. Antonie van Leeuwenhoek, 18, 349-356.

20. Tomlinson, W. J., and Grocott, R. G. 1945 Canine histoplasmosis; pathologic study of three reported cases and first case found in Canal Zone. Am. J. Clin. Path., 15, 501-507.

21. Draheim, J. H., Mitchell, J. R., and Elton, H. W. 1951 Histoplasmosis: fourth case report from the Canal Zone. Am. J. Trop. Med., 31, 753-760.

22. Tucker, H. A. 1952 Histoplasmosis on the Isthmus of Panama. Summary of epidemiological survey (1949-1950) and report of fourth local case (1951). Am. J. Trop. Med. Hyg., 1, 462-469.

23. Ajello, L. 1954 Occurrence of Histoplasma capsulatum and other human pathogenic molds in Panamanian soil. Am. J. Trop. Med. Hyg., 3, 897-904.

24. Larsh, H. W., Hinton, Agnes, and Furcolow, M. L. 1953 Laboratory studies on Histoplasma capsulatum. III. Efficiency of the flotation method in isolation of Histoplasma capsulatum from soil. J. Lab. Clin. Med., 41, 478-485.

25. Broders, A. C., Dochat, G. R., Herrell, W. E., and Vaughn, L. D. 1943 Histoplasmosis producing vegetative endocarditis. Review of literature, with report of a case. J. Am. Med. Assoc., 122, 489-492.

26. Broders, A. C., Dochat, G. R., Herrell, W. E., and Vaughn, L. D. 1944 Unusual case of histoplasmosis. Proc. Staff Meet. Mayo Clin., 19, 123-128.

27. Weiss, E. D., and Haskell, B. F. 1952 Anorectal manifestations of histoplasmosis. Am. J. Surgery, 84, 541-544.

28. Cain, J. C., Bevins, E. J., and Downing, J. E. 1947 An unusual pulmonary disease. Arch. Intern. Med., 79, 626-641.

29. Cain, J. C. 1949 Epidemic pulmonary disease. Med. Clin. N. Amer., 33, July, 1099-1110.

30. Grayston, J. T., and Furcolow, M. L. 1952 Acute febrile illness with disseminated pulmonary infiltrations - A syndrome caused by Histoplasma capsulatum. Tuberculology, 13, 9-14.

31. Schwartz, B., and Spitz, L. J. 1952 Histoplasmosis in epidemic form. A follow-up study. Arch. Intern. Med., 89, 541-546.

32. Furcolow, M. L., and Grayston, J. T. 1952 Occurrence of histoplasmosis in epidemics. Trans. 48th Ann. Meet. Natl. Tuberc. Assoc, 1-9.

33. Furcolow, M. L., and Larsh, H. W. 1952 Direct isolation of Histoplasma capsulatum from soil: probable etiological relationship to Camp Gruber pneumonitis. Proc. Soc. Exptl. Biol. Med., 80, 246-248.

34. Grayston, J. T., and Furcolow, M. L. 1953 The occurrence of histoplasmosis in epidemics - Epidemiological studies. Am. J. Publ. Hlth., 43, 665-676.

35. Emmons, C. W., and Ashburn, L. L. 1948 Histoplasmosis in wild rats: Occurrence and histopathology. Publ. Hlth. Repts., 63, 1416-1422.

36. Cole, C. R., Chamberlain, D. M., and Prior, J. A. 1951 Incidence, symptomatology, and diagnosis of canine histoplasmosis. Proc. Book, Am. Vet. Med. Assoc., 88th Ann. Meet., 179-181.

37. Furcolow, M. L. 1949 Development of calcification in pulmonary lesions associated with sensitivity to histoplasmin. Publ. Hlth. Repts., 64, 1363-1393.

38. Friedman, V., Brown, J. E., and Turner, E. V. 1950 A case of proven systemic histoplasmosis with apparent recovery. Ohio St. J. Med., 46, 44-45.

39. Furcolow, M. L. 1950 Tuberculin negative, histoplasmin positive disseminated pulmonary calcification. Postgrad. Med., 8, 15-20.

