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DIFFERENCES IN THE DISTRIBUTION OF SENSITIVITY TO HISTOPLASMIN AND ISOLATIONS OF *HISTOPLASMA CAPSULATUM*[1]

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Comstock, G. W. (Training Center for Public Health Research, Box 2067, Hagerstown, Md. 21740) C. N. Vicens, N. L. Goodman, and S. Collins. Differences in the distribution of sensitivity to histoplasmin and isolations of *Histoplasma capsulatum*. *Amer. J. Epid.*, 1968, *88*: 195–209.—In 1963, 7,787 junior and senior high school students in Washington County, Maryland were tested with histoplasmin. Among 1,450 students who were lifetime residents of a single house, there was marked geographic variation in the prevalence of histoplasmin reactors, ranging from 10 to 90% with no apparent correlation with any topographic or geographic features except for low prevalence within the city of Hagerstown. Marked variation in the frequency of histoplasmin sensitivity within short distances made it unlikely that meterologic factors were involved and suggested that infection was usually acquired close to home. No association was found with the presence of chickens or domestic birds on the premises. Students who lived on alkaline soils were more likely to have been infected than those who lived on acid soils. Positive soil cultures for *Histoplasma capsulatum* did not show a similar pattern of associations, being more frequent from premises with chickens and in areas with acid soils. The discrepancies between skin sensitivity and soil isolations could result from some characteristic of the organism, such as spore size, which varied with soil conditions.

The geographic distribution of histoplasmosis has been both a puzzle and a challenge to investigators during the quarter-century since it first became apparent that infection with *Histoplasma capsulatum* was very common in some

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The initial suggestion that Washington County, Maryland might be a fruitful location for studies on histoplasmosis was made by Dr. Carroll E. Palmer, who continued to give helpful advice throughout the study. The authors are also grateful to Dr. Philip E. Sartwell for his suggestions and criticisms.

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parts of the United States and very rare in others (1–3). Large scale studies by the Tuberculosis Program of the U.S. Public Health Service showed that counties in which the majority of young adult lifetime residents reacted to histoplasmin were heavily concentrated in the east central portion of the country, a triangular area with its apices near Omaha, Nebraska, Columbus, Ohio and Natchez, Mississippi (3–5). Elsewhere in the nation, there were few such counties; most of them were scattered in eastern Texas and western Louisiana, along the Appalachian mountains from Bristol, Tennessee to Gettysburg, Pennsylvania, and on Maryland's Eastern Shore. But infection was not uniformly distributed within the major areas of high and low prevalence, and subsequent studies of counties and towns have shown considerable differences in the frequency of reactors to histoplasmin even within these small areas (6–15). Indeed, variation appears to be the hallmark of the geographic distribution of histoplasmin sensitivity. The cause or causes of this variation are still undetermined.

Studies of the distribution of *H. capsulatum* have been handicapped by the difficulties of isolating the organism. Although it is believed to be a normal inhabitant of the soil, it can be found in only a small proportion of soil samples even in apparently endemic areas. Nevertheless, accumulated experience indicates *H. capsulatum* is most likely to be demonstrable in soil that is moist, shaded, moderately acid and enriched by bird or bat manure (15–19). Although these factors have often been found to be associated with human infections, particularly in epidemic situations (19–21), there have been instances in which human and animal infections could not be correlated with isolations of *H. capsulatum* (11, 15).

The present study was suggested by the observation that histoplasmin reactor rates among U.S. Navy recruits from counties to the south and east of Washington County, Maryland were very high while those from counties to the north and west were low (22). Washington County appeared to straddle a steep gradient of histoplasmin sensitivity, a situation expected to provide a wide range of reactor rates in its population. An additional consideration in selecting this area was the unusually complete information about all residents of the county that had just been collected in a private census. The combined findings of a histoplasmin skin test survey, the census and a preliminary soil sampling for *H. capsulatum* have provided evidence that factors associated with the ability to isolate the organism from the soil are not necessarily the same as those associated with human sensitivity to histoplasmin. This finding has important implications for investigations of the epidemiology of histoplasmosis.

