ENVIRONMENTAL FACTORS INFLUENCING THE OCCURRENCE OF HISTOPLASMA CAPSULATUM AND MICROSPORUM GYPSEUM IN SOIL[1, 2]

L. D. ZEIDBERG AND LIBERO AJELLO

Tennessee Department of Public Health, Williamson County Tuberculosis Study, Franklin, Tennessee, and Communicable Disease Center, Public Health Service, Atlanta, Georgia

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The ecological concept of disease as defined by Gordon and Augustine (1948) emphasizes the influence of the environment on the occurrence and distribution of morbid conditions among aggregations of individuals. That environmental factors may profoundly affect agents of disease as well as human and animal hosts is well known. That alterations in the environment may disturb the balance between agent and host in favor of one or the other has been demonstrated again and again. Yet, in considering the ecology of disease, emphasis is often placed almost exclusively on the relationship of man to his environment, whereas the influence of the environment on the etiological agent is either forgotten or ignored.

Much may be learned in the laboratory concerning the cultural requirements of microorganisms. Nevertheless, under artificial, controlled laboratory conditions it is not possible to duplicate the many physical, chemical, and biological forces that affect the survival and reproduction of organisms in their natural habitat. However, by investigating the natural occurrence and distribution of agents of disease, by studying the conditions and circumstances under which they appear in one place and not in another, the true relationship of an organism to its environment eventually may be established. Such knowledge may serve not only to explain differences in the geographical distribution of disease but also may indicate the most probable natural reservoirs of infection and potential endemic areas of disease.

As part of the general epidemiological studies of histoplasmosis in Williamson County, Tennessee, soil was cultured for *Histoplasma