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A STUDY OF STRAINS OF HISTOPLASMA CAPSULATUM

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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 #55 #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.

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