MATERIALS AND METHODS

To conduct the histoplasmin survey, a team of nurses thoroughly experienced in intracutaneous testing was assigned to Washington County by the Tuberculosis Program of the U.S. Public Health Service, which also provided antigens and supplies for the survey. The Washington County Health Department assigned nurses to assist with the testing, and the Washington County Tuberculosis Association provided educational materials and most of the clerical help at the survey sites. The survey was done in November, 1963. All of the 10,227 junior and senior high school students in Washington County enrolled in 19 public and parochial schools were offered skin tests with tuberculin (5 TU PPD-S) and histoplasmin (1:100 H-42). Coccidioidin tests (1:100 Cutter #99096) were also given to students in Hancock, Clear Spring and Williamsport. The prevalence of histoplasmin sensitivity was expected to be low in the western area around Hancock, intermediate farther to the east at Clear Spring, and high in Williamsport on the south central border of the county. This expectation was subsequently confirmed; consequently, testing with coccidioidin in only these three areas was thought to be sufficient to indicate the frequency and nature of cross-reactions between histoplasmin and coccidioidin in the county.

Tuberculin was administered intracutaneously on the volar surface of the right forearm, histoplasmin similarly on the left forearm. Coccidioidin was also given on the left, half of the tests being given above the site of injection of histoplasmin and half below. Glass syringes and platinum needles were used. The needles were sterilized by flaming between each test. Syringes were used for only one antigen.

At approximately 72 hours after testing, the test sites were carefully examined. The transverse diameter of any induration was measured and recorded in millimeters. Eighty-six per cent of the students had their tests read by the same nurse. When more than one reader was needed to complete the day's workload, a second nurse helped with the readings, but insofar as possible saw only those children who had lived outside the county at some time. For children with two tests on the left forearm, the readers were not allowed to know which reaction was due to coccidioidin and which was due to histoplasmin.

Tests were read for 7,787 students, 76 per cent of the total enrollment. Less than 2 per cent were non-white, and all but 2 per cent were between the ages of 12 and 19.

All survey records were matched against lists from a special census done in the summer of 1963 by the Johns Hopkins School of Hygiene and Public Health, National Cancer Institute and Washington County Health Department. Information from the census included the type of house and water supply, and the presence of domestic animals on the premises. Also included was the date when families moved into their present homes. If both these data and the residence history taken during the survey showed that not more than one year had been spent in another dwelling, the student was classified as a lifetime one-house resident.

Detailed maps available from the census and previous work by the National Cancer Institute allowed very accurate location of homes throughout the county. The type of soil on which the houses of the subjects were located was determined from soil maps published by the U.S. Department of Agriculture, based on examinations by soil scientists of every field and parcel of land in the county (23).

In 1965, it was decided to begin studies involving soil cultures for *H. capsulatum*. As an initial step, locations which seemed likely to yield positive cultures were selected. These were 14 elementary schools in areas of high prevalence of histoplasmin sensitivity and all homes, 86 in number, in which two or more lifetime residents had been tested and in which all were positive histoplasmin reactors. From each site, soil was collected from numerous areas. Samples were taken from the superficial

TABLE 1

Correlation of sizes of reactions to coccidioidin and histoplasmin among students in selected schools in Washington County, Maryland

	Mm induration to histoplasmin												Total	
	0–	2–	4–	6–	8–	10–	12–	14–	16–	18–	20–	22–	Number	Per cent
Mm induration to coccidioidin														
0–	501	1	26	53	123	194	147	68	18	3	2		1,136	96.1
2–					4	4	4						12	1.0
4–	2			1	3	7	3	2	1	1	1	1	22	1.9
6–	1			1	1		1						4	0.3
8–	1				1	2	2						6	0.5
10–														
12–	1												1	0.1
14–														
16–	1												1	0.1
Totals														
Number	507	1	26	55	132	207	157	70	19	4	3	1	1,182	
Per cent	42.9	0.1	2.2	4.7	11.2	17.5	13.3	5.9	1.6	0.3	0.2	0.1		100.0

layers of the soil. Whenever possible, the selected areas were moist, shaded and likely to be contaminated with bird droppings. Between 10 and 15 pounds of soil were collected from each site, and treated as a single sample after mixing.

Each sample was examined for *H. capsulatum* in two ways. After being allowed to run in cages in which the floor was covered with soil, mice were sacrificed and examined for *H. capsulatum*. The soil was also examined by intraperitoneal injection into mice, after the soil had been exposed to high humidity in a closed vessel (24). Characteristics of sites were not disclosed to the laboratory group until the examinations had been completed.

