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Incidence of skin sensitivity to histoplasmin in 1,586 University of Nebraska students : with a review of the literature

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INCIDENCE OF SKIN SENSITIVITY TO HISTOPLASMIN IN 1586 UNIVERSITY OF NEBRASKA STUDENTS. (WITH A REVIEW OF THE LITERATURE)

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PREFACE

A study involving the skin testing of some 1800 persons must require the services of many people. The author is deeply irdebted to Minnie Schaefer, R.N., for setting up and operating the actual skin testing procedure; Bess Brown, R.N., and the personnel of the University Hospital Central Supply Department, who were invaluable in cleaning and sterilizing our equipment at night between test days; to the nurses of the Student Health Center on the Lincoln campus who handled the passing out of questionaires and assisted in the skin test procedure; and to Samuel Fuenning,M.D. who coordinated the skin testing with the hurried days of entrance physical examination in Lincoln. My warm thanks are expressed to Harry W. McFadden, M.D. who has patiently advised me through the long months of preparing to skin test, calculating statistics and firally through the numerous drafts of this paper.

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INTRODUCTION

This paper reports the first large survey of skin hypersensitivity to histoplasmin in the State of Nebraska. Several investigators have made nation-wide surveys which included residents of Nebraska. The number of Nebraskans studied has always been small and skin sensitivity of 4-25% positive reactors has been reported. This study was undertaken to further clarify the picture of Histoplasmosis and to attempt to establish another boundary of the area where Histoplasmosis is prevalent.

In a study in Omaha, Nebraska; Murphy, Moragues, Russum, E. Clement and G. Clement have reported positive skin reaction of 3% in children and 10% in adults.(60). Conlin and Hankins have reported a fatal case in Nebraska (16) and the clinical diagnosis of Histoplasmosis has been recorded for six patients at University Hospital, University of Nebraska College of Medicine. Thus, Histoplasmosis is present but the exact prevalence of infection is not clear.

OUR TEST METHOD

Our study panel consisted of a group of students entering the University of Nebraska, Lincoln, Nebraska, during September 1955. Each student received a tuberculin and histoplasmin skin test. A chest X-ray was taken of each person.

The histoplasmin was from lot HKC-5 standardized by the method of Howell (101). The histoplasmin was furnished by M.L. Furcolow. The histoplasmin was injected intradermally in 0.1cc doses on the volar surface of the right arm. The tuberculin was intermediate strength Purified Protein Derivative furnished by E.A. Rogers of the State of Nebraska, Department of Health. The tuberculin was given intradermally in 0.1cc doses on the volar surface of the left arm. The results of the histoplasmin skin testing are reported in this paper. The tuberculin results and the radiographic findings are subjects of separate papers.

A histoplasmin skin test was considered positive when the zone of reaction showed 5mm or more of induration; a doubtful reaction had less than 5mm of induration, and a negative reaction had no induration. The tuberculin skin test was considered positive with 5mm or more of induration, doubtful with less than 5mm of induration, and negative with no induration. All the skin tests were

read in 48 hours. The skin tests were read by the authors of the three papers.

Each skin test was given using a separate tuberculin syringe and separate needle to avoid the possibility of transmitting homologous serum jaundice (102). The syringes and needles for the two antigens were handled separately to avoid any mixing of the antigens and thus obscuring the results. All loading, cleaning and sterilizing of syringes was done by a team of registered nurses.

Our test population was divided into five groups: Residents of Nebraska, Mostly Residents of Nebraska, Non-residents of Nebraska, Foreign students, and Wanderers. A residents was defined as a person who had never lived outside of the State of Nebraska for a period longer than six months. The classification Mostly Nebraska Resident was used for those people who did not fit the definition of Resident, yet had spent the majority of their life in Nebraska. The non-residents were students from other states. The foreign students were from outside the United States. A wanderer was a person who did not classify under the other categories. Generally these persons had lived in the United States but the majority of their life had not been spent in one state.

The definition of a Resident is arbitrary and was suggested by other investigations reported in the liter-

ature. We used this definition after finding that the known migratory habits of people in the United States were evident in the young age group of this test population.

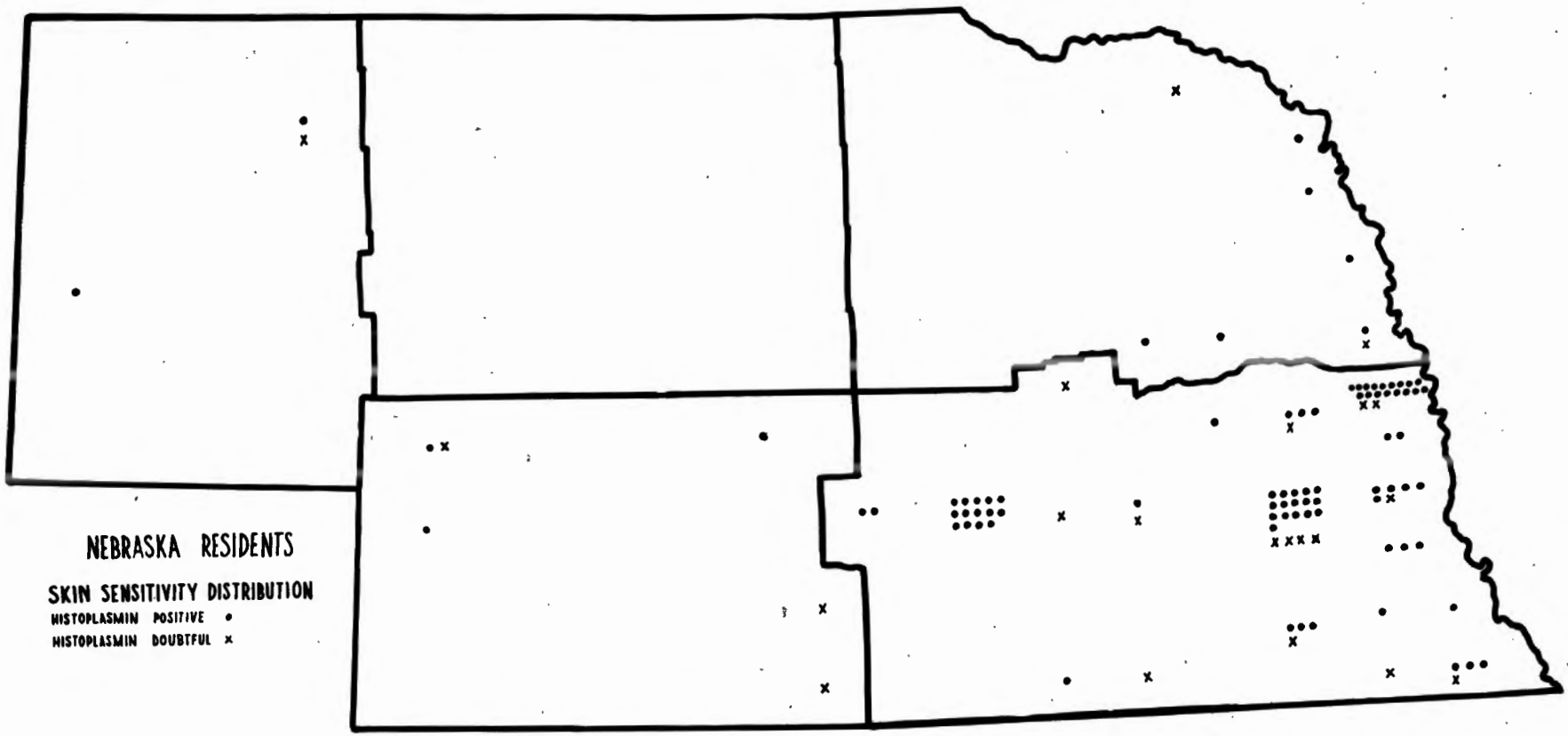
The Nebraska Residents and Mostly Nebraska Residents were subdivided into groups by the county in which they had lived the majority of their lifetime. Some students did not fit into this classification and were placed in a group of Nebraska Residents at Large. For statistical analysis, the doubtful histoplasmin reactors were grouped with the positive reactors (49a).

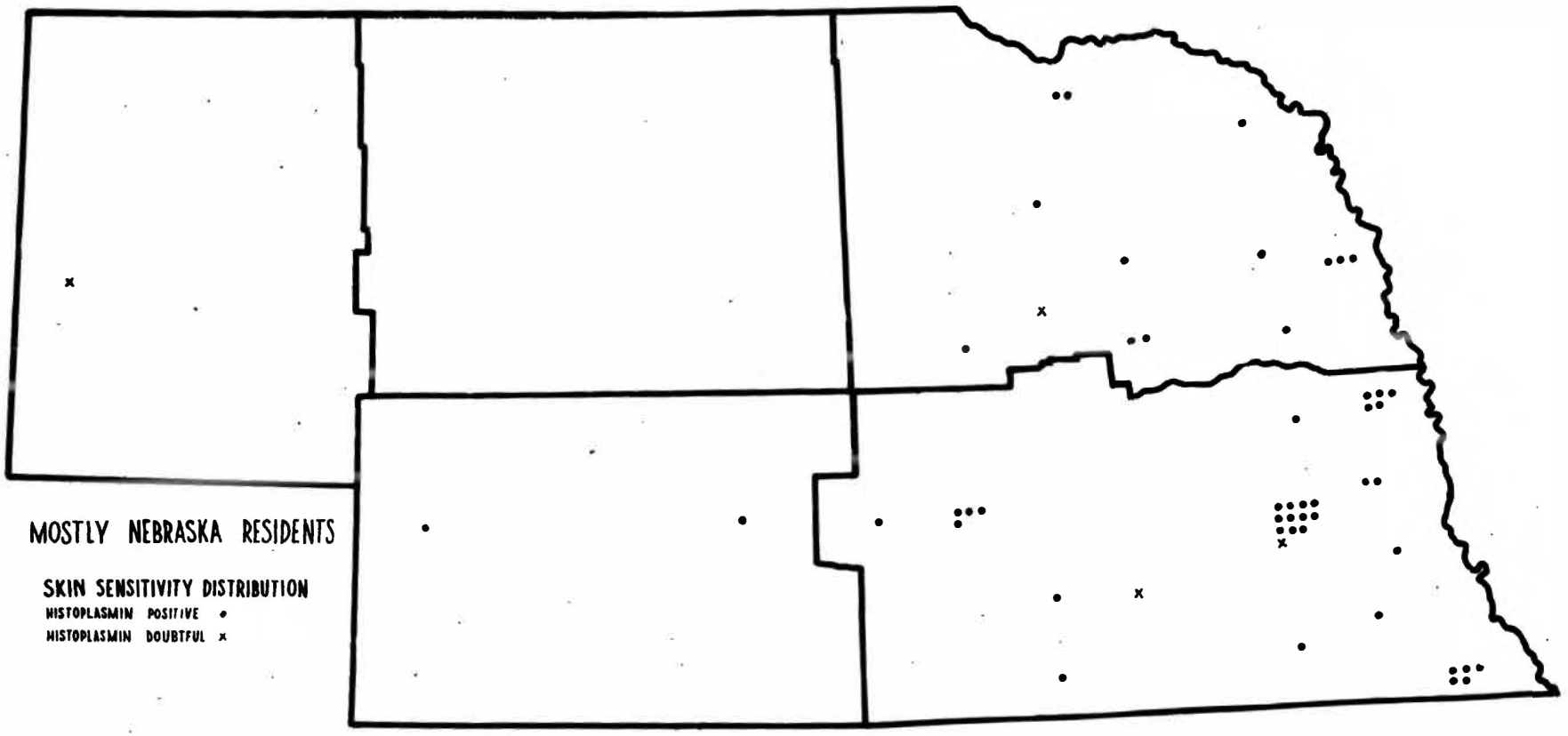
SURVEY RESULTS

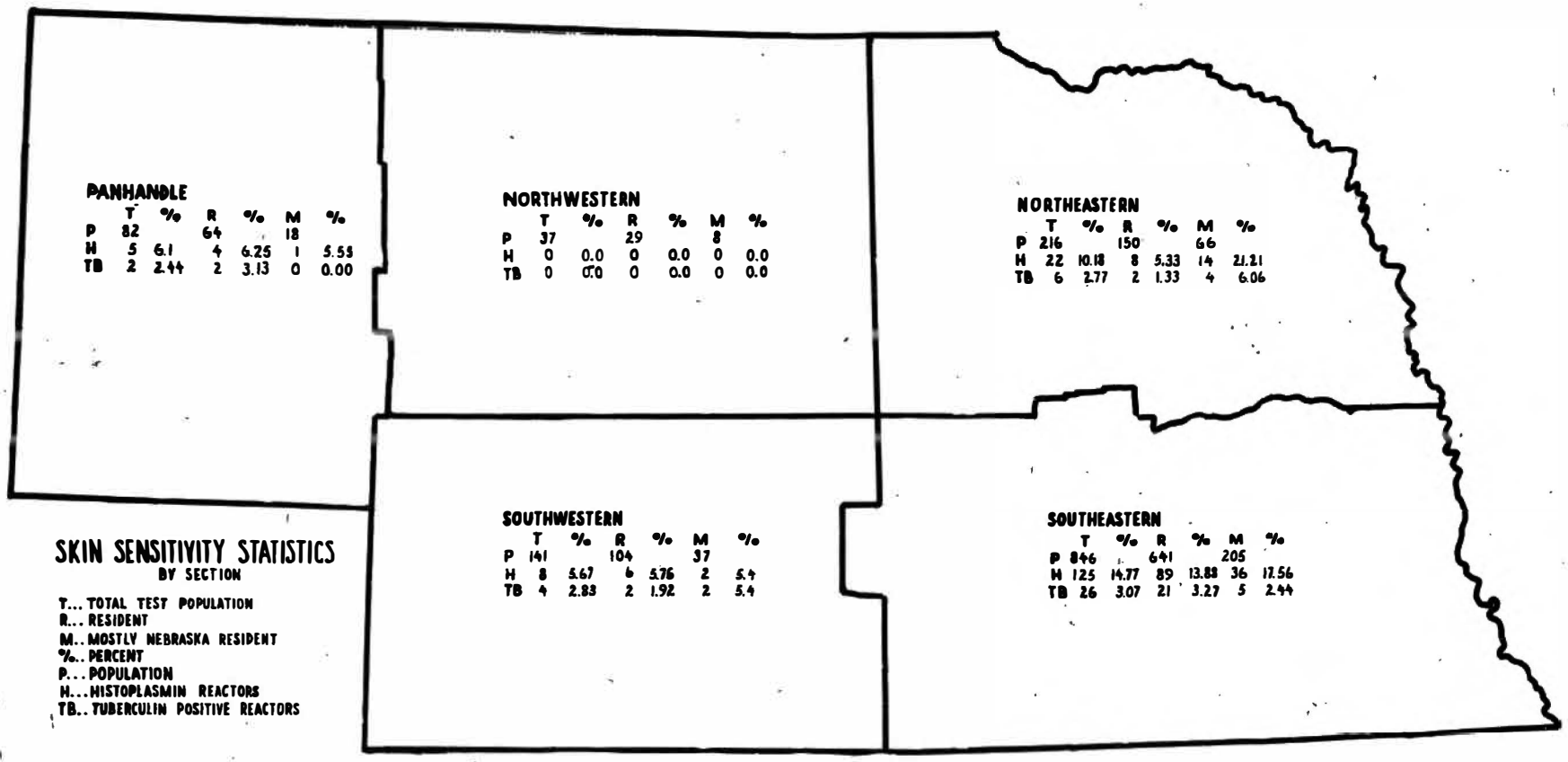
There was a total of 1,586 students in our series who could be divided into groups as follows: Residents of Nebraska- 1000, Mostly Nebraska Residents- 334, Outstate Residents- 181, Foreign students- 20, and Wanderers- 51. Two hundred and eighteen, or 13.74% of the total study group had reactions to histoplasmin. When the positive reactors were divided into groups, 109 or 10.9% of the Residents had skin reactions to histoplasmin. Of the Mostly Nebraska Residents, 53 or 15.87% had reactions. The outstate residents, foreign students and wanderers had 41 (22.65%), 2 (10%), and 13 (25.49%) reactions respectively. (Appendix I and II).