40. Furcolow, M. L., Guntheroth, W. G., and Willis, M. J. 1952 The frequency of laboratory infections with Histoplasma capsulatum: Their clinical and X-Ray characteristics. J. Lab. Clin. Med., 40, 182-187.

41. Furcolow, M. L., Mantz, H. L., and Lewis, I. 1947 The roentgenographic appearance of persistent pulmonary infiltrates with sensitivity to histoplasmin. Publ. Hlth. Repts., 62, 1711-1734.

42. Christie, A. 1950 Histoplasmosis and pulmonary calcification. Ann. N. Y. Acad. Sci., 50, 1283-1298.

43. Christie, A., and Peterson, J. C. 1946 Benign histoplasmosis and pulmonary calcification. Am. J. Dis. Child., 72, 460-464.

44. Mochi, A., and Edwards, P. Q. 1952 Geographical distribution of histoplasmosis and histoplasmin sensitivity. Bull. World Hlth. Org., 5, 259-291.

45. High, R. H., Zwerling, H. B., and Furcolow, M. L. 1947 Disseminated pulmonary calcification. A report of 113 cases. Publ. Hlth. Repts., 62, 20-29.

46. Palmer, C. E. 1945 Nontuberculous pulmonary calcification and sensitivity to histoplasmin. Publ. Hlth. Repts., 60, 513-520.

47. Klingberg, W. G. 1950 Generalized histoplasmosis in infants and children. J. Pediat., 36, 728-741.

48. Bunnell, I. L., and Furcolow, M. L. 1948 A report on ten proved cases of histoplasmosis. Publ. Hlth. Repts., 63, 299-316.

49. Wheeler, W. E., Friedman, V., and Saslaw, S. 1950 Simultaneous non-fatal systemic histoplasmosis in two cousins. Am. J. Dis. Child., 79, 806-819.

50. Kirsch, E. 1951 Beobachtung einer Histoplasmose mit Sektionsbefund. Z. Tropenmed. Parasitol., 3, 86-93. (Abstract in Rev. Med. Vet. Mycology, 1, No. 2136, 500-501.)

51. Kurung, J. M. 1952 The isolation of Histoplasma capsulatum from sputum. Am. Rev. Tuberc., 66, 578-587.

52. Moffit, G. W. 1956 The isolation of Histoplasma capsulatum from sputum as a diagnostic procedure. J. Lab. Clin. Med., 47, 499-502.

53. Haley, L. D. 1952 Pulmonary mycoses and the diagnostic laboratory. Am. J. Med. Techn., 18, 1-19.

54. Campbell, C. C. 1950 The isolation and identification of pathogenic fungi. Am. J. Med. Techn., 16, 57-72.

55. Lehman, R. M. 1950 Technique used in demonstrating Histoplasma capsulatum. Am. J. Med. Techn., 16, 18-24.

56. Johnson, J. E., Jr., and MacCurdy, J. M. 1952 Pulmonary histoplasmosis diagnosed by scalene biopsy. Am. Rev. Tuberc., 66, 497-500.

57. Weed, L. A., and Baggenstoss, A. H. 1951 The isolation of pathogens from tissues of embalmed human bodies. Am. J. Clin. Path., 21, 1114-1120.

58. Kligman, A. M., and Mescon, H. 1950 The periodic acid-Schiff stain for the demonstration of fungi in animal tissue. J. Bacteriol., 60, 415-421.

59. Kligman, A. M., Mescon, H., and DeLamater, E. D. 1951 The Hotchkiss-McManus stain for the histopathologic diagnosis of fungus diseases. Am. J. Clin. Path., 21, 86-91.

60. Kligman, A. M., and Lewis, F. S. 1953 A method for preparing permanent stained mounts of pathogenic fungi for microscopic examination. J. Bacteriol., 65, 148-150.

61. Starr, G. F., Dawe, C. J., and Weed, L. A. 1955 Use of periodic acid-Schiff stain in identification of pathogenic fungi in tissues. Am. J. Clin. Path., 25, 76-83.