RESULTS

Association of histoplasmin sensitivity with personal and household characteristics. Of the 1,182 students tested with coccidioidin, only 6 had reactions to this antigen which were larger than their reactions to histoplasmin, as shown in table 1. Even if clerical error could be excluded, it does not appear that infection with *Coccidioides immitis* is a significant problem in Washington County. More important, there was no indication of the type of sensitivity found by Palmer, Edwards and Allfather to be characteristic of the area around east Texas, with small reactions to both histoplasmin and coccidioidin suggesting that the primary source of sensitivity to these antigens was infection with some other organism (25).

The distribution of all the tested students by size of reaction to histoplasmin was clearly bimodal, as is shown in figure 1. Slightly less than half of the students had no induration to histoplasmin. Among those with some induration, the mean reaction size was 10.3 mm, a value almost identical to that found among Navy recruits from states in the so-called endemic area (25). Although it would be justifiable with a distribution such as this to define a positive reactor as a person with 2 or more mm of induration, it was decided to abide by the usual, if somewhat arbitrary, definition of a positive reactor as a person with 5 or more mm of induration.

There were appreciable differences in the prevalence of histoplasmin sensitivity according to the residence history taken at the time of the survey. This is shown in table 2. Students who had lived less than one year outside of Washington County had the highest frequency of positive reactions, somewhat more than half of them being classified as positive. Those who had moved into the county but had not lived outside of Maryland had a lower frequency of positive reactions; those coming from other states ranked lowest in this respect, only one-third having positive reactions to histoplasmin. Lifetime residents of the county who had moved from place to place had a different frequency of sensitivity than those who had lived in only one area. Students coming from a high-prevalence area to a low-prevalence area were more likely to be positive reactors than the residents of the low-prevalence area. The reverse was true for students moving from a low- to a high-prevalence area.

To obtain the best possible index to factors possibly related to the geographic distribution of histoplasmin sensitivity, the subsequent analysis is restricted to children who were classified as lifetime residents of one house. The geographic distribution of histoplasmin sensitivity based on this group of 1,450 students is shown in figure 2. The basic units for this figure are socio-economic areas in the city of Hagerstown and election districts in the rest of the county. The fundamental pattern is not changed by the use of enumeration districts or elementary school districts. Only 10 per cent of the children in the westernmost district were infected. Approximately

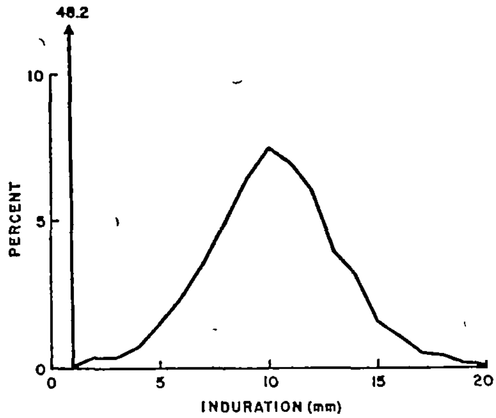

FIGURE 1. Percentage distribution of sizes of histoplasmin reactions among 7,787 students tested in Washington County, Md., 1963.

TABLE 2

Prevalence of histoplasmin reactors by residence history at time of survey

Residence other than Washington County	Total tested	5+ mm to histoplasmin	
		Number	Per cent
Totals*	7,787	3,934	50.5
Less than 1 year elsewhere	5,925	3,271	55.2
Other counties of Maryland	370	162	43.8
Other states or countries	1,489	501	33.6

* Includes 3 with no residence history.

one-third of those in the two northeastern districts were reactors, but there was a sharp division in these areas. Children living in the eastern portion of each district had almost no sensitivity; however, it was high among children from the western half of these districts. The city of Hagerstown is the rectangular area of moderate to low prevalence toward the upper right portion of the county. Throughout most of the city, prevalence ranged from 33 to 45 per