As will be discussed in more detail later, there is a fringe area around the "endemic area" of Histoplasmosis. In an effort to determine whether Nebraska or part of Nebraska lies within this fringe area, the state was divided into five sections and the statistics for each section calculated. (Appendix IVa). Figure 1 shows the geographical distribution of skin reactors for Residents of Nebraska and Figure 2 shows the distribution for the Mostly Nebraska Residents.

When the statistics are calculated by section (Fig. 3 and Appendix IVb) it is seen that the Southeastern section has a total test population of 846 with 125


NEBRASKA RESIDENTS
SKIN SENSITIVITY DISTRIBUTION
HISTOPLASMIN POSITIVE •
HISTOPLASMIN DOUBTFUL ×


MOSTLY NEBRASKA RESIDENTS
SKIN SENSITIVITY DISTRIBUTION
HISTOPLASMIN POSITIVE •
HISTOPLASMIN DOUBTFUL ×



SKIN SENSITIVITY STATISTICS
BY SECTION

T... TOTAL TEST POPULATION
R... RESIDENT
M.. MOSTLY NEBRASKA RESIDENT
%.. PERCENT
P... POPULATION
H... HISTOPLASMIN REACTORS
TB.. TUBERCULIN POSITIVE REACTORS

(14.77%) histoplasmin reactors. Of these, 641 were residents, 89 (13.88%) had histoplasmin reactions. There were 205 Mostly Nebraska Residents, 36 (17.56%) of whom had histoplasmin reactions.

The total test population of the Northeastern section was 216. Twenty-two (10.18%) of these had skin reactions. Of the 150 Residents, 8 (5.33%) had histoplasmin reactions, while 14 (21.21%) of the 66 Mostly Nebraska Residents had skin sensitivity.

One hundred and forty-one students of the test population were from the Southwestern section. Eight (5.67%) of these showed sensitivity to histoplasmin. Six (5.76%) of the 104 Residents had reactions, while 2 (5.4%) of the 37 Mostly Nebraska Residents had histoplasmin reactions.

The total test population of the Northwestern section was 37. Twenty-nine of these were Residents and 8 were Mostly Nebraska Residents. There were no histoplasmin reactions in the students from this area, making the percentage 0.0 in all three categories.

The Panhandle section demonstrated 5 (6.10%) skin reactors for the test population of 82. Of the 64 Residents, 4 (6.25%) had skin sensitivity, while 1 (5.55%) of the 18 Mostly Nebraska Residents had skin reaction to histoplasmin.

When the test population is divided into male and

female groups it is found that there are 683 males and 317 females. among the Residents. Sixty of the males and 25 of the females had histoplasmin skin sensitivity. In the Mostly Nebraska group, there are 265 males and 69 females. Among the men, 39 had skin sensitivity as did 6 of the women. (Appendix V).

Appendix VI shows the age distribution and the skin reactions for both the Residents and Mostly Nebraska Residents. As was expected from the type of test population studied, there was no wide distribution of ages and no statistical evaluation was attempted.

THE HISTORY OF HISTOPLASMOSIS

The history of the disease Histoplasmosis began in 1906 when Samuel T. Darling described what he believed to be a protozoan parasite. The organism had been found at autopsy and was described: "There is seen to be a general infection by a parasite having a prediliction for the endothelial and epithelial cells"(22a). Darling proposed the name Histoplasma capsulatum for this organism. The discoverer wrote further in 1908 and 1909 (22b,c) stating that clinically splenomegaly was the noticeable sign of the disease and noted that at autopsy the lungs had findings similar to miliary tuberculosis, with the exception that granulomas were not as numerous.

Darling's Histoplasmosis was first diagnosed in the United States by Riley and Watson in 1926 (86a,b,118). This case showed the characteristic pathological at autopsy noted by Darling. In 1945, Parsons and Zarafonetis (70) collected from the literature a series of 71 cases. Soon afterwards Iams (41a) assembled 81, and Payne and Furcolow (31k) have since reported fatal cases. Until 1945, Histoplasmosis was generally considered a systemic, fatal fungus disease

Interest in Histoplasmosis was increased greatly by C.E. Smith (105b) in 1943 while he was working on coccidioidomycosis. While carrying out skin test surveys with

coccidioidin, the possibility of a cross reaction with some other antigen was suggested. Theorizing that another fungus might be the cause, Smith was reminded that in previous work an antigen from Histoplasma capsulatum had fixed complement in a patient with coccidioidal granuloma. Further investigation showed that the cross-reactions with coccidioidin were found in the endemic area of Histoplasmosis. An antigen from Histoplasma capsulatum was prepared and was found to evoke reactions in persons with non-tuberculous pulmonary calcification.

This observation by Smith offered a solution to the problem of nontuberculous pulmonary calcification which had been widely debated for many years. Numerous articles profferred theories concerning persons with pulmonary calcification who did not react to intradutaneous injection of tuberculin. (17,18,33a,63).

The actual area where pulmonary calcification was noted in tuberculin negative persons was well outlined by Long (52) when reviewing the X-rays of 53,400 inductees. He outlined an area in the states of Tennessee, Kentucky, Arkansas, Illinois, Indiana, and Ohio, and he noted: "In general, the calcification noted in the films of men from this region was considerably larger and more extensive than that in men from other parts of the country. Also, disseminated 'miliary' calcifications, variously

believed to represent healed residuals of post-primary hematogenous dissemination of tuberculosis or perhaps in some case a healed funguos infection of the lungs, seemed relatively more frequent in this area." During this same year Gass in Tennessee was adding further evidence through skin testing against tuberculosis as the cause of much of the pulmonary calcification (30b).

The fungi were not the only etiologic agents investigated as the cause of calcification. Ascaris infection and abnormal calcium metabolism were among the many agents suggested (32b,67).

Christie and Peterson, working in Tennessee, reported the first ante-mortem diagnosis of Histoplasmosis in 1945. They "proved" their diagnosis by skin testing with Histoplasmin. This achievement led them to further studies from which they concluded: "1. That either the response to the skin test was the result of previous infection with Histoplasma capuslatum or to infection with some other fungus having a common or closely related antigen; 2.That infection with this unknown fungus was much more common than would be explained on the basis of any clinically known fungus infection, including the fungi commonly responsible for dermatomycosis; 3. That infection with such and agent must at times be almost symptomless. It follows of course, that Histoplasmosis might exist in benign form,

and that all the people who had positive tests might have had an unrecognized infection. Many of those routinely tested who reacted positively to histoplasmin were cases with pulmonary calcifications and negative tuberculin reactions."(15a,b).

After such findings as these, Palmer et al in 1945 undertook a large study of histoplasmin reactivity in a group of student nurses and found a higher correlation between histoplasmin sensitivity than tuberculin sensitivity, when compared with pulmonary calcification. This led them to the decision that there was probably a subclinical type of Histoplasmosis which caused pulmonary calcification.(69a,d,123a,b). The area which they outlined for high skin sensitivity was in the Mississippi river basin, the same area outlined earlier by Long.

Furcolow (32j) in 1950 demonstrated by culture that Histoplasma capsulatum was the cause of two cases of pulmonary calcification, which he felt helped prove that tuberculosis was not as frequent a cause of pulmonary calcification as histoplasmosis. By 1952, (5b,4), most students of the disease assumed that Histoplasmosis was a common cause of pulmonary calcification. The statistics showing decreasing mortality and morbidity from tuberculosis with continued high incidence of pulmonary calcification added further proof.

THE ORGANISM HISTOPLASMA CAPSULATUM

Darling first described Histoplasma capsulatum : "The microorganism was present in enormous numbers in the tissues, generally intracellular in large mononuclear endothelial cells in the liver, spleen, lymph nodes, submucous nodes in the ileum and colon, and in the hyaline nodes in the lungs. The organism itself is a small, round or oval microorganism, 1 to 4 microns in diameter, possessing a polymorphous chromatin nucleus, basophilic cytoplasm and achromatic spaces all enclosed within an achromatic refractile capsule."(22a). The organs and cells he found affected, we now recognize as belonging to the reticulo-endothelial system. The descriptions found in the most recent articles are almost identical to the original.

da Roche lima (104b) in 1912, after studying Darling's slides, suggested that Histoplasma capsulatum might be a fungus. In 1933, De Monbreum (23) and Hansmann and Schenken (37), working independently, succeeded in identifying the organism as a fungus and described its cultural characteristics.

Silverman (104b) has collected the works of numerous men and gives an excellant description of the mycology. "Histoplasma capsulatum belongs to the Moniliacae of the Fungi Imperfecti and is a biphasic fungus which, in tissues

and on culture media at 37 degrees centigrade, appears as a small yeast. At room temperature a mold develops (mycelial or saprophytic phase.) The colony, after six to ten days on Sabouraud's dextrose agar, is small, white and cottony in appearance. The colony enlarges fairly fast and becomes slowly tan to dark brown in back. The hyphas are generally thin. Septae and occasionally racquet mycelia are observed. The latter are found more often in cultures changing from the yeast phase to mycelial growth than in cultures kept constantly at room temperature. The number but not the diagnostic importance of the tuberculate spores has been over estimated in the past. Since the particle size has been shown to be of importance in penetration into the alveoli of the lung, it is indeed of interest to establish which spore size is prevalent. The fungus is very resistent to physical exposure. It has been kept for four months in dried sterile soil, in water, ice, and is reported as viable after heating at 62 degrees centigrade for ten minutes."

The fungus has been grown on Sabouraud's dextrose agar, blood agar, liquid media, chorioallantoic membrane of the chick embryo, brain-heart infusion blood agar, potatoe and dextrose agar, bark and soil. It has been demonstrated that biotin and sulfide or sulfhydryl groups are necessary for the growth of the yeast phase. (32m, 35b, 104b).

Loosli reports the best success with enriched blood agar with penicillin and streptomycin added, and instructs that: "blood, sputum, bronchial secretions, exudates, fluid specimens and bone marrow aspirates are streaked directly on the surface of the previously prepared media. Biopsy material, sputum, and other exudates should first be ground in a small amount of antibiotic broth before the homogenate is streaked on the media"(53d). Menges and Furcolow have reported that a humidity of 100% and temperatures of 20-30 degrees centigrade are needed for growth of the fungus.(57).

Wright, Giemsa and hematoxylin and eosin stains have been used by most investigators. The periodic acid-Schiff method, using either the Hotchkiss-McManus or Bauer stain, gives the most consistently satisfactory results(15e,73,80a,104b).

THE DISEASE HISTOPLASMOSIS

As more and more investigators such as Zwerling and Palmer (123a) published their work, there appeared to be an answer forthcoming to the problem of non-tuberculous pulmonary calicification. The answer seemed to be that a fungus, probably Histoplasma capsulatum, was the causative agent. This hypothesis presupposed a disease spectrum for Histoplasmosis which ranged from a benign, almost symptomless form to the better known fatal systemic form. Bunnell and Furcolow (8a,32k) reinforced this theory by proving that Histoplasmosis existed in a benign or subclinical form and could have an end stage of miliary pulmonary calcification (104a).

"Histoplasmosis is a disease which varies in the degree of parasitization from a single isolated primary lesion, completely asymptomatic, to one which is progressively disseminated by the blood stream and results in an overwhelming generalized parasitization of the host with marked cachexia and death occurring in a matter of weeks." (74). At present this disease spectrum divides itself into three general classifications. Most authors in their investigations have noted various subtypes which fit well in the more general classes.

The spectrum of Histoplasmosis is 1. Non-fatal, asymptomatic infection; 2. Non-fatal, symptomatic infect-

ion, and 3. Fatal, symptomatic infection. Within these classes are all the various gradations of infection.

The non-fatal, asymptomatic infection is diagnosed only by the finding of pulmonary calcification in conjunction with positive skin reaction to histoplasmin. Many persons who have converted from negative to positive skin reactions give a history of no illness. It is now felt that these people have probably suffered from an illness similar to a "cold which hangs on" which in reality was an extremely benign histoplasmosis infection.