62. Grayston, J. T. 1952 A study of the complement fixation reaction in histoplasmosis. J. Lab. Clin. Med., 40, 90-101.

63. Saslaw, S., and Campbell, C. C. 1949 A collodion agglutination test for histoplasmosis. Publ. Hlth. Repts., 64, 424-429.

64. Saslaw, S., and Campbell, C. C. 1950 Studies on the stability of the histoplasmin collodion agglutination test. J. Lab. Clin. Med., 35, 780-785.

65. Saslaw, S. and Campbell, C. C. 1948 A method for demonstrating antibodies in rabbit sera against histoplasmosis by the collodion agglutination technique. Proc. Soc. Exptl. Biol. Med., 68, 559-562.

66. Van Pernis, P. A., Benson, M. E., and Hollinger, P. H. 1941 Specific cutaneous reactions with histoplasmosis. Preliminary report of another case. J. Am. Med. Assoc., 117, 436-437.

67. Christie, A., and Peterson, J. C. 1945 Pulmonary calcification in negative reactors to tuberculin. Am. J. Publ. Hlth., 35, 1131-1147.

68. Campbell, C. C., and Saslaw, S. 1949 The use of yeast phase antigens in a complement fixation test for histoplasmosis. III. Preliminary results with human sera. Publ. Hlth. Repts., 64, 551-560.

69. Christie, A. 1951 Histoplasmosis and pulmonary calcification - geographic distribution. Am. J. Trop. Med., 31, 742-752.

70. Palmer, C. E. 1946 Geographic differences in sensitivity to histoplasmin among student nurses. Publ. Hlth. Repts., 61, 475-487.

71. Christie, A., and Peterson, J. C. 1946 Histoplasmin sensitivity. J. Pediat., 29, 417-432.

72. Beadenkopf, W. G., Loosli, C. G., Lack, H., Rick, F. A., and Slattery, R. V. 1949 Tuberculin, coccidioidin,

histoplasmin sensitivity in relation to pulmonary calcifications. (A survey among 6,000 students at the University of Chicago). Publ. Hlth. Repts., 64, 17-29.

73. Zeidberg, L. D., Dillon, A., and Gass, R. S. 1951 Some factors in the epidemiology of histoplasmin sensitivity in Williamson County, Tennessee. Am. J. Publ. Hlth., 41, 80-89.

74. Furculow, M. L., and Sitterley, J. 1951 Further studies of the geography of histoplasmin sensitivity in Kansas and Missouri. J. Kan. Med. Soc., 52, 584-589.

75. DeMonbreun, W. A. 1939 The dog as a natural host for Histoplasma capsulatum. Report of a case of histoplasmosis in this animal. Am. J. Trop. Med., 19, 565-587.

76. McLeod, J. H., Emmons, C. W., Rose, S., and Burke, F. G. 1946 Histoplasmosis. A report of four cases, two in siblings. Histoplasmin test and other diagnostic procedures. J. Pediat., 28, 275-295.

77. Prior, J. A., and Cole, C. R. 1951 Studies on the communicability of histoplasmosis. Am. Rev. Tuberc., 63, 538-546.

78. Robinson, V. B., McVicker, D. L., and Peterson, J. C. 1951 Some aspects of the epizootiology of histoplasmosis in two Boxer breeding kennels. J. Am. Vet. Med. Assoc., 119, 195-200.

79. Ferebee, S. H., and Furcolow, M. L. 1947 Histoplasmin sensitivity among siblings. Publ. Hlth. Repts., 62, 834-847.

80. Olson, B. J., Wright, W. H., and Nolan, M. O. 1941 An epidemiological study of calcified pulmonary lesions in an Ohio county. Publ. Hlth. Repts., 56, 2105-2126.

81. Zeidberg, L. D. 1954 A theory to explain the geographic variations in the prevalence of histoplasmin sensitivity. Science, 119, 654.

82. Beadenkopf, W. G., and Loosli, C. G. 1951 Histoplasmosis, tuberculosis and coccidioidomycosis. J. Am. Med. Assoc., 146, 621-624.

83. Monge M., C. 1955 La fiebre de Tingo Maria. Anales de la Facultad de Medicina, Lima, Universidad Nacional Mayor San Marcos, 38, 1080-1082.