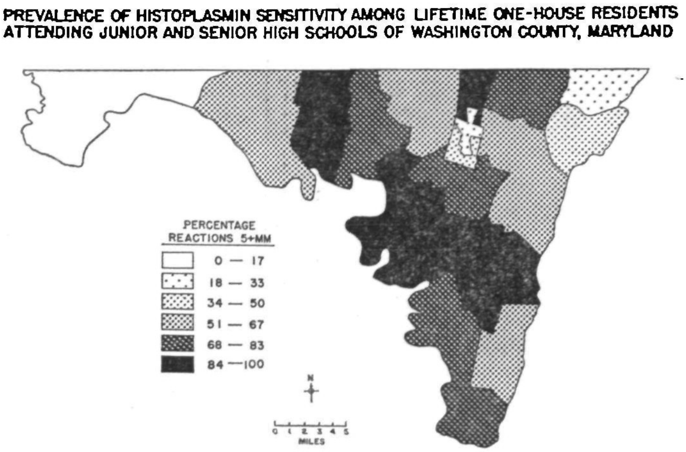

FIGURE 2. Map of Washington County showing prevalence of histoplasmin sensitivity by geographic subdistricts.

cent, except for the northernmost area and a keystone-shaped segment of the election district to the north of the city, which is a residential suburb, where the prevalence was only about 25 per cent. Except for the fact that these two low-prevalence areas are residential and wooded, there are no obvious physical differences from the farmland to the north with scattered houses and some trailer homes along the main highway. In this rural part of the election district directly north of the city, 96 per cent of the children were reactors. In addition, it can be seen that there are other bands of very high prevalence across each arm of the L-shaped county.

No features of climate, topography or agriculture can be related to these extreme variations in the frequency of histoplasmin sensitivity. Washington County is located at the narrowest portion of western Maryland where at one point only a few miles of the county separate Pennsylvania from West Virginia. The southern boundary of the county is formed by the Potomac river, and all of the county lies within its watershed, with most of the creeks meandering southward to the Potomac. The eastern edge of the county is formed by the western slopes of the Blue Ridge Mountains, containing the highest point of the county (elevation, 2,145 feet). At their foot is the Hagerstown Valley, part of the Great Limestone Valley of eastern states. It is approximately 20 miles wide at this point, with an elevation of 300 to 600 feet. The valley floor is generally level to rolling, with local ridges and frequent outcroppings of limestone. The western half of the county, as measured along the straight northern border, extends into the eastern slopes of the Appalachian Mountains, and consists of a series of narrow valleys and ridges, ranging up to 2,000 feet high. Most of the county is well drained, and there are no swamps or marshes.

Sixty per cent of the acreage in Washington County is suited for regular cultivation, 15 per cent is limited, and 12 per cent is fit only for pasture. The remaining 13 per cent is mostly rough and rocky, and some of it is severely eroded and mountainous (23). Dairy farming and orchards comprise the major portions of the county's agriculture, the former located mostly in the Hagerstown Valley, and the latter concentrated in the westernmost district and the northern half of the Blue Ridge slopes in the east. Poultry raising, while common, is not a major component of local agriculture (26).

The 1,450 lifetime residents of one house came from 1,121 households. There were 851 children who were the only members of their household to be tested. The remaining 599 came from 270 households, and as could be predicted from the geographic distribution of reactors, there was a marked tendency for all children in a household to react similarly to histoplasmin.

At all ages, boys were slightly more likely than girls to be positive reactors, the overall prevalence for boys being 56.7 per cent, and for girls 54.5 per cent. Prevalence increased markedly with age, ranging from 46.4 per cent among those born in 1951 to 65.1 per cent among those born in 1946. The probability of a resident becoming infected, as estimated from the age-prevalence curves, varied markedly with geographic area. Where the overall prevalence was more than 83 per cent, the estimated rate of acquiring new infections was nearly 20 per cent per year. In areas where only 18 to 33 per cent of the students were infected, the estimated infection rate was between 1 and 2 per cent per year. In the westernmost area, there was no increase in prevalence with age.

The use of crude prevalence ratios did not seem appropriate for the analysis of the relationship of histoplasmin sensitivity to various housing factors. Because 36 per cent of the residents of the city of Hagerstown and 63 per cent of the non-city residents were reactors, it is obvious that anything associated with rural living would automatically show a positive correlation with prevalence of histoplasmin sensitivity. To minimize this built-in association, the following procedure was adopted. The geographic districts were stratified with respect to frequency of histoplasmin sensitivity in the same subgroups used for figure 2, namely 0–17, 18–33, 34–50, 51–67, 68–83 and 84–100 per cent. It was reasoned that any factor that might be causally associated with histoplasmin sensitivity should be manifest within each stratum, and that a stratum-adjusted rate would be the best summary of the strength of that association. Stratum-adjusted rates are used for tables 3 through 7. In these and subsequent tables, the totals are not always the same because persons for whom pertinent information was not available have been omitted.