The non-fatal, symptomatic infection is also commonly called the benign type and is often divided into an acute and chronic phase, the latter usually being the long period of recovery. This infection causes symptoms very similar to the condition that is known as atypical pneumonia or is sometimes like influenza. The people affected by proven epidemics and while working in laboratories have these symptoms: "Sudden onset, generalized malaise, followed in12-36 hours by chills, fever, and prostration. Chest complaints are characteristically mild, with mild discomfort and non-productive cough. Pronounced weakness was uniformly present, and occasionally dyspnea. Fever and acute illness lasted a few days to three weeks, weakness lasted up to months" (32t).

Ordinarily patients recover from these first two

types, but a few progress to the fatal symptomatic infection.

Peterson and Christie (74) list these presenting symptoms in their relative order of frequency for the fatal infection:

"1. Fever
2. Abdominal enlargement
   a. hepatomegaly
   b. splenomegaly
3. Cough
4. Weight loss
5. Diarrhea
6. Pallor
7. Vomiting
8. Dyspnea
9. Purpura
10. Oral ulcers"

The fever commonly shows no characteristic pattern. Anemia and leukopenia are often present and the disease often manifests itself as ulceration of the skin or mucous membrane, especially in the oropharynx, larynx, small intestine or colon. Necrosis of the adrenals may be noted and lesions of the bone have been reported.

This fatal part of the spectrum probably progresses from an illness similar to atypical pneumonitis. The lesions in the lungs may develop necrosis and even cavitation and later disseminate. The possibility of other primary sources of dissemination has not been investigated.

The fatal form may be acute, similar to a fulminating septicemia, or chronic, as commonly seen with tuberculosis. In childhood it may simulate leukemia or it may

be interpreted as ulcerative colitis. The fatal type is seen more often in children under two and in persons over fifty. (5b,15e,32t,50,74,104b).

"The respiratory system is probably the portal of entry in the majority of cases. Cough is not uncommon. It is generally non-productive unless there is associated cavitation. It may be associated with chest pain. Hemoptysis is rare. Some patients have demonstrated dyspnea. Night sweats have been present in occasional cases. In adults granulomatous lesions of the upper respiratory passages have occurred with considerable frequency (in fatal cases). Nasal and laryngeal ulcerations of a chronic nature are repeatedly described."(104b).

Furcolow, Mantz and Lewis (32d) give the following characteristics of persistent pulmonary infiltrations in persons positive to histoplasmin and negative to tuberculin: "Only a few were limited to the lymph nodes, a few were of the disseminated type and approximately two-thirds were nodular, sharply circumscribed foci. The remaining one-fourth were diffuse patchy infiltrations which with poorly defined borders sometimes developed into nodular lesions."

"Disseminated infiltrates may be uniform, millet-seed in size and may range from a few millimeters in diameter to a large conglomerate patchy area. In some of the

infiltrates a central core of calcification may be seen." (32h,t,120).

Fine widespread reticulations have been observed in the lungs, as has gross cavitation. The absence of pathologic pulmonary shadows does not exclude pulmonary involvement. The limitations of size of lesions, shape, sharpness of margins, location and total number which apply to any disease militate against roentgen recognition of disease in Histoplasmosis as well. (69c,104b,123a).

"The clinical importance of such lesions lie in the fact that they must be differentiated from atypical pneumonia and tuberculosis which they mimic radiologically in many particulars. The larger infiltrative lesions can be differentiated from atypical pneumonia only by their persistence over long periods of time and by the proper interpretation of histoplasmin and tuberculin reaction as well as complement-fixation and cold agglutination tests"(15e).

As we have already mentioned, pulmonary calcification first led investigators to the further study of Histoplasmosis. In a person with a healed primary complex it is impossible to differentiate between histoplasmosis and tuberculosis as the cause.(123a) However, disseminated bilateral calcification is more frequently associated with a positive reaction to histoplasmin than with one to tuberculin (123b). It has also been reported that the

mulberry primary focus type of calcification is more commonly associated with a positive histoplasmin skin test. (104a). Furcolow and High (32b) have set up the following classification when evaluating calcifications: "1. Miliary calcification- calcification is small, round, uniform in size, numerous and widely and symetrically scattered throughout each lung field. 2. Multiple bilateral calcification- calcareous deposits fewer in number, often irregular in outline, of varying size, often distributed in an asymmetric pattern. The two above groups to be divided into subgroups by presence or absence of nodal involvement."

Another interesting phase of the pulmonary infection has been the discovery that solitary lung nodules may contain Histoplasma capsulatum. In the past, most of these non-neoplastic nodules were considered to be tuberculomas. Now it appears that a majority may be granulomas of Histoplasmosis (34,46,56,104b).

Puckett (80b) has recently published a series of 67 cases which had been referred with diagnoses such as carcinoma, tuberculosis, coccidioidomycosis and "asymptomatic X-ray findings". These cases represent what Puckett feels is the intermediate stage of Histoplasmosis. All the patients underwent resection of pulmonary tissue. No Histoplasma capsulatum could be cultured preoperatively or postoperatively but the organism was identified in all

with periodic acid-Schiff stain. The type and location of the lesions, which were usually fibrocavitary and often apical in position, suggested to the author the possibility that this pathology might be a reinfection rather than a primary one. He said: "The majority (of lesions) were located in the immediate subpleural region and approximately one-half were associated with a round or stellate pleural placque that was fused to the more peripheral portion of the lesion. In no instance were there adhesions between parietal pleura over parenchymal lesions containing Histoplasma capsulatum."

As has been noted earlier, the reticulo-endothelial system is the actual site of infection by Histoplasma capsulatum. Various degrees of involvement have been recorded in the literature, most of the cases reported being of the fatal type. It would appear safe to assume, however, that there is some degree of involvement systemically in the reticulo-endothelial system even in the asymptomatic, benign infection. The clinical picture has been described as one with emaciation, pyrexia, anemia, leukopenia and splenomegaly (93). Being very closely connected with the reticulo-endothelial system, the liver and spleen are very frequently involved, especially in the disseminated type of infection. The sinusoids of the liver have been reported at times to be so packed with engorged Kupffer

cells as to raise the question of the proper operation of these cells. At other times this same situation seems to have caused central necrosis such as is found in tuberculomas. These findings have suggested the use of liver biopsy in diagnosis.

Splenomegaly is more common in children and is caused by hyperplasia of histiocytes which may be distended by numerous organisms. Atrophy of the splenic follicles and calcifications have been reported (97). Another organ found affected quite frequently is the adrenal gland (104b).

Gastrointestinal lesions of both ulcerating and granulomatous type have been demonstrated. The lesions have been identified in the oropharyngeal and perianal regions as well as in the intestine. The symptoms have varied widely with location of the lesion. (10,14b,59, 104b,106,119). The work done on the possibility of Histoplasma capsulatum involving the appendix and thereby causing symptoms which may lead to the diagnosis of appendicitis has been discussed both pro and con and at present seems to remain undecided (28d,81, 87).

The hematologic work reported has been on the fatal systemic type so little is known about the reaction of the blood in the asymptomatic type. The findings are not constant but generally show an anemia and leukopenia (104b). There have also been reports of Histoplasmosis causing

calcification of the pericardium (6) and vegetative endocarditis (8). Lesions have been found in bone (15c) and the bone marrow is affected in a manner similar to that of the liver (104b). Macular chorioretinitis has been diagnosed following the intradermal injection of histoplasmin (47). Genital histoplasmosis is similar to that affecting other areas of the skin (21).

Involvement of the central nervous system has been observed. Here it gives a picture similar to that of tuberculosis meningitis. The process is most pronounced at the base of the brain and the granulomatous reaction is similar microspocially. This infection may result in pleocytosis and reduction of sugar content of the cerebrospinal fluid (94,100,109).

Perhaps the most interesting and important clinical feature of Histoplasmosis is its similarity to other diseases. The almost identical symptomatology and similar pattern of pathogenesis with tuberculosis was the main reason for the first interest in the disease and remains a strong reason for continuing the study of this fungus. Its similarity to coccidioidomycosis originally led Smith to work on a skin antigen for testing for Histoplasmosis and to the suggestion of its importance.(4,93b) Because it affects the same system in the body it has been confused with sarcoidosis, lymphoblastoma and Hodgkins dis-

ease. (13,29,62,76,85). The symptoms may suggest a neoplasm, or one of the viral diseases such as ornithosis, and being a fungus causes symptoms similar to coccidioidomycosis, blastomycosis, torulosis (58), and possibly actinomycosis and nocardiosis. Because the reticulo-endothelial system is relatively poorly understood, one is led to the speculation that there is more than a similarity of system involved by these diseases. However, little study has been made along the line that there may be a closer relationship.

Man is not alone in being afflicted by this fungus. It has also been found in rats, cattle, dogs, horses, cats, skunks, foxes, opossums, bears and racoons (28b, 32g,i,p,y,104b).

The incubation period of Histoplasmosis is probably five to fifteen days (104b) but the specific means of transmission of the disease has not been demonstrated (101,71). Recently several authors have pointed out the strong probability that the infection is air borne and that infection is by means of inhalation (28c,32s,42b). Several other epidemiological points such as the possibility of a carrier state, reinfection, and person to person infection have been suggested but have been neither proved nor disproved. (5b,104a).

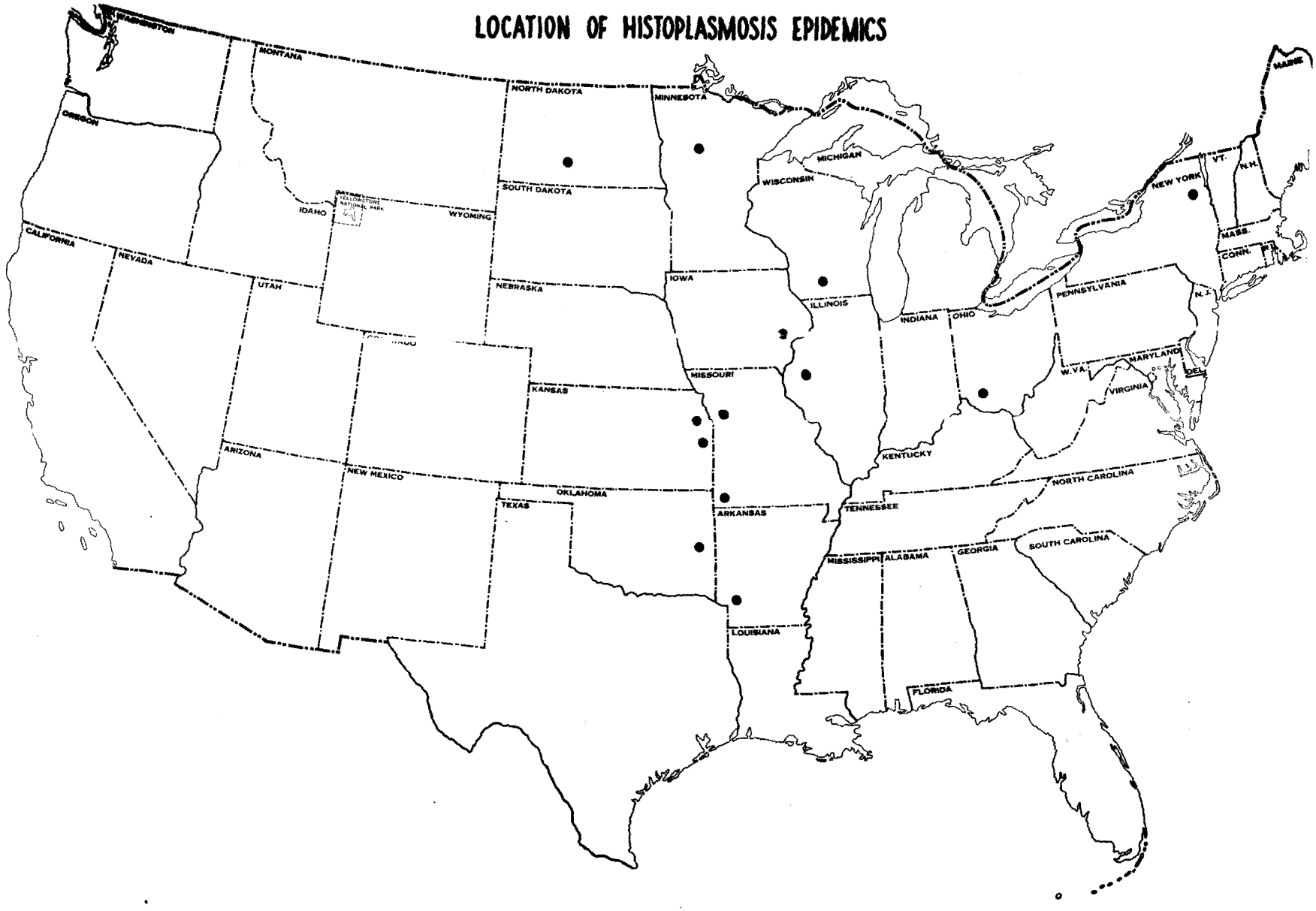
The organism itself has frequently been isolated

from soil (28c,32n,r,53b,c), and a theory of location of the organism by types of soil has been advanced (121c,d). Histoplasma capsulatum has also been found in areas inhabited by chickens and pigeons (32y,84,121b). Recently, it has been isolated from the air in two places where epidemics have been reported (104b).

Laboratory experiments have shown that a humidity of 100% is required for growth and that temperatures of 20-30 degrees centigrade are necessary (32m,121a) The laboratory has also been used to illustrate that some of the procedures used in studying Histoplasmosis, such as floatation techniques, are not exacting enough (61).

Furcolow et al (1953) have proved thirteen epidemics (Figure 4) by means of the following criteria: "1. Isolation of HIstoplasma capsulatum from the soil at the point source of the epidemic. 2. The presence of positive serological tests for Histoplasmosis. 3. The presence of positive histoplasmin skin tests. 4. The development of miliary calcification. At least three of these requirements to be met by each epidemic."(11,30,32q,t,x,42,43,53c,89, 96,117,121c). Recently Silverman has reported a fourteenth epidemic (104b).