84. Garcia R., O. 1955 "La fiebre de Tingo Maria" - Histoplasmosis. ibid., 1083-1091.

85. Arellano Z., R. C., and Galvez B., J. 1955 El agente etiologica de la fiebre de "Tingo Maria." ibid., 1092-1098.

86. Bouroncle C., A. 1955 Sensibilidad a la histoplasmina en algunas localidades del Peru. ibid., 1099-1112.

87. Lazarus, A. S., and Ajello, L. 1955 Aislamiento de Histoplasma capsulatum de suelo de una cueva en el Peru. Revista de Medicina Experimental, Lima, 9, 5-15.

88. Salvin, S. B. 1953 Immunization of mice against Histoplasma capsulatum. J. Immunol., 70, 267-270.

89. Salvin, S. B. 1955 Further studies on immunization of mice against Histoplasma capsulatum. Am. J. Hyg., 61, 72-81.

90. Schaefer, J., and Saslaw, S. 1954 Some factors affecting resistance of mice to experimental histoplasmosis. Proc. Soc. Exptl. Biol., N. Y., 85, 223-225.

91. Salvin, S. B., and Ribi, E. 1955 Structural elements of Histoplasma capsulatum and their role in immunization against experimental disease. In Sternberg, T. H., and Newcomer, V. D., Ed. Therapy of Fungus Diseases. An International Symposium. Little, Brown and Co., Boston. page 279.

92. Beamer, P. R., Schulz, D. M., Walcher, D. N., and Vellios, F. 1955 Histoplasmosis: Pathogenesis and immunology in relation to therapy. ibid., pp. 280-286.

93. Kier, J. H., Campbell, C. C., Ajello, L., and Sutliff, W. D. 1954 Acute bronchopneumonic histoplasmosis following exposure to infected garden soil. J. Am. Med. Assoc., 155, 1230-1232.

94. Castaneda, M. R. 1947 A practical method for routine blood cultures in brucellosis. Proc. Soc. Exptl. Biol. Med., 64, 114-115.

95. Marwin, R. M. 1949 An improved blood culture bottle utilizing a solid-and-liquid medium. Am. J. Clin. Path., 19, 697-698.

96. Emmons, C. W., Morlan, H. B., and Hill, E. L. 1949 Histoplasmosis in rats and skunks in Georgia. Publ. Hlth. Repts., 64, 1423-1430.

97. Grayston, J. T., Loosli, C. G., and Alexander, E. R. 1951 The isolation of Histoplasma capsulatum from soil in an unused silo. Science, 114, 323-324.

98. Grayston, J. T., Lester, Wm., Jr., Tanzi, F., Combs, L. W. and Alexander, E. R. 1952 Some epidemiological and clinical aspects of pulmonary histoplasmosis in a farm family. Trans. Assoc. Am. Phys., 65, 159-167.

99. Loosli, C. G., Grayston, J. T., Alexander, E. R., and Tanzi, F. 1952 Epidemiological studies of pulmonary histoplasmosis in a farm family. Am. J. Hyg., 55, 392-401.

100. Moore, M. 1934 Posadasia Pyriformis, and P. capsulata, two causative organisms of Darling's histoplasmosis, in the United States. Ann. Mo. Bot. Gardens, 21, 347-348.

101. Moore, M. 1935 Morphological and physiological study of two species of Posadasia: P. capsulata (Darling) Moore and P. pyriformis Moore. ibid., 22, 353-360.

102. Howell, A., Jr. 1940 Studies on Histoplasma capsulatum and similar form-species. II. Effect of temperature. Mycologia, 32, 671-680.

103. Howell, A., Jr. 1941 Studies on Histoplasma capsulatum and similar form-species. III. Effect of hydrogen-ion concentration. ibid., 33, 103-117.

104. Dodd, K., and Tompkins, E. H. 1934 A case of histoplasmosis of Darling in an infant. Am. J. Trop. Med., 14, 127-134.