TABLE 3

Adjusted prevalence of histoplasmin reactions among lifetime residents of one house, by year house was built*

Year built	Total tested	5+ mm to histoplasmin	
		Number	Adjusted per cent*
Before 1900	410	275	58.1
1900–1944	632	324	56.4
1945–1954	361	168	47.8

* See text for method of adjustment.

The prevalence of histoplasmin sensitivity among students according to the age of the house in which they lived is shown in table 3. There is a modest trend toward increased likelihood of being a reactor to histoplasmin for residents of old houses. This finding might signify that dusts containing *Histoplasma capsulatum* are more common in or around old houses, possibly as

TABLE 4

Adjusted prevalence of histoplasmin reactions among lifetime residents of one house, by material used for outside walls

Material for walls	Total tested	5+ mm to histoplasmin	
		Number	Adjusted per cent
Stone	65	51	60.2
Wood & stone	72	44	58.2
Asbestos shingle	325	176	54.9
Brick	387	210	54.0
Wood	361	189	53.8
Concrete	118	53	49.6

TABLE 5

Adjusted prevalence of histoplasmin reactions among lifetime residents of one house, by type of basement floor

Basement floor	Total tested	5+ mm to histoplasmin	
		Number	Adjusted prevalence
Concrete	852	436	52.5
Other	453	264	55.5

TABLE 6

Adjusted prevalence of histoplasmin reactions among lifetime residents of one house, by source of drinking water

Source of water	Total tested	5+ mm to histoplasmin	
		Number	Adjusted prevalence
Spring	105	78	62.8
Deep well	273	168	53.2
City system	727	319	51.8
Cistern	200	132	51.5
Shallow well	74	45	49.6

the result of accumulated exposures to birds or bats over the years, or that old houses were more likely to have been built in areas of the county where conditions are favorable for the growth of or infection by the organism. Relationship of material used for house walls to histoplasmin sensitivity is shown in table 4. Prevalence was highest among residents of stone houses, and lowest among residents of houses with concrete walls. This finding may reflect the same factors as age of house, for with the exception of a number of log buildings still in use, many of the older homes are constructed of stone while concrete walls are found only in more recently built homes. Because of findings associated with two epidemics of histoplasmosis, it was thought that conditions favorable to infection with *H. capsulatum* might be found in basements without concrete floors (21). However, as shown in table 5, the evidence did not favor this hypothesis.

H. capsulatum is reported to be able to grow in water (27, 28), and bird droppings are thought to improve the likelihood of its growth (18). Because many cisterns are filled with rainwater from roofs, subject to contamination with spores and bird droppings, it seemed possible that infections with histoplasmosis might be more common among students whose drinking water came from cisterns. This turned out not to be so, as can be seen in table 6. Students whose drinking water came from springs showed a higher than average prevalence of positive reactions, which may reflect the same factors as those associated with old houses, for the early homes were often located near springs. Another possibility is that the soil near springs may be damp, and dampness is believed to favor the growth of *H. capsulatum* (6, 17, 29–31).

A final attribute available from the census records was the presence or absence of certain domestic animals on the property. Their relationship to the

TABLE 7

Adjusted prevalence of histoplasmin reactions among lifetime residents of one house, by presence or absence of domestic animals on premises

Animal	Animal present			Animal absent		
	Total tested	5+ mm. to histoplasmin		Total tested	5+ mm to histoplasmin	
		Number	Adjusted prevalence		Number	Adjusted prevalence
Horses	79	63	62.6	1,350	715	54.0
Chickens or birds	410	262	60.2	1,018	515	52.4
Cattle	241	180	59.5	1,188	598	53.0
Cats	674	415	56.7	754	362	51.9
Dogs	889	514	56.2	539	263	51.4

frequency of reactors is shown in table 7. In all instances, the presence of these animals—horses, chickens or birds, cattle, cats and dogs—was associated with a slightly increased prevalence of histoplasmin sensitivity, whether considered singly or in combination. However, in no instance was this association marked, and horses were as closely associated with histoplasmin sensitivity as chickens or birds. This finding lends little support to the widely held theory that bird droppings are highly associated with histoplasmosis (18).