LOCATION OF HISTOPLASMOSIS EPIDEMICS



THE METHODS OF STUDY AND DIAGNOSIS

The clinical and laboratory diagnosis of Histoplasmosis is a difficult matter. The more difficult because it so closely mimics other diseases. There are many laboratory aids available for assistance in diagnosis. Saslay and Prior (91h) have summed them up: "1. A high index of clinical suspicion in the endemic area. 2. Careful clinical evaluation of the patient with awareness of the protean manifestations of the disease. 3. Positive skin test may serve to include the disease in the differential diagnosis. 4. Complement fixation and collodion agglutination tests are usually of value in the early phase of the disease. 5. Repeated cultures of blood, bone marrow, ground biopsy material and bronchial aspirates often yield positive results. 6. Biopsies of the lymph nodes, cutaneous ulcers, lung, liver or spleen frequently assist in diagnosis". As Silverman has pointed out, all the evidence is circumstantial except that of culture but the evidence is still good (104b).

Skin testing has been the basis for many studies which have served to increase our knowledge of Histoplasmosis, especially the epidemiology, and to a limited extent the diagnosis of the disease. The first investigators developed their skin test antigens from the mycelial phase of the fungus (15a,28a,90c,92,105b,113). Since that

time, work has been done in an attempt to develop an antigen from the yeast phase because it is this phase which is found in the tissues of man. However, this work has not developed a better antigen to date (12b,15a,26,75,91f). The name histoplasmin was first used by Christie in 1945 (15a) and has been universally accepted. The first histoplasmins were made by the men using them and no standardization was done. In 1948, Howell (101) developed a method by which he standardizes all histoplasmins to a 1:1000 dilution of histoplasmin lot H-15. This work is important because good comparisons can not be made unless the histoplasmin used in collecting data has been standardized.

When histoplasmin first came into wide use there was some question as to the specificity. The investigators who have done the most extensive work on Histoplasmosis have pointed out that they feel the antigen is specific by analogy (5b,15d,69a,b,d,123a) and have done experiment-ation to show specificity to be good (32c). Smith (105b) has shown that sensitivity lasts for many years. There is, as with other antigens, a group of persons who give doubt-ful reactions to histoplasmin. Loosli, after exhaustive statistical work, has shown that these reactors should be included with the positive reactors when compiling data, while doubtful reactors to tuberculin should be classed

as negative reactors. Others have found that there is some reversion to negative from the group of positive reactors (121a). Most authors such as Iams (41b) feel that the specificity is good because of the good correlation with pulmonary calcification. The skin hypersensitivity is suppressed by critical illness as are the reactions of other skin test antigens (14a,15d,32f). Cross and Howell have isolated an immunologically active polysaccharide from histoplasmin (20).

The specificity of histoplasmin has also been questioned because of cross reactions with other fungal antigens. It was the cross reaction with coccidioidin which st called attention to Histoplasmosis (105a). Blastomycosis, coccidioidomycosis and infection with haplosporangium have all been noted as showing a cross reaction. Further investigation has indicated that a high concentration of histoplasmin must be used to obtain a cross reaction with Blastomycosis. If a low concentration of the antigen is used the antigen is specific for the fungus from which it is derived (9a,26,39a,93). Smith has shown that a concentration of coccidioidin ten times that routinely used for testing is necessary for a cross reaction with Histoplasmosis.

Christie wrote in 1951 (15d): "There are those who believe there is some difference between histoplasmin

sensitivity and histoplasmosis. We want at the onset to state that we believe histoplasmin sensitivity to be merely an allergic reaction induced by infection with Histo-plasma capsulatum. The condition is, as in tuberculosis and tuberculin sensitivity, no reflection of the state or activity of infection with Histoplasma capsulatum, although conversion is an important clinical fact undoubtedly signifying recent infection. The sensitivity appears in a few weeks after infection begins and may persist for many years after the infection has apparently subsided. It is true that the antigenic complexes which have been available for use as histoplasmin do show some cross reaction with other infections, principally other mycosis, but we do not believe that this fact is of any real importance in the interpretation of histoplasmin sensitivity.

Numerous methods of using histoplasmin in skin testing have been reported (4,5a,c,7,15a,b,19,32a,l,p,v,u,z, 33c,36,44,54,61b,69a,b,c,d,77,78a,93,99,107,112,121a). Now that most of the histoplasmin used in skin testings has been standardized by the method of Howell (101) the use of the antigen has gained much significance. One-tenth cubic centimeter of the standardized histoplasmin is injected intradermally in the volar surface of the forearm. The test is read in 48 hours and a positive reaction is considered to be one with 5mm or more of induration.

Erythema is usually recorded but is not considered in the actual reading. A doubtful reaction has less than 5mm of induration and a negative reaction has no induration. The doubtful reactors are of importance only in large surveys for statistical information. A doubtful reaction in a clinically suspect patient would require further study by other means.

Roentgenographic examination of the chest has an extremely important role in the study of Histoplasmosis. By means of the film showing pulmonary calcification it has been possible to correlate skin tests with a known result of disease. For many years calcification was considered to be caused only by tuberculosis, however large studies have shown that identical types of pulmonary findings may be caused by the fungi. As has been noted already, the best correlation of pulmonary calcification has been found among histoplasmin reactors in an area often designated as the Mississippi river basin. From the clinical aspect the X-ray may be the first laboratory aid used. The findings, being either calcification or atypical pneumonitis, may ther call for further investigations.

Serologic studies are used in many instances as aids in diagnosis. In 1948 complement fixation tests were proved successful in guinea pigs with Histoplasmosis(110). Soon afterward, the same tests were also proven with human sera

These first tests were performed using histoplasmin as antigen. Because the yeast phase was the form found in humans is was postulated that a yeast phase antigen would be useful in the complement fixation test instead of the mycelial phase used to develop histoplasmin. This hypothesis was proved successfully and was found to work on human sera (32e,90a,91a,c,104a). The investigators working on the complement fixation test have found that repeated skin tests will increase the titer in the sera (12c). It is felt that the complement fixation test is useful in determining acute histoplasmosis and sometimes chronic histoplasmosis. The titers remaining over a period of years (5c,12d,32x,90). There is a cross reaction with the other fungi in the complement fixation test, notably blastomycosis. However, it has been shown that the titers remain highest for the homologous infection (110,9a,12a,d, 90b).

"For practical purposes we believe that the presence of a positive complement fixation test at levels considered critical by the specific laboratory where the test is performed warrants further clinical and cultural investigation. The suspicion of active disease will certainly arise if the titer of the serologic reaction should be rising on serial tests. However, the importance of performing the tests on sera obtained at different periods of ill-

ness, if at all possible on one day, using identical conditions and antigen, cannot be overemphasized."(104b)

There has also been a precipitin test developed for Histoplasmosis. This test has shown that it is reliable in both animal and human sera. The men who have developed the test feel that it is best for the diagnosis of acute Histoplasmosis while the complement fixation test is best for the more chronic infection. The titers for the precip- itin test develop earlier in the disease (32w,71,90b,91a). Norden has reported an agglutination test with sheep red blood cells and Saslaw and Campbell feel that their col- lodion agglutination test may be useful and is more stable than complement fixation tests. As in the other serolog- ical tests there is a cross reaction with the other fungi (12e,65,78c,91b,d,e,g,104a). Silverman (104b) feels that serology has not been used on enough verified cases as yet. He points out the variance in techniques and states that serology is reliable only within the limits of the individual laboratory using the test and their success with it. He also brings up the question of the actual meaning of a rising or a falling titer.

Christie (15e) has said: "In the individual case the only way of establishing a diagnosis of active histoplasmosis is either the isolation of the fungus from the lesions, the observation of acquisition of histoplasmin sen-

sitivity, the conversion evaluated in relationship to the lesion, or by the observation of rising titers of humoral antibody." Most authors feel that the only means of a positive diagnosis of Histoplasmosis is by the culture of Histoplasma capsulatum. They consider the other methods as aids but not positive proof. The different techniques of culture have been mentioned earlier. Histo-plasma capsulatum has been cultured directly from lesions as well as from gastric contents and from the blood (49).

Many other means of study of Histoplasmosis have been tried. Randall has achieved culture of the organism in tissue (82a,b). Schwartz and Barsky had little success with bone marrow aspiration for the fungus (95), but Rohn and Bond (88) were convinced that it was a useful test in pediatric age patients. Tissue biopsy has often been successful both for indentifying the organism in stained sections and for culture. The biopsy site has most often been a cutaneous lesion or a lymph node (14a,29,45,100, 111). Alexander and Baker (2) were able to diagnose a case of Histoplasmosis by biopsy of the liver and spleen. Vollmer (115) attempted to develop a patch test for Histoplasmosis similar to that for Tuberculosis, but failed.

TREATMENT

At the present time there is no known specific treat-ment for Histoplasmosis. Sulfonamides became available at approximately the time this fungus was found to be other than a rare disease; they have proved of no benefit. The same is true for penicillin and the broad spectrum antibiotics (35,51,98a,b). Neoarsphenamine, Stilbamidine, 2-hydroxy stilbamidine and ethyl vanillate have shown some promise, especially *in vitro*, but have not proven themselves clinically (27,51,64, 98b,c,122). The injection of cortisone in experimentally infected animals served only to give a wider dissemination of the disease (114). Fungicidin obtained from *Streptomyces aureus*, which is also known as nystatin, has given good results in exper-imentally infected animals (11e,83) but its use has not been reported in humans. Surgical removal of infected tissue, particularly lung tissue, has been successful (80b).

THE GEOGRAPHIC DISTRIBUTION OF SKIN SENSITIVITY

As has been noted previously, the original interest in Histoplasmosis was created when it was found that the area of highest skin sensitivity to histoplasmin correlated with the area of highest pulmonary calcification. It had long been known that many of the persons with pulmonary findings were tuberculin negative. Christie (15a) first noted the high correlation in Tennessee in a small study group. He found that a group including histoplasmin positive and histoplasmin doubtful reactors correlated best. Goddard, Edwards and Palmer (69d) in analyzing their study of student nurses found: "It should be noted that, although the calcification rates do not approach the magnitude of the rates of histoplasmin reactors, the relative decrease in the percentage of student nurses with pulmonary calcification in the arrayed cities, is of comparable magnitude to the relative decrease in the rate of histoplasmin reactors. The difference in frequency of calcification in nurses who react to one antigen but not to the other is striking. Pulmonary calcification in histoplasmin reactors is over three times as frequent as is is in tuberculin reactors- 33.2 percent as compared with 10.7 percent. Among the total group of over 16,000 nurses studied, 1,550 were found to have shadows interpreted as definite and/or probable pulmonary calcification on their chest films.

Among the 1,550 nurses, reactors to histoplasmin alone account for 1,052 nurses; reactors to both antigens for 219; reactors to tuberculin alone for 203; and nonreactors to both antigens for 76. In percentage terms, the frequencies are 68, 14,13 and 5 respectively. Considering the total frequency of reactors to each antigen, 82 percent of the nurses having calcification reacted to histoplasmin and 27 percent to tuberculin." Everyone who has studied the skin reactions and pulmonary findings closely have found that the correlation of pulmonary calcification is better with histoplasmin sensitivity. (3,5a,b,24,40,32j, 36,78b,107,69d).

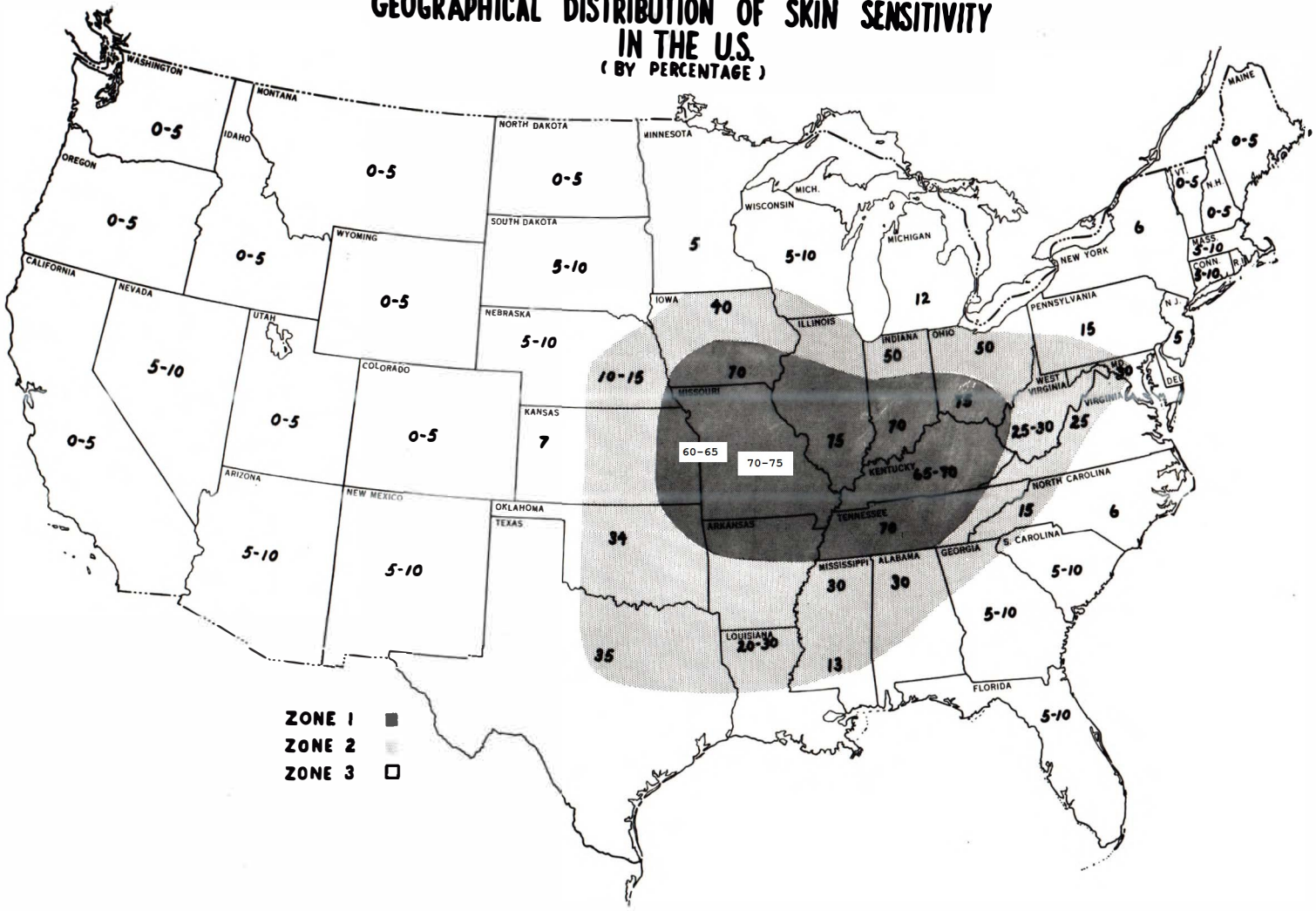
Some of the most important work done on Histoplasmosis has been the national surveys to attempt to define the exact geographical distribution of histoplasmin sensitivity. As Palmer said (69b): "The primary point which this paper seeks to establish is not the actual level of sensitivity to histoplasmin in different areas in the United States but rather the relative levels of sensitivity in different localities." The rates of skin sensitivity found have varied from 0 to 84% within the United States. The area with the highest sensitivity is generally conceded to include Tennessee, Kentucky, Missouri, and parts of Illinois, Indiana, Ohio, Iowa and probably Arkansas. The inclusion of this large an area in the so-called

"endemic area" naturally assumes there will be a fairly wide deviation from local area to local area. The various data published concerning these states shows a variance of as much as 25% from investigator to investigator (5a, 69b,105b,121c).