105. Hansmann, G. H., and Schencken, J. R. 1933 A unique infection in man caused by a new yeast-like organism, a pathogenic member of the genus Sepedonium. Am. J. Path., 10, 731-738.

106. Howell, A., Jr., and Kipkie, G. F. 1950 Studies on experimental histoplasmosis. IV. A comparison of the virulence of five strains of Histoplasma capsulatum by intracerebral inoculation of male dba line 1 mice. J. Lab. Clin. Med., 36, 547-554.

107. Rowley, D. A., and Huber, M. 1955 Pathogenesis of experimental histoplasmosis in mice. II. Comparison of the intravenous and intraperitoneal routes of infection; comparison of the pathogenicity of four strains of Histoplasma capsulatum. J. Infect. Dis., 97, 27-34.

108. Rowley, D. A., and Pine, L. 1955 Some nutritional factors influencing growth of yeast cells of Histoplasma capsulatum to mycelial colonies. J. Bacteriol., 69, 695-700.

109. Campbell, C. C., and Saslaw, S. 1951 Failure of streptomycin to enhance the infectivity of Histoplasma capsulatum in mice. Publ. Hlth. Repts., 66, 16-19.

110. Campbell, C. C., and Saslaw, S. 1949 Enhancement of growth of certain fungi by streptomycin. Proc. Soc. Exptl. Biol. Med., 70, 562-563.

111. Emmons, C. W. 1949 Histoplasmosis in animals. Trans. N. Y. Acad. II, 2, 248-254.

112. Menges, R. W., Furcolow, M. L., Larsh, H. W., and Hinton, A. 1952 Laboratory studies on histoplasmosis.

I. The effect of humidity and temperature on the growth of Histoplasma capsulatum. J. Infect. Dis., 90, 67-70.

113. Scheff, G. 1945 Biochemical and immunological properties of Histoplasma capsulatum, No. 650. Yale J. Biol. Med., 18, 41-54.

114. McLaurin, A., Beamer, P. R., and Tuttle, D. M. 1941 Morphologic, biochemical, and immunologic studies on several strains of Histoplasma capsulatum. Am. J. Path., 17, 751-752.

115. Lewis, G. M., and Hopper, M. S. 1948 An introduction to medical mycology. Third ed., Year Book Publ., Inc., Chicago. pp. 12, 276-277.

116. Robinson, H. M., Gigge, F. H., and Bereston, E. S. 1953 Fluoresence of Microsporum audouini-infected hair. II. Cultural studies. Arch. Derm. Syph., Chicago, 68, 311-313.

117. Tidwell, W. L., Heather, C. D., and Merkle, C. 1955 An autoclave-sterilized medium for the detection of urease activity. J. Bacteriol., 60, 701-702.

118. Beamer, P. R., Smith, E. B., and Barnett, H. L. 1944 Histoplasmosis. Report of case in infant and experimental observations. J. Pediat., 24, 270-280.

119. Carmichael, J. W. 1955 Frozen storage for stock cultures of fungi. Mycologia, 48, 378-381.

120. Ritter, C. 1954 Studies of the viability of Histoplasma capsulatum in tap water. Am. J. Publ. Hlth., 44, 1299-1304.

121. Nielsen, G. E., and Evans, R. E. 1954 A study of the sporulation of Histoplasma capsulatum. J. Bacteriol., 68, 261-264.

122. Iljin, W. S. 1953 Causes of death of plants as a consequence of loss of water: Conservation of life in desiccated tissues. Bull. Torrey Bot. Club, 80, 166-177.

123. Hilsont, P., and Verge, J. 1954 Recherches sur le diagnostic allergique de la lymphangite epizootique du cheval par l'histoplasmine. Ann. Inst. Pasteur, 87, 737-738.

124. Dubois, A., Janssens, P. G., and Brutsaert, P. 1952 Un cas d''histoplasmose africaine. Avec une note mycologique sur Histoplasma duboisii n. sp. par R. Vanbreuseghem. Ann. Soc. Belg. Med. Trop., 32, 569-583.

125. Vanbreuseghem, R. 1953 Histoplasma duboisii and African histoplasmosis. Mycologia, 45, 803-816.