Association of histoplasmin sensitivity with soil characteristics. Because it is generally agreed that *H. capsulatum* is an inhabitant of the soil, it seemed reasonable to assume that its presence might be correlated with certain soil characteristics. And if this were so, children whose homes were located on soils conducive to the presence of the organism should be more likely to be infected than children whose homes were located on unfavorable soils. Thus, a clue to soils that might be best suited to the growth of *H. capsulatum* could be provided by the prevalence of histoplasmin sensitivity among children living in areas with different soil types.

Because the locations of all houses had been accurately recorded for the 1963 census, the type of soil on which they were situated could be determined from detailed soil maps published by the U.S. Department of Agriculture, except for houses in Hagerstown and its built-up suburbs (23). The analysis was restricted to homes of children who had been lifetime residents of one house. These homes were located on 75 different soil types but many soils were represented by very small numbers of children, and only 15 soil types were represented by 20 or more children each. The prevalence of histoplasmin reactors among children residing on these 15 soils ranged from 88.0 per cent to 16.7 per cent, with no pattern discernible to inspection.

There was less but still considerable variation in the prevalence of reactors living on the great soil groups. These results are shown in table 8. The highest prevalence was found among residents on gray-brown podzolic soils, and the lowest among those living on red-yellow podzolic soils. One difference in these two soil groups is the degree of acidity, the former being neutral or slightly acid, the latter tending to be strongly acid.

The prevalence of reactors according to the parent material of the soils is

TABLE 8

Prevalence of histoplasmin reactions among lifetime residents of one house in rural areas, by great soil group

Great soil group	Total tested	5+ mm to histoplasmin	
		Number	Per cent
Gray-brown podzolic	566	407	71.9
Alluvial	36	24	66.7
Gray-brown & red-yellow podzolic	48	29	60.4
Lithosols	124	66	53.2
Red-yellow podzolic	122	51	41.8

TABLE 9

Prevalence of histoplasmin reactions among lifetime residents of one house in rural areas, by parent material for soil

Parent material	Total tested	5+ mm to histoplasmin	
		Number	Per cent
Micaceous schists & phyllites	27	24	88.9
Limestones	323	240	74.3
Limestone with sandstone or shale	213	158	74.2
Quartzite	48	29	60.4
Sandstones	32	19	59.4
Shales	122	59	48.4
Crystalline rock materials	25	12	48.0
Metabasalt	64	26	40.7
Shales and sandstones	74	29	39.2

shown in table 9. Soils derived from limestones tended to have a higher proportion of reactors than almost all of the others.

A similar conclusion emerged when the prevalence of reactors was studied according to the agricultural capability of the soils. There was no correlation with suitability for different types of crops or other agricultural uses. Of the soil capability units represented by 20 or more tested children, the units with the highest and lowest proportion of reactors were both steep and eroded. For the high-prevalence unit, it was noted that "rockiness consists of limestone outcroppings", while the low-prevalence unit was not associated with limestone. Only 15 children lived on soils considered to be limited because of wetness and poor drainage, but it should be noted that 11 or 73 per cent of them were reactors, while reactors comprised only 60 per cent of those living on soils limited by erosion.

In keeping with the foregoing findings, there was a marked correlation of the prevalence of reactors with pH of the soil (table 10). Only a third of the lifetime residents on strongly acid soils were positive reactors, while among those on alkaline soils almost 90 per cent reacted to histoplasmin.

No discernible correlation of prevalence of reactors could be noted with the suitability of the soil for forestry, irrigation or sewage disposal. A similar lack of correlation was noted with various engineering characteristics of the soils such as weight bearing capacity, dispersion, and shrink-swell potential.

TABLE 10

Prevalence of histoplasmin reactions among lifetime residents of one house in rural areas, by pH of soil type on which house was located

Median pH	Total tested	5+ mm to histoplasmin	
		Number	Per cent
7.0 and over	27	24	88.9
6.5–6.9	77	57	74.0
6.0–6.4	234	180	65.6
5.5–5.9	156	102	65.5
Under 5.5	181	66	36.5

Soil cultures for Histoplasma capsulatum. None of the soil specimens from schools was positive. From the 86 specimens collected around houses in which all the tested lifetime residents were positive histoplasmin reactors, there were 11 which yielded *H. capsulatum.* There was a marked association of a history on the 1963 census regarding the presence or absence of chickens and domestic birds with the proportion of positive isolations (table 11). *H. capsulatum* was isolated from 26 per cent of the sites with chickens or birds, and from only 2 per cent of those without birds.