Numerous authors have attempted to section the country into several zones of skin reactivity. We would propose that there are actually only three large areas (Figure 5). The first area includes the states noted above. Here reactivity has generally been reported as higher than 70% and includes areas with as high as 84% positive skin tests. The second area would include a distribution varying in width, around the perimeter of the first area. The percentages in this zone would run from 10-50%. This area has not been well maped out but a few pilot studies when assembled with our own survey would seem to indicate the presence of such a zone (4,5c,9b,32l,p,38,61a,b,66, 68,72,74,78a,91h,103). The third area would include the rest of the United States where the reports of skin test positive persons run from 0-10%.

The numerous reports in the literature on the geographic distribution prove one important fact. Even in the so-called "non-endemic" zone, there is a skin reactivity rate. Thus there is always necessity to be aware of the disease and its manifestations, especially its ability

GEOGRAPHICAL DISTRIBUTION OF SKIN SENSITIVITY IN THE U.S.
(BY PERCENTAGE)



to cause radiographic findings similar to tuberculosis (1,8,15b,d,19,25,31,32u,z,44,48,54,69d,77,79,99,108,112, 116,121a).

Histoplasmosis has also been reported in Canada, Africa, South and Central America, Europe and possibly in Asia and Australia (35,104b).

In interpreting the geographic data collected by various authors there seems to be little agreement on how long a period of time serves to classify a person as a lifetime resident of a county or state. The definition has varied from fifty percent of the time to five-sixths. One author uses the definition to mean a person who has never been absent from his home area for a period of longer than six months. All of these definitions are relative because, to date, no one knows how long a period of time is necessary for a person to become a reactor. Some reports would indicate that the inhalation of the infecting organism for a period of minutes is sufficient (3,5a,24, 32a,z,53a,68,69b,78a).

Concerning the distribution of skin sensitivity with sex, all investigators agree with Beadenkopf and Loosli (5a): "A slight but consistently higher prevalence of sensitivity to (tuberculin, coccidioidin, histoplasmin) was found among males in all age groups."(5am15a,32z,53a) Most authors have had results similar to those of Christie

and Peterson (15a): "The reactions to histoplasmin were encountered in the youngest age group to the extent that 41.7% of those under 5 years gave positive reactions. The percent positive increased to a maximum of 92.6% in the children 15-19 years of age." (5a,15a,b,24,32a,1,53a,54, 121a). Also race and urban-rural dwelling statistics have all been similar to those of Beadenkopf and Loosli (5b): "The racial similarity of the histoplasmin rates is signicantly unlike the great racial difference in tuberculin sensitivity rates and suggests that economic factors are not so important in the acquisition of a H. capsulatum infection. Also, unlike tuberculosis, the histoplasmosis infection rate for urban residents is not higher than that of rural dwellers. Indeed an indication of higher rural has been reported."(5b,32a,1,78a).

"An analysis of published data on the prevalence of histoplasmin sensitivity by age for children in Kansas, Missouri and middle Tennessee supports the hypothesis that a constant conversion rate, unvarying with age and time, operates among those negative to histoplasmin up to about 18 years of age (the oldest age in study). This conversion rate does not appear to begin operating at the same age for all groups of children (Which the authors can not explain.). It is pointed out that the existence of subgroups within a population with widely different conversion rates

can result in a composite conversion rate that decreases with age even if the conversion rate for each subgroup is constant." (9b,32o,v,55).

Zeidberg (121c) in 1954 offered a theory on the geographic distribution of Histoplasmosis: "Simply stated, the proffered theory is this: That red-yellow podzolic* soils, because of their characteristics, provide the best soil environment for Histoplasma capsulatum. Red-yellow podzolic soils are distributed in certain well defined geographic areas in many parts of the world. These soils are characteristically acid and relatively shallow. They are found in warm-temperate regions where humidity is high and the average precipitation is between 40-80 inches. In the United States, red-yellow podzolic soils are distributed over an area that corresponds quite closely with the region in which the highest prevalence of histoplasmin sensitivity has been observed. The correlation is by no means perfect."

-* Defined in appendix VII

DISCUSSION

In the past ten years Histoplasmosis has become a much better understood disease entity. An "endemic area" has been fairly well mapped out. Surrounding this area another area exists where Histoplasmosis is not as common a cause of pulmonary calcification but is present to a large enough extent to be clinically important. This fringe area has been well mapped out in some states.

We have undertaken this study in an effort to evaluate to what extent Histoplasmosis is present in Nebraska. It was felt that the fringe area probably extended into the southeastern part of the state. Although our test group was not ideal, it was large enough to give some impression as to the prevalence of Histoplasmosis in Nebraska.

Our test population with a total of 13.74% skin sensitivity to histoplasmin falls within the area which we call the "Fringe area". After dividing the state into five sections we found that with a percentage of 14.77% the southeastern section had a much higher incidence of skin sensitivity to histoplasmin than did the Northwestern, Southwestern, Northwestern and Panhandle areas with 10.18%, 6.57%, 0.0% and 6.10% respectively. From these figures we concluded that only the Southeastern section of Nebraska actually lies within the fringe area. When

first looking at these figures, it appears that the Northeastern section of the state may also fall in the fringe area. This is true for the total population, but is due entirely to the incidence of sensitivity in the Mostly Nebraska Residents from that section.

By further dividing our Nebraska test population into Residents and Mostly Nebraska Residents we were able to show that among residents overall skin reactivity was 10.9%. When considered by residence in one of the five sections, we found that the Southwestern section had a percentage of 13.88, while the other areas dropped to: Northeastern 5.33%, Southwestern 5.76%, Northwestern 0.0%, and Panhandle 6.25%. This illustrates much more clearly a higher incidence of skin reactivity to histoplasmin in Southeastern Nebraska.

The evaluation of the Mostly Nebraska Residents by the five sections shows that they too follow a marked difference in distribution with the Southeastern section having 17.56%, Northeastern 21.21%, Southwestern 5.4%, Northwestern 0.0% and the Panhandle 5.55%. This being a much smaller test population, a single skin reaction gave a much greater difference in the statistics for each section. We feel that this might account in part for the results in the Northeastern section. In addition, many of the persons classified as Mostly Nebraska Residents

were young men who had served with the armed forces. It was difficult to determine exactly where their travels had taken them. It had to be assumed that many of them at one time or another were in the endemic area. Although all these factors had an influence all the positive reactions cannot be accounted for in this way. It is possible that a focus of infection is in this area or that statistics are misleading by the small test population.

Statistically our test population does not follow exactly the population of the state. Lancaster county being the site of the University of Nebraska has a large test population. Also the Northwestern and Panhandle sections have small test populations. We do not propose that the actual incidence of Histoplasmosis follows exactly our statistical results. We do feel that Histoplasmosis is present and therefore of clinical importance.

We did not do a complete statistical survey of the Outstate Residents, Foreign students and Wanderers. The Outstate students were mostly from Iowa, Kansas, and South Dakota. They appeared to follow closely the results of much larger studies than ours. Therefore we did not feel that we needed to repeat the work with our small test groups from those states. The Wanderers with a percentage of 25.49% illustrate that one cannot dismiss Histoplasmosis if outside the endemic area. A clinician

serving one of these persons would find it very important to know their skin sensitivity if attempting to diagnose a chest complaint. The Foreign students with positive skin reactions to histoplasmin had resided in or near the endemic area. None of this group were from countries where Histoplasmosis has been reported.

We found no difference in the skin sensitivity between males and females. Perhaps this is due to the overall rural population in Nebraska. We did not attempt to analyze the age distribution of skin sensitivity because most of the test population fell in the 17-19 year age group for Residents and 17-24 year age group for the Mostly Nebraska Residents

Clinically physicians in Nebraska, as in all states, should always consider the diagnosis of Histoplasmosis when evaluating pulmonary complaints as well as the non-specific symptoms such as chronic cold with cough, malaise, chills and fever. The diagnostic aids available are skin test antigens, X-ray, complement fixation tests, precipitin tests, periodic acid-Schiff stain for tissues and actual culture of the organism. The finding of Histoplasma capsulatum in solitary lung nodules has just begun to be studied but appears to be a much more common finding than would be expected. Resection of lung tissue represents the only successful treatment for Histoplasmosis reported to date.

CONCLUSIONS

1. Histoplasmosis is an important disease and should always be included in the differential diagnosis of pulmonary disease. It also can mimic several other diseases which affect the reticulo-endothelial system and in its mildest form may be passed off as a bad cold or influenza.

2. There is a disease spectrum for Histoplasmosis which is divided: . Non-fatal, asymptomatic infection; 2. Non-fatal, symptomatic infection; and 3. Fatal, symptomatic infection.

3. There are three zones of skin sensitivity to histoplasmin: Area one with 70% or more positive skin tests; Area two with 10-50% positive skin tests which surrounds area one; and Area three where less than 10% of persons have positive skin tests.

4. Histoplasmosis is present in Nebraska. The southeastern part of the state is within the "ffinge area" which surrounds the endemic area of Histoplasmosis.

5. Absence from the state for a period of longer than six months does not increase the incidence of skin sensitivity markedly.

6. When considering the possibility of Histoplasmosis one should remember that many persons are living in Nebraska who have lived elsewhere and may have skin sensitivity to histoplasmin.

7. The radiologic finding of a solitary lung nodule should bring to mind the possible diagnosis of Histoplasosis and successful treatment by sectional removal of lung tissue.

APPENDIX I

TOTAL STUDIES

	TP	H		T		BA		
			d	p	d	p	d	
Residents	1000	84 (87)	21 (22)	23 (26)	9 (10)	3	1	859
Most-Nebr. Residents	334	46 (49)	4	9 (12)	5	3	0	267
Outstate Residents	181	38	3	2	4	0	0	134
Foreign	20	2	0	5	1	0	0	12
Wanderers	51	9 (11)	1 (20)	1 (3)	1 (2)	2	1	36
Total	1586	179	29	40	20	8	2	1308

Key: TP-total population, H- histoplasmin, T- tuberculin, BA- reactors to both antigens, N- negative, p- positive, d- doubtful.

Parentheis indicate the actual number of skin reactions after the reactors to both antigens have been included.

APPENDIX II

HISTOPLASMIN AND TUBERCULIN REACTORS BY GROUP

	H	%	T	%	TP
Residents	109	10.9	26	2.6	1000
Most.-Nebr. Residents	53	15.87	12	3.59	334
Outstate Residents	41	22.65	2	1.10	181
Foreign	2	10.00	5	25.00	20
Wanderers	13	25.49	3	5.88	51
Total	218	13.74	48	3.02	1586

Key: H- histoplasmin reactors, T- tuberculin reactors, % - percentages, TP- Test population

APPENDIX III

STATISTICS BY COUNTY FOR NEBRASKA RESIDENTS AND MOSTLY NEBRASKA RESIDENTS

C	P	TP			N	H p	H d	T p	T d	BA p	BA d
Adams	28,855	12	R	9	9						
			M	3	3						
Antelope	11,624	10	R	6	6						
			M	4	3	1					
Arthur	803	0									
Banner	1,325	0									
Blaine	1,203	2	R	1	1						
			M	1	1						
Boone	10,721	7	R	5	5						
			M	2	1		1				
Box Butte	12,279	16	R	11	11						
			M	5	5						
Boyd	4,911	1	R	1	1						
			M	0	0						
Brown	5,164	4	R	1	1						
			M	3	2				1		
Buffalo	25,134	21	R	11	9	2					
			M	10	9	1					
Burt	11,536	13	R	9	8	1					
			M	4	1	3					
Butler	11,432	12	R	11	10	1					
			M	1	1						
Cass	16,361	21	R	17	11	5	1				
			M	4	2	2					
Cedar	13,843	12	R	7	6		1				
			M	5	5						
Chase	5,176	11	R	9	9						
			M	2	2						
Cherry	8,397	10	R	8	8						
			M	2	2						
Cheyenne	12,081	8	R	6	6						
			M	2	2						
Clay	8,700	12	R	6	6						
			M	6	5	1					
Colfax	10,010	16	R	13	11	1			1		
			M	2	2				1		
Cumming	12,994	6	R	4	3			1			
			M	2	1	1					

APPENDIX III (con't.)