Because of the manner in which the sampling sites were selected, there was relatively little variation in the great soil types represented. However, the variation in pH was sufficient to make analysis worthwhile. The results are shown in table 12, both according to the median pH of the soil type reported by the U.S. Department of Agriculture (23), and by the pH found on examination of the sample collected. Because the sampling extended into the built-up suburbs of Hagerstown, it was not possible to estimate the soil type for 12 of the sampled sites. However, in contrast to the association of alkaline pH with histoplasmin sensitivity, there was no evidence of any association of median pH with the frequency of isolations.

TABLE 11

Isolations of Histoplasma capsulatum *from selected sites in Washington County, by presence or absence of chickens or other domestic birds on premises*

Chickens or domestic birds	Sites cultured	Isolations	
		Number	Per cent
Present	38	10	26.3
Absent	43	1	2.3

TABLE 12

Isolations of Histoplasma capsulatum *from selected sites in Washington County, by pH of soil type on which house was located and by pH of sample collected*

	pH of soil type			pH of sample		
	Sites cultured	Isolations		Sites cultured	Isolations	
		Number	Per cent		Number	Per cent
7.0 and over	4	1	25.0	26	0	0.0
6.5–6.9	16	3	18.8	22	2	9.1
6.0–6.4	33	2	6.1	15	3	20.0
5.5–5.9	11	2	18.2	15	5	33.3
Under 5.5	10	1	10.0	8	1	12.5
Totals	74	9	12.2	86	11	12.8

Furthermore, when pH was determined by actual measurement of the samples, it was found that isolations were most frequent from soils of moderate acidity, a result in keeping with those of others (16, 32).

DISCUSSION

A number of fortunate circumstances combined to strengthen the present study. First was the ability to limit the analysis to a large number of children who were lifetime residents of single houses which could be accurately located on detailed maps. Basing residence history on questions asked in different ways at two different times increased the certainty that the sample represented a highly stable population. The study variables were collected entirely independently. Skin tests were measured without knowledge of residence or other historical information. Because most of the tested children attended consolidated schools representing large geographic areas, the reader of the histoplasmin tests could identify the area of residence only within very broad limits. Information about housing, soil types, and exposure to animals was collected independently of the skin test survey. Soil specimens were identified only by code numbers, so that soil isolations were performed without knowledge of the characteristics of the sites from which an individual specimen had come.

A major strength was the wide range of frequencies of histoplasmin reactors among junior and senior high school children in Washington County. The distribution of sensitivity within the county bore no relation to any obvious topographic feature. Areas of very high and low prevalence were found along the Potomac river valley; high and low prevalence areas were also found along the Blue Ridge mountains. The low prevalence of sensitivity within the city of Hagerstown and one of its built-up suburbs to the north afforded the only clearcut association with any discernible geographic feature. The other built-up suburbs east and southwest of the city showed moderate to high prevalence of sensitivity among the lifetime residents. There was no correlation with the major type of farming practiced.

The marked variation in sensitivity within short distances in itself indicates that climatic or meteorologic factors do *not directly affect the probability* of human infections except in the sense of imposing broad limitations upon the growth or infectivity of the organism. Man's disturbance of the soil, indicated as the cause of human infections in urban epidemics (12), could not be associated with the observed patterns of histoplasmin sensitivity in Washington County. The only major disturbances of soil during the lifetime of the tested children involved construction of portions of three highways, an airport runway, a large industrial plant, a shopping center and two high schools. The airport runway is located in the northwest corner of the very high-prevalence area north of Hagerstown, and with generally westerly winds could have been related to the sources of these infections. All of the other sites are either located within low-prevalence areas, or are so situated that prevailing wind conditions should have exposed high and low prevalence areas equally. Home construction with its attendant soil disturbance has been most marked in the suburbs to the north, east and southwest of Hagerstown. The considerable variation in prevalence in these areas has already been noted. Most striking is the keystone-shaped suburb to the north of the city, which has remained a low-prevalence area in spite of considerable construction in a wooded section, in marked contrast to the very high prevalence around it. It is also noteworthy that the prevalence of histoplasmin sensitivity among boys is only very slightly greater than among girls, in spite of the fact that the farm boys are heavily exposed to dust during plowing and harvesting.

A further inference can be drawn from the striking variation in frequency of histoplasmin sensitivity relative to the *location of the children's homes*. Even children who have lived in one house all their lives lead highly mobile lives. If exposures away from home were important determinants of the likelihood of becoming infected, such marked variations could not be demonstrated by place of residence. Something very close to home, applying equally to boys and girls, must be the major source of infections in this part of the country, and possibly elsewhere too.

Although studies in Washington County based on isolations of *H. capsulatum* are limited, confidence in the results is increased because they are consistent with those of others (16, 18, 32). The presence of chickens or other domestic birds on the premises greatly increased the likelihood that *H. capsulatum* could be demonstrated in the soil. Isolations were also more frequent when the soil samples had a moderately low pH.

A striking finding of this study is that human infections, as indicated by reactions to the histoplasmin skin test, did not follow the pattern observed for soil isolations. Positive histoplasmin reactions were only slightly more common on premises where chickens or other domestic birds were kept, and were more likely to be found among children who lived on alkaline than on acid soils. Sufficient numbers of children were examined to make it highly unlikely that these observations resulted from chance. It thus appears that environmental factors favoring sensitization of humans by *H. capsulatum* must be different from the factors favoring isolation of the organism from soil samples with current technics.

To reconcile the discrepancies between the observed distribution of *H. capsulatum* and the distribution of histoplasmin sensitivity requires speculation, which to some extent has been put forth by others (33). Let us assume that conditions favoring the growth of *H. capsulatum* also reduce the likelihood that a given dose of organisms will cause an infection among humans living in the area, and that conditions making growth more difficult but not impossible increase the likelihood of the same numbers of organisms causing human infection. Let us further assume that laboratory isolation of the organism is possible from sites where growth conditions are favorable, but that our present technics do not allow successful isolations under conditions where growth is difficult. Among the various characteristics of the organism which might be related both to the ease of isolation and to its ability to infect humans is the formation of spores of varying sizes.

It is generally accepted that inhaled particles larger than 5 microns in diameter rarely reach the pulmonary alveoli, being trapped in the respiratory passages along the way. Particles less than 0.1 micron reach the alveoli readily but usually pass out again with the exhaled air. The optimal size for alveolar retention appears to be on the order of 1 micron, depending presumably on density of the inhaled particle as well as on its size (34–35). The spores of *H. capsulatum* are rarely larger than 16 microns in diameter. Most are less than 5 microns but considerable variation in the distribution of spore sizes has been reported (33, 37, 38). In one series of 14 strains cultured on artificial media for 1 month, the proportion of spores approximating 1.6 microns in diameter varied from 9 to 83 per cent (33). The infectivity of these strains, if the spores were to become airborne, would presumably show similar variation.

The numbers of spores produced in a given situation could also affect infectivity. Even with a growth situation that produced a very high proportion of spores too large to reach the alveoli, the numbers of small spores could reach levels high enough to result in a high risk of infection if vast numbers of spores were formed. This sort of situation could fit the epidemics of histoplasmosis where it is often relatively easy to isolate the organism and exposed humans are very likely to become infected.

This line of speculation is partially

substantiated by the few available facts. Experience with dusts and other organisms makes it reasonable to believe that small spores are most likely to cause airborne infections. Unfortunately, almost nothing is known about the types of spores found under natural conditions, largely because only the large tuberculated spores are readily recognizable microscopically, and because the effect of culturing technics on spore formation and size is not known. It does appear from one study that marked variation in spore size may occur within the endemic area of the United States (33). But another study that also showed variation in spore sizes between strains is not consistent with the hypothesis set forth in this paper. Emmons found that strains isolated from south Georgia where human infections are rare produced large numbers of small spores, while strains from Loudoun County, Virginia, an area with a high frequency of human infections, had a higher proportion of large spores (37).

However, the major purpose of this speculation is not to suggest that spore size is *the* important determinant of the pathogenesis and epidemiology of histoplasmosis. By emphasizing again that conditions favoring the isolation of the organism from the soil are not necessarily the same as those favoring human infection, it is hoped that investigations into the basic biology of *H. capsulatum* will be encouraged. It is likely that much more will need to be learned about the fundamental growth habits of the organism before the epidemiology of histoplasmosis can be fully understood.

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