C	P	TP		N	H p	H d	T p	T d	BA p	BA d
Custer	19,170	19	R 17	15	1		1			
			M 2	2						
Dakota	10,401	10	R 7	6	1					
			M 3	2				1		
Dawes	9,708	2	R 1	1						
			M 1	1						
Dawson	19,393	21	R 16	16						
			M 5	4	1					
Deuel	3,330	9	R 8	7						1
			M 1	1						
Dixon	9,129	3	R 1	1						
			M 2	1	1					
Dodge	24,265	29	R 20	20						
			M 9	7	1		1			
Douglas	281,020	129	R103	60	16	2	4		1	
			M 26	21	4				1	
Dundy	4,354	2	R 1	1						
			M 1	1						
Fillmore	9,610	12	R 8	7				1		
			M 4	3		1				
Franklin	7,096	8	R 6	6						
			M 2	2						
Frontier	5,282	4	R 4	4						
			M 0							
Furnas	9,285	13	R 11	11						
			M 2	1			1			
Gage	28,052	42	R 32	27	3	1	1			
			M 10	8	1		1			
Garden	4,114	3	R 2	2						
			M 1	1						
Garfield	2,912	3	R 3	3						
			M 0							
Gosper	2,734	5	R 3	3						
			M 2	2						
Grant	1,057	3	R 3	3						
			M 0							
Greeley	5,575	12	R 7	6				1		
			M 5	4	1					
Hall	32,186	44	R 32	18	14					
			M 12	8	4					
Hamilton	8,778	20	R 16	15		1				
			M 4	4						

APPENDIX III (con't.)

C	P	TP			N	H p	H d	T p	T d	BA p	BA d
Harlan	7,189	9	R	6	5		1				
			M	3	3						
Hayes	2,404	0									
Hitchcock	5,867	3	R	2	2						
			M	1	1						
Holt	14,859	9	R	7	7						
			M	2	1			1			
Hooker	1,061	1	R	1	1						
			M	0							
Howard	7,226	10	R	7	6			1			
			M	3	2				1		
Jefferson	13,623	7	R	6	6						
			M	1	1						
Johnson	7,251	11	R	7	6	1					
			M	4	3	1					
Kearney	6,409	15	R	14	13			1			
			M	1	1						
Keith	7,449	9	R	7	4	1	1		1		
			M	2	2						
Keya Paha	2,160	1	R	1	1						
			M	0							
Kimball	4,283	2	R	2	2						
			M	0							
Knox	14,820	15	R	8	8						
			M	7	5	2					
Lancaster	119,742	273	R	203	174	15	4	7	2	1	
			M	70	55	11	1	2	1		
Lincoln	27,280	22	R	13	12			1			
			M	9	9						
Logan	1,355	3	R	3	3						
			M	0							
Loup	1,348	1	R	1	1						
			M	0							
McPherson	825	0	R								
Madison	24,338	17	R	14	14						
			M	3	1	1		1			
Merrick	8,812	11	R	9	9						
			M	2	2						
Morril	8,263	10	R	10	10						
			M	0							

APPENDIX III (con't.)

C	P	TP			N	H p	H d	T p	T d	BA p	BA d
Nance	6,512	7	R	6	5		1				
			M	1	1						
Nemaha	10,973	9	R	7	5	1	1				
			M	2	2						
Nuckolls	9,609	8	R	5	4	1					
			M	3	2	1					
Otoe	17,056	16	R	12	9	3					
			M	4	3	1					
Pawnee	6,744	8	R	6	5		1				
			M	2	2						
Perkins	4,809	8	R	5	4	1					
			M	3	1	1		1			
Phelps	9,048	13	R	7	5		1		1		
			M	6	6						
Peirce	9,045	5	R	4	3				1		
			M	1	1						
Platte	19,910	14	R	7	6	1					
			M	7	5	1				1	
Polk	8,044	7	R	6	5			1			
			M	0							
Red Willow	12,977	11	R	10	10						
			M	1	1						
Richardson	16,886	20	R	11	7	3	1				
			M	9	4	5					
Rock	3,026	3	R	3	3						
			M	0							
Saline	14,046	21	R	19	17			2			
			M	2	1			1			
Sarpy	15,693	7	R	6	4	2					
			M	1	1						
Saunders	16,923	28	R	24	20	3	1				
			M	4	3					1	
ScottsBluff	33,939	21	R	15	14	1					
			M	6	5	1					
Seward	13,155	18	R	12	12						
			M	6	6						
Sheridan	9,539	10	R	8	5	1	1	1			
			M	2	2						
Sherman	6,421	3	R	2	2						
			M	1	1						
Sioux	3,124	1	R	1				1			
			M	0							
Stanton	6,387	6	R	3	3						
			M	3	3						

APPENDIX III (con't.)

C	P	TP			N	H		T		BA	
						p	d	p	d	p	d
Thayer	10,563	10	R	7	6		1				
			M	3	3						
Thomas	1,206	0									
Thurston	8,590	8	R	5	4					1	
			M	3	3						
Valley	7,252	8	R	8	8						
			M	0							
Washington	11,511	7	R	6	4	1	1				
			M	1	1						
Wayne	10,129	2	R	2	2						
			M	0							
Webster	7,395	6	R	4	4						
			M	2	2						
Wheeler	1,526	3	R	3	3						
			M	0							
York	14,346	18	R	17	14	1	1				
			M	1	1						
At large		12	R	11	9	2					
			M	1	1						
Totals		1334	R1000			87	22	26	10	3	1
			M 334			49	4	12	5	3	0

Key: C- county, P- population (1950 census), TP- test population, N- negative, H- histoplasmin reactors, T- tuberculin reactors (positive only), BA- reactors to both antigens, p- positive, d- doubtful, R- residents, M- Mostly Nebraska Residents.

APPENDIX IVa

COUNTIES IN THE FIVE SECTIONS

Southeastern - Adams, Buffalo, Butler, Cass, Clay, Douglas, Fillmore, Franklin, Gage, Hall, Hamilton, Howard, Jefferson, Johnson, Kearney, Lancaster, Merrick, Nance, Nemaha, Nuckolls, Otoe, Pawnee, Polk, Richardson, Saline, Sarpy, Saunders, Seward, Sherman, Thayer, Webster, York.

Northeastern - Antelope, Boone, Boyd, Burt, Cedar, Colfax, Cuming, Dakota, Dixon, Dodge, Garfield, Greeley, Holt, Knox, Madison, Pierce, Platte, Stanton, Thurston, Valley, Washington, Wayne, Wheeler.

Southwestern - Chase, Custer, Dawson, Dundy, Frontier, Furnas, Gosper, Harlan, Hayes, Hitchcock, Keith, Lincoln, Perkins, Phelps, Red Willow.

Northwestern - Arthur, Blaine, Browne, Cherry, Custer, Grant, Hooker, Keya Paha, Logan, Loup, McPherson, Rock, Thomas.

Panhandle - Banner, Box Butte, Cheyenne, Dawes, Deuel, Garden, Kimball, Morrill, Scotts Bluff, Sheridan, Sioux.

APPENDIX IVb.

STATISTICS BY SECTION

S	T	%	R	%	M	%
Southeastern						
P	846		641		205	
H p	108		74		34	
H d	17		15		2	
H	125	14.77	89	13.88	36	17.56
T p	26	3.07	21	3.27	5	2.44
T d	6		4		2	
Northeastern						
P	216		150		66	
H p	19		6		13	
H d	3		2		1	
H	22	10.18	8	5.33	14	21.21
T p	6	2.77	2	1.33	4	6.06
T d	2		2		0	
Southwestern						
P	141		104		37	
H p	5		3		2	
H d	3		3		0	
H	8	5.67	6	5.76	2	5.40
T p	4	2.83	2	1.92	2	5.40
T d	2		2		0	
Northwestern						
P	37		29		8	
H p	0		0		0	
H d	0		0		0	
H	0	0.0	0	0.0	0	0.0
T p	0	0.0	0	0.0	0	0.0
T d	1		0		1	
Panhandle						
P	82		64		18	
H p	2		2		0	
H d	3		2		1	
H	5	6.10	4	6.25	1	5.55
T p	2	2.44	2	3.13	0	0.00
T d	1		1		0	

Key: S- section, T. total, %- percentage, R- Resident, M- Mostly Nebraska Resident, P- population, H p- Histoplasmin, H d- Histoplasmin doubtful, H- Total Histoplasmin reactors, T p- Tuberculin positive, T d- Tuberculin doubtful.

APPENDIX V

MALE-FEMALE SKIN SENSITIVITY DISTRIBUTION

	H		T		BA		N	T
	p	d	p	d	p	d		
RESIDENT								
Male	60	17	14	7	2	0	583	683
Female	25	4	7	3	1	0	277	317
MOSTLY NEBRASKA RESIDENTS								
Male	39	5	9	4	3	0	205	265
Female	6	1	1	1	0	0	60	69

Key: H- histoplasmin, T- tuberculin, BA- reaction to both antigens, N- negative, T- total, p- positive, d- doubtful.

APPENDIX VI

AGE DISTRIBUTION

A	H		T		BA		N	T
	p	d	p	d	p	d		
RESIDENT								
16	1						10	11
17	28	7	6	4			305	350
18	39	10	9	3	2	1	419	444
19	3	1		1			64	69
20	3						18	21
21	1	1					11	13
22	4						11	15
23	3		1	1			9	14
24	3						5	8
25							2	2
26	1	1	1		1		4	8
27	1							1
28				1				1
29							1	1
31							1	1
51	1							1
?	1							1
Mostly Nebraska Resident								
16	1	1		1				3
17	4	1	2				40	47
18	8		2	3			64	77
19	3						18	21
20			1				12	13
21	5		2		1		17	23
22	6						46	52
23	7	1	2	2	1		31	44
24	7		3				19	29
25	3			1	1		16	21
26	2		1				4	7
27	1						4	5
28	1						1	2
29	1		1					2
30							1	1
43	1							1
?	1							1

APPENDIX VII

DEFINITION OF PODZOLIC SOIL

from The American College Dictionary - Random House

a forest soil, notably acidic, having an upper layer that is greyish-white or ash-covered and depleted of colloids and iron and aluminum compounds, and a lower layer, brownish in color, in which those have accumulated; an infertile soil difficult to cultivate, found over vast areas in northern Northa America and Eurasia.

BIBLIOGRAPHY

1. Absher, W.K. Correlated study of tuberculin, Histoplasmin, and coccidioidin sensitivities in the Rocky Mountain Area. Am. Rev. Tuberc. 59:643-649, 1949.

2. Alexander, J.T and Baker, F. Acute disseminated Histoplasmosis: a report of a case diagnosed by needle biopsy of the spleen and liver. Med. Ann. Dist. Columbia 22:359,1953.

3. Armstrong, J.W. Histoplasmosis: Study of reactors to Histoplasmin. Am. J. Pub. Health 39: 1136-1140,1949.

4. Aronson, J.D et al. The geographic distribution of histoplasmin sensitivity in Pennsylvania. Pennsylvania M.J. 56:543-7,1953.

5. a)Beadenkopf, W.G., et al. Tuberculin, coccidioidin and histoplasmin sensitivity in relation to pulmonary calcifications: A survey of 6,000 students at the University of Chicago. Pub. Health Rep. 64:17-32,1949. b) Beadenkopf, W.G. and Loosli, C.G. Histoplasmosis, tuberculosis and coccidioidomycosis.JAMA 146:621-624, 1951. c) Beadenkopf, W.G. et al. Histoplasmin and tuberculin sensitivity among Illinois residents. Am. J. Hyg. 57:328-343,1953.

6. Billings, F.T. and Couch, O.A. Pericardial calcification and histoplasmin sensitivity. Ann. Int. Med. 42: 654-8,1955.

7. Bond, JO. Histoplasmin and tuberculin sensitivity. Pub. Health Rep. 69:1141-4,1954.

8. Broder, A.C. et al. Histoplasmosis producing vegatative endocarditis. JAMA 122:489-91,1943.

9. a) Bunnell, I.L. and Furcolow, ML. A report of ten proved cases of histoplasmosis. Pub Health Rep. 63: 299-316,1948. b) Idem. Variations in histoplasmin sensitivity in certain cities in eastern Kansas. Pub. Health Rep. 63: 1298-1305,1948.

10. Burton, C.T. and Wallenborn, P.A. Histoplasmosis of the larynx. Virginia M. Monthly 80:665-8,1953.

11. Cain, J.C., Devins, E.T. and Downing, J.E. An unusual pulmonary disease. Arch. Int. Med. 79:626-641,1947.

Bibliography 2

12. a)Campbell, C.C. and Saslaw, S. The use of yeast phase antigens in a complement fixation test for histoplasmosis, II. Results with ground antigen. J. Lab. Clin. Med. 33:1207,1948. b) Campbell, C.C. Antigenic fractions of Histoplasma capsulatum. Am. J. Pub. Health 43:712,1953. c) Idem. Effect of histoplasmin skin testing on serological results. Proc. Soc. Exp. Biol. 82:4,1953. d) Idem. Serological diagnosis with respect to histoplasmosis, coccidioidomycosis and blastomycosis and the problem of cross reactions. J. Lab. Clin. Med. 42:896,1953. e) Campbell, C.C., Hodges, E.P. and Hill, G.B. Therapeutic effect of nystatin (fungicidin) in mice experimentally infected with Histoplasma capsulatum. Antibiotics and Chemotherapy 4:406,1954.

13. Cawley, E.P. and Curtis, A.C. Histoplasmosis and lymphoblastoma; are these diseases related? Invest. Dermat. 11:443-453,1948.

14. a) Charr, R. Some nontuberculous disease of the lungs. M. Clinic N. Am. 31:1475-1492,1947. b) Idem. Clinical Histoplasmosis in Pennsylvania. Pennsylvania M.J. 56: 548-52,1953.

15. a) Christie, A. and Peterson, J.C. Pulmonary calcification in negative reactors to tuberculin. Am. J. Pub. Health 35:1131,1945. b)Idem. Pulmonary calcification and sensitivity to histoplasmin, tuberculin, and haplosporangin. JAMA 131:658-660,1946. c) Christie, A. Histoplasmosis and pulmonary calcification: Geographic distribution. Am. J. Trop. M. 31:742-52,1951. e)Idem. The disease spectrum of human histoplasmosis. Tr. As. Am. Physicians 64:147-54,1951.

16. Conlin, F.M. and Hankins, C.R. Histoplasmosis, case. Nebraska M.J. 32:101-103,1947.

17. Crabtree, C.F. Tuberculosis studies in Tennessee. Am. Rev. Tuberc. 28:1-31,1933.

18. Crimm, P.D. and Short, D.M. Tuberculin anergy in cases with pulmonary calcification. Am. Rev. Tuberc. 39:64,1939

19. Cronk, G.A. Pulmonary calcification and histoplasmin sensitivity in New York state. N,Y.J. Med. 51:1919,1951.

20. Cross, F.W. and Howell, A. Studies of fungus antigens, II,Preliminary report of the isolation of an immunologically active polysaccharide from histoplasmin. Pub. Health Rep. 63:179-183,1948.

21. Curtis, A.C. and Cawley, E.P. Genital histoplasmosis. J. Urol. 57: 781-787,1947.

22. a)Darling, S.T. A protozoan general infection producing pseudotubercles in the lungs and focal necrosis in the liver, spleen and lymph nodes. JAMA 46:1283,1906. b)Idem. Histoplasmosis: A fatal infectious disease resembling Kala-azar found among natives of tropical America. Arch. Int. Med. 2:107,1908. c) Idem. The morphology of the parasite (Histoplasma capsulatum) and the lesions of histoplasmosis, a fatal disease of tropical medicine. J. Exper. Med. 11:515,1909.

23. De Monbreum, W.A. Cultivation and Cultural characteristics of Darling's Histoplasma Capsulatum. Am. J. Trop. Med. 14:93,1934.

24. Derifield, R.S. and Cole, R.L. Skin sensitivity to histoplasmin in Iowa. J. Iowa Med. Soc. 41:16,1951.

25. Dickie, H.A. Histoplasmin and tuberculin sensitivity in relation to pulmonary calcifications among Uni. of Wisconsin students. Ann. Int. Med. 28:1087-1093,1948.

26. Dyson, J.E. and Evans,E.E. Skin test antigens from yeast phase cultures of Blastomyces dermatitidis and Histoplasma capsulatum. Mich. Uni. Med. Bull. 20:3,1954

27. Ellis,Jr.,FF., Scott, R.J. and Miller, J.M. Treatment of progressive disseminated histoplasmosis with ethyl vanillate and propamidine. Antibiotics 2:347-50,1952.

28. a) Emmons, C.W., Olson, B.J. and Eldridge,W.W. Role of fungi in pulmonary diseases; cross reactions of histoplasmin. Pub. Health Rep 60:1383-1394,1945. b)Emmons, C.W. Histoplasmosis in wild rats; occurrence and histopathlogy. Pub. Health Rep 63:1416-1422,1948. c) Idem Isolation of Histoplasma capsulatum from soil. Pub. Health Rep. 64:892-896,1949. d) Idem. Histoplasmosis. Bull. N.Y. Acad. Med. 31:637-638,1955.

29. Ende, N. Hodgkins disease associated with Histoplasmosis

Cancer, N.Y. 5:763-9,1952.

30. Feldman, H.A. and Sabin, A.A. Pneumonitis of unknown etiology in a group of men exposed to pigeon excreta. J. Invest. Clin. 27:533,1948.

31. Forbes,B.G. et al. Histoplasmin and tuberculin sensitivity in Texas infants and children. Pub. Health Rep 68:320,1953.

32. a) Furcolow,M.L., High,R.H. and Allen,M.F. Epidemiologic aspects of sensitivity to histoplasmin and tuberculin. Pub. Health Rep 61:1132-1144,1946. b)Furcolow,M.L. and High,R.H. Disseminated pulmonary calcification; 113 cases. Pub. Health Rep 62:20-29,1947. c) Furcolow, M.L and Forebee,S.H. Histoplasmin sensitivity among siblings. Pub. Health Rep 62:834-47,1947. d) Furcolow, M.L., Mantz,H.L. and Lewis,I. Roentgenographic appearance of persistent pulmonary infiltrates associated with sensitivity to histoplasmin. Pub. Health Rep. 62: 1711-1718,1947. e)Furcolow,M.L.,Bunnell,I.L. and Tennenberg,D.J. A complement-fixation test for histoplasmosis.II.Preliminary results with human sera. Pub. Health Rep 63:169-173,1948. f)Furcolow,M.L.,Emge,M.L. and Bunnell,I Depression of tuberculin and histoplasmin sensitivity associated with critical illness. Pub. Health Rep 63:1290-1298,1948. g)Furcolow,M.L. and Ruhe,J.S. Histoplasmin sensitivity among cattle. Am. J. Pub. Health 39:719,1949. h)Furcolow,M.L. Development of calcification in pulmonary lesions associated with sensitivity to histoplasmin. Pub. Health Rep. 64:1363-1393,1949. i)Furcolow,M.L.,Menges,R.W. and Ruhe,J.S. Experimental histoplasmosis in a dog; a nonfatal case. Pub. Health Rep. 65:628-631,1950. j)Furcolow,M.L. Tuberculin negative, histoplasmin positive, disseminated Pulmonary calcification. Postgrad. Med. 8:15-20,1950. k) Furcolow,M.L. Histoplasmosis, further observations; mycology and bacteriology. Pub. Health Rep. 65:965-994, 1950. l)Furcolow,M.L. and Sitterley,J. Further studies of the geography of histoplasmin sensitivity in Kansas and Missouri. J. Kansas Med. Soc. 52:584-9,1951. m) Furcolow,M.L. et al. Laboratory studies of histoplasmosis:I. The effect of humidity and temperature on the growth of Histoplasma capsulatum. J. Infect Dis. 90: 67-70,1952. n)Furcolow,M.L. Direct isolation of Histoplasma capsulatum from soil; Probable etiological relationship to Camp Gruber pneumonitis. Proc. Soc. Exp.

Biol. and Med.,N.Y. 80:246,1952. o)Furcolow,M.L. et al The frequency of laboratory infections with Histoplasma capsulatum:Their clinical and X-ray characteristics. J. Laborat. Clin. Med. 40:182-7,1952. p)Furcolow,M.L. et al Comparison of histoplasmin sensitivity rates among human beings and animals in Boone County, Mo. Am. J. Pub. Health 42:926-9,1952. q)Furcolow,M.L. et al. Nontuberculous chest disease: Occurrence of histoplasmosis in epidemics. Tr.Nat. Tuberc. Assn. 48:83-91,1952 r)Furcolow,M.L. et al. Laboratory studies of Histoplasma capsulatum: III. Efficiency of flotation method in isolation of Histoplasma capsulatum from soil. J. Lab. Clin. Med. 41:3,1953. s)Furcolow,M.L. and Grayston,J.T. The occurrence of histoplasmosis in epidemics: epidemiological studies. Am. J. Pub. Health 43:665-76,1953. t)Furcolow,M.L. et al. Occurrence of histoplasmosis in epidemics: etiological studies. Am. Rev. Tuberc. 68: 307,1953. u)Furcolow,M.L. et al. Incidence of tuberculin histoplasmin and blastomycin reactors among a group of school children. Am. J. Pub. Health 43:2,1953. v) Furcolow,M.L. et al.Tuberculin-histoplasmin conversion rates in Kansas City as an indication of the prevalence of the infection. Tr. Nat. Tuberc. Assn. Vol.49,1953. w) Furcolow,M.L. et al. Precipitins in human histoplasmosis. J. Laborat. Clin. Med. 43:259-274,1954. x) Furcolow,M.L. et al. Serological studies on an outbreak of pulmonary disease at Camp Gruber, Oklahoma. Arch. Int. Med. 93:906,1954. y)Furcolow,M.L. et al. An outbreak of histoplasmosis involving animals and man. Am. J. Vet. Res. 15:520-4,1954. z) Furcolow,M.L. et al. Histoplasmin, coccidioidin, tuberculin sensitivity among school children in two Texas counties. Pub. Health Rep 70:12-8,1955.

Gass, R.S. Tuberculosis studies in Tennesse. Am. Rev. Tuberc. 38:441,1938. b)Gass,R.S. and Harrison,E.F. Pulmonary calcification and tuberculin sensitivity among children in Williamson County Tennessee. Am. Rev. Tuberc. 47:379,1943. c)Gass,R.S and Zeidberg,L.D. Diagnosis aids in histoplasmosis. Am. Rev. Tuberc. 70: 360-2,1954.

34. Good,C.A., Clagett,O.T. and Weed,L.A. Granuloma of the lung: a problem in differential diagnosis. Tr. Nat. Tuberc. Assn. 1951.

35. Green,P. Pulmonary calcification and histoplasmin sen-

Sitivity. Canad. M.J. 63:143-46,1950.

36. Groover,M.R. et al. Sensitivity of skin to histoplasmin in differential diagnosis of pulmonary disease. Arch. Int. Med. 80:496-513,1947.

37. Hansmann,G.H. and Schenken,J.R. A unique infection in man caused by a new yeastlike organism, a pathogenic member of the genus Sepedonium Am. J. Path 10:731,1934.

38. HighR.H. Non-tuberculous pulmonary calcification and histoplasmin sensitivity. Pennsylvania M.J. 50:384,1947

39. a)Howell,A. Fungus antigens: Quantitative studies of cross reactions with histoplasmin and blastomycin in guinea pigs. Pub. Health Rep. 62:631-651,1947. b) Idem. The efficiency of methods of isolation of Histoplasma capsulatum. Pub. Health Rep. 63:173-176,1948.

40. Hudson,E.H., Moore,F.R. and Phillips,E.A. Comparative sensitivity to histoplasmin and tuberculin in Ohio University students. J. Lancet 68:142-157,1948.

a)Iams,A.M., Tene,M.M. and Flanagan,H.F. Histoplasmosis in children. Review of the literature with report of a case. Am. J. Dis Child. 70:229,1945. b) Iams,A.M. Histoplasmin skin test. Ann. New York Acad. Sc. 50: 1380,1950

42. a) Ibach,M.J. and Furcolow,M.L. Isolation of Histoplasma capsulatum form air. Science 119:71,1954. b) Ibid. Epidemic histoplasmosis in airborne Histoplasma capsulatum. Proc. Soc. Exp. Biol. Med. 85:72,1954.

43. Idstrom, L.G. Primary atypical pneumonia. U.S. Army Med. Dept. 81:88,1944.

44. Ireland,H.D. Histoplasmin and old tuberculin reactors in Kent County, Michigan. J. Michigan M. Soc. 50: 619-620,1951.

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

46. Katz,S. Histoplasmoma GP 10:73,1954.

47. Krause,A.A. and HopkinsW.G. Histoplasmosis; Ocular manifestations. Am. J. Ophth. 34:564-566,1951.

48. Krug,E.S. and Glenn,H.R. Comparative studies in tuberculosis and histoplasmosis among 2000 students entering Pennsylvania State College. J. Lancet 68:206-208, 1948.

49. Kunstadter,R.H., Whitcomb,F.C. and Milizer,A. Primary Histoplasmosis with recovery of Histoplasma capsulatum from blood and bronchial secretions. J. Labor. Clin. Med. 34:1290-1291,1949.

50. Lee,J.R. et al. Acute benign histoplasmosis. Am. Rev. Tuberc. 69:625-30,1954.

51. Locket,S., Atkinson,E.A. and Grieve,W.S.M. Histoplasmosis in Great Britain; a description of a second case of dissmenited histoplasmosis; treatment with ethyl vanillate. British M.J. 4841:857-60,1953.

Long,E.R and Stearns,W.H. Physical examination at induction. Standards with respect to tuberculosis and their application as illustrated by a review of 53,400 X-ray films of men in the Army of the United States. Radiology 41:44-50,1943.

a)Loosli,C.G. and Beadenkopf,W.G. Epidemiological apects of histoplasmin, tuberculin and coccidioidin sensitivity. Am. J. Hyg. 53:33-57,1951. b)Loosli,C.G. Grayston,J.T. and ALEXander,E.R. Isolation of Histoplasma capsulatum from soil in an unused silo. Science 114:323-4,1951. c) Loosli,C.G. Some epidemiological and clinical aspects of pulmonary histoplasmosis in a farm family. Tr. Assoc. Am. Physicians. 65:159,1952. d)Loosli,C.G. Histoplasmosis: Some clinical, epidemiological and laboratory aspects. Med. Clin. N. Amer. 171(Jan) 1955.

54. Lynch,J.F. and Alpern,E.B. Results of histoplasmin skin testing in children from St. Louis, Mo. area. J. Ped/ 38:51-54,1951.

55. Manos,N.E. Histoplasmin sensitivity conversion rates. Am. J. Hyg. 58:119-31,1953.

56. Matthiessen,D.E. The surgical significance of solitary circumscribed lung nodules in histoplasmosis; a report of two cases. Am. Rev. Tuberc. 69:829-36,1954.

57. Menges,R.W. and Furcolow,M.L. Effect of Humidity and Temperature on the growth of Histoplasma capsulatum. J. Inf. Dis. 90:67-70,1952.

58. Mider, P.E. Systemic Infection with Cryptococcus neoformans (Torula histolytica) and Histoplasma capsulatum in the same patient. Arch Path. 43: 102-110,1947.

59. Morgan,H.J. Histoplasmosis in the gastrointestinal tract. J. Tennessee M. Assn. 45:488-91,1952.

60. Murphy,J.H.,Moragues,V.,Russum,B.C.,Clement,E. and Clement,G. Exhibit of Clinical and Pathological Features of Histoplasmosis. Omaha Mid-West Clinical Soc. Unpublished data.

61. a) Murphy,R.J. and Davis,W.H. Histoplasmin sensitivity in selected areas of North Carolina with report of a case of probable histoplasmosis in a child. N. Carolina M.J. 13:556,1952. b)Murphy,R.J. and Peck,W.M. Preliminary report of histoplasmin and other antigens sensitivity in North Carolina. Am. J. Pub. Health 41:1521-1525,1952.

62. Murray,J.F. and Brandt,F.A. Histoplasmosis and malignant lymphoma. Am. J. Path. 27:783-799,1951.

63. Nelson,W.E., Mitchell,A.G. and Brown,E.W. The intracutaneous tuberculin reaction associated with calcified intrathoracic lesions. Am. Rev. Tuberc. 37:311,1938

64. Nejedly,R.E. and Baker,L.A. Treatment of localized histoplasmosis with 2-hydroxystilbamidine. Arch. Int. Med. 95:37-40,1955.

65. Norden, A. Agglutination of sheep erythrocytes sensitized with histoplasmin. Proc. Soc. Exp. Biol. Med. 70:218-220,1949.

66. Oliver,R.K. Histoplasmosis; a roentgenographic survey of some Alabama counties; report of two cases of pulmonary cavitation. A.M.Assn. Alabama 24:113-21,1954.

67. Olson,B.J., Wright,W.H. and Nolan,MO. An epidemiological study of calcified pulmonary lesions in an Ohio county. Pub. Health Rep. 56:2105,1941.

68. O'Neal,R.M. Histoplasmin sensitivity in Mississippi; A new boundary. Pub Health Rep. 68:714-6,1953.

69. a)Palmer,C.E. Nontuberculous pulmonary calcification and sensitivity to histoplasmin. Pub. Health Rep. 60: 513-520,1945. b) Palmer,C.E. et al. Geographic differences in sensitivity to histoplasmin among student nurses. Pub. Health Rep. 61:475-487,1946. c)Palmer, C.E., Edwards,L.B. and Lewis,I. Studies of pulmonary findings and antigen sensitivity among student nurses. III. Pulmonary infiltrates and mediastinal adenopathy observed among student nurses at the beginning of training. Pub. Health Rep. 63:1569-1600,1948. d) Palmer,C.E., Goddard,J.C. and Edwards,L.B. Studies of pulmonary findings and antigen sensitivity among student nurses. IV. Relationship of pulmonary calcifications with sensitivity to tuberculin and histoplasmin. Pub. Health Rep 64:820-846,1949. e) Palmer,C.E. and Peterson,O.S Studies of pulmonary findings and antigen sensitivity among student nurses. V. Doubtful reactions to tuberculin and to histoplasmin. Pub. Health Rep. 65: 1-32,1950. f) Palmer,C.E. et al. Studies of pulmonary findings and antigen sensitivity to student nurses. VI. Geographic differences in sensitivity to tuberculin as evidence of nonspecific allergy. Pub. Health Rep. 65: 1111-1131,1950.

70. Parsons,R.J. and Zarafonetis,C.J.D. Histoplasmosis in man; report of 7 cases and review of 71 cases. Arch. Int. Med. 75:1-23,1945.

71. Pates,A.L. Precipitin reactions in experimental histoplasmosis. Science 108:383-385,1948.

72. Payne,N.S., Henninger,G.R. and Sutton,L.E. Histoplasmosis: Occurrence in Virginia. Virginia Med. Monthly 79:430-5,1952.

73. Peabody,J.W. Demonstration of fungi by periodic acid-Schiff stain in pulmonary granulomas. JAMA 157:885-8, 1955.

74. Peterson,J.C. and Christie,A. Histoplasmosis. Ped. Clin. N. Amer. Feb:127-49,1955.

75. Pine,L. Histoplasma capsulatum; growth of yeast phase in liquid media. J. Bact. :671-9,1954.

76. Pinkerton,H. Histoplasmosis: Three fatal cases with disseminated sarcoid-like lesions. Arch Int. Med. 90: 456,1952.

77. Pitt,C.K. Histoplasmosis and incidence of positive histoplasmin and tuberculin skin tests in children of North Alabama. South. M.J. 45:1172,1952.

78. a) Prior,J.A. and Allen,M.F. Geographic distribution of histoplasmin and tuberculin reactors among Ohio State University freshman and student nurses in training in Columbus, Ohio hospitals. Pub. Health Rep. 62:1608-18,1947. b)Prior,J.A. et al. Geographic distribution of pulmonary calcification among university students in Ohio. Pub. Health Rep. 65:1132,1950. c) Prior,J.A. et al. Effect of repeated histoplasmin skin tests on skin reactivity and collodion agglutination. Am. Rev. Tuberc. 66:588-93,1952.

79. Pryor,H.B. Histoplasmosis in California children. J. Ped. 34:12-19,1949.

80. a)Puckett,T.F. Pulmonary histoplasmosis; a study of two cases with identificiation of Histoplasma capsulatum in resected lesions. Am. Rev. Tuberc. 67:453-76,1953. b) Puckett,T.F. Pulmonary Histoplasmosis. Am. J. Surg. 90:93-100,1955.

81. Rafferty,A., Trafas,P.C. and McClure,R.D. Histoplasmosis, common cause of appendicitis and mesenteric adenitis. Ann. Surg. 132:720-728,1950.

a)Randall,C.C. and Hackney,A.L. Observations on human tissue cultures naturally infected by Histoplasma capsulatum. Am. J. Path. 29:861-9,1953. b)Randall,C.C. Histoplasma capsulatum in tissue culture. Proc. Soc. Exp. Biol. Med. 77:150,1951.

83. Raubitscheck,F.,Acker,R.F. and Waksman,S.A. Production of an antifungal agent of the fungicidin type by Streptomyces areus. Antibiotics and Chemotherapy 2:179,1952.

84. Reeves,R.T. Pulmonary histoplasmosis. Am. J. Roent. 72:769-71,1954.

85. Reimann,H.A. and Price,A.H. Histoplasmosis resembling sarcoidosis. Tr. Assn. Am. Physicians. 62:112-5,1949.

86. a) Riley,W.A. and Watson,C.T. Darlings histoplasmosis in the United States. Minnesota Med. 9:97,1926. b) Riley,W.A. Histoplasmosis of Darling, case originating in Minnesota. Am. J. Trop. Med. 6:271,1926.

87. Robertson,J.W Histological survey of human appendices for Histoplasma capsulatum. J. Albert Einstein M. Center 2:147,1954.

88. Rohn,R.J. and Bond,W.H. The value of routine bone marrow culture for Histoplasma capsulatum in pediatric hematology. Blood, Balt. 8:329-45,1953.

89. Rutledge,L.H. An unusual type of pulmonary disease involving six members of a family. Minnesota Med. 33: 694-699,1950.

90. a)Salvin,S.B. Complement fixation studies in esperimental histoplasmosis. Proc. Soc. Exp. Biol. Med. 66:342-345,1947. b)Salvin,S.B. and Hottle,G.A. Serologic studies on antigens from Histoplasma capsulatum, Darling. J. Immunol. 60:57-66,1948. c)Salvin,S.B. Factors influencing histoplasmin formation. J. Bact. 56:541-546,1948. d)Salvin,S.B. and Furcolow,M.L. Serologic studies in human histoplasmosis. Fed. Proc. 12:457,1953.

91. a)Saslaw,S. and Campbell,C.C. Use of yeast phase antigens in complement-fixation; Preliminary results with rabbit sera. J. Lab. Clin. Med. 33:811-8,1948. b)Idem. Method for demonstrating antibodies in rabbit sera against histoplasmin by colloid agglutination technic. Proc. Soc. Exp. Biol. Med. 68:559-62,1948. c)Idem. Use of yeast phase antigens in complement fixation test for histoplasmosis. IIIPreliminary results with human sera. Pub. Health Rep. 64:551,1949. d)Idem. Comparison between histoplasmin and blastomycin by colloidion agglutination technic. Pub. Health Rep. 64:290-294,1949. e) Idem. Collodion agglutination test. Pub. Health Rep. 64:424-29,1949. f)Idem. Serologic studies. Am. J. Pub. Health 40:427 35,1950. g)Idem. Stability of histoplasmin collodion agglutination test. J. Lab. Clin. Med. 35: 780-785,1950. h)Saslaw,S. and Prior,J.A. The relationship of proved histoplasmosis to histoplasmin sensitivity in Ohio. Ohio State M.J. 48:229-31,1952.

92. Scheff,G.J. Biochemical and immunological properties of Histoplasma capsulatum. Yale J. Biol. Med. 18:41, 1945.

93. Scherr,G.H. The laboratory diagnosis of mycotic infections. I. Histoplasmosis. Nebraska M.J. 39:339-41, 1954.

94. Schulz,D.M. Histoplasmosis of the central nervous system. JAMA 151:549,1953.

95. Schwartz,S.O. and Barsky,S. Report of 193 marrow biopsy specimens cultured for Histoplasma capsulatum. Blood 7:545-9,1952.

96. Schwartz,B. and Spitz,L.J. Histoplasmosis in epdimeic form; a follow up study. Arch. Int. Med. 89:541,1952.

97. Schwartz,J.,SIlverman,F.N., Adriano,S.M., Straub,M., Levine,S. The relation of splenic calcification to Histoplasmosis. N. Eng. J.M. 252:887-91,1955.

98. a)Seabury,J.H. and Drygas,H.H. Histoplasmosis; penicillin in treatment, 2 cases. Ann. Int. Med. 25:340, 1946. b)Seabury,J.H. and Srtis.D. In vitro susceptibility of histoplasmosis to therapeutic agents. Proc. Soc. Exp. Biol. Med. 61:15,1946. c)Seabury,J.H. Stilbamidine in treatment of histoplasmosis. Ann. Int. Med. 31:520-23,1949.

99. Seastrunk,J.G. and Wildon,C. Histoplasmin sensitivity in Columbia, South Carolina. J. South Carolina M.A. 45:37-39,1949.

100. Shapiro,J.L., LUx,J.J. and Profkin,B.E. Histoplasmosis of the central nervous system. Am. J. Path. 31: 319-35,1955.

101. Shaw,L.W., Howell,A. and Weiss,E.S. Biological assay of lots of histoplasmin and the selection of a new working lot. Pub. Health Rep. 65:583-604,1950.

102. Sherwood,P.M. An outbreak of syringe hepatitis with jaundice in hospitalized diabetic patients. Ann. Int. Med. 33:380-96,1950.

103. Shively,J.A. and Tirman,W.S. Pulmonary calcification and histoplasmin sensitivity in Northern Indiana. J. Indiana State M. Assn. 44:951:1951.

104. a)Silverman,F N. Pulmonary calcification, tuberculosis? Histoplasmosis Am. J. Roent. 64:747-64,1950. b) Silverman,F.N., Schwarz,J. and Lahey,M.E. Histoplasmosis. Am. J. Med. 19:410-459,1955.

105. a)Smith,C.E. Coccidioidomycosis. Med. Clin. N. Amer. 27:790-807,1943. b)Idem. Histoplasmin sensitivity and coccidioidal infection. Am. J. Pub. Health 39:722,1949.

106. Snoke,P.O. and Held,G.J. Oral histoplasmosis, report of a case. J. Oral Surg. 11:241,1953.

107. Sontag,L.W. and Allen,J.E. Lung calcifications and hstoplasmin-tuberculin skin sensitivity. J. Ped. 30: 657-667,1947.

108. Spitz,L.J. Histoplasmosis in non-epidemic regions. Am. J. Med. 15:624,1953.

109. Sprofkin,B.E., Shapiro,J.L. and Lux,J.J. Histoplasmosis of the central nervous system: a case report of histoplasma meningitis. J. Neuropath. 14:288-96,1955.

110. Tennenberg,D.J. and Howell,A. A complement fixation test for histoplasmosis. Pub. Health Rep 63:163,1948.

111. Thomas,W.A. and Mitchell,J.H. Histoplasmosis, diagnosis from biopsy of cutaneous nodule. Am. J. Med. 2:538-43,1947.

112. Troxell,M.A. A histoplasmin and tuberculin study of psychiatric pa ients abnormal chest reentgenograms. Minnesota Med. 36:235,1953.

113. Van Pernis,P.A. Specific cutaneous reactions with histoplasmosis Preliminary report of another case. JAMA 117:436,1941.

114. Vogel,R.A., Michael,M. amd Timpe,A. Cortisone in experimental histoplasmosis. Am. J. Path. 31:535,1955.

115. Vollmer,H. Histoplasmin patch test. Am. J. Dis. Child 78:65-66,1949.

116. Waring,J.I. and Gregg,D.B. Pulmonary calcifications and sensitivity to histoplasmin in Charleston, South Carolina. Am. J. Dis. Child. 73:139-142,1947.

117. Washburn,A.M., Tuohy,J.H. and Davis,E.L. Cave sickness: A new disease entity? Am. J. Pub. Health 38: 1521-1526,1948.

118. Watson,C.T. A case of histoplasmosis originating in Minnesota. Arch Path. L:667,1926.

119. Weiss,E.D. and Haskell,B.F. Anorectal manifestations of histoplasmosis. Am. J. Surg. 84:541-543,1952.

120 Zahn,D.W. Pulmonary infiltration associated with sensitivity to histoplasmin, case. Am. Rev. Tuberc. 59: 636-642,1949.

121. a)Zeidberg,L.D. Some factors in the epidemiology of histoplasmin sensitivity in Williamson County, Tenn. Am. J. Pub. Health. 41:80-8,1951. b)Zeidberg,L.D. and Azello,L. Environmental factors influencing the occurrence of Histoplasma capsulatum and Microsporum gypseum in soil, J. Bact.,Balt. 68:156-9,1954. c) Idem. A theory to explain the geographic variations in the prevalence of histoplasmin sensitivity. Am. J. Trop. M. Hyg. 3:1057-65,1954. d)Idem. A theory to explain the geographic variations in the prevalence of histoplasmin sensitivity. Science 119:3097,1954.

122. Zinneman,H.H. and Hall,W.H. Chronic pharyngeal and laryngeal histoplasmosis successfully treated with ethyl vanillate. Minnesota Med. 36:249-52,1953.

123. a)Zwerling,H.B. and Palmer,C.E. Pulmonary calcifications; Roentgenographic observations in relation to histoplasmin and tuberculin reactions. Radiology 47: 59-63,1946. b). Idem. Pulmonary calcification in relation to sensitivity to histoplasmin. JAMA 134:691-692,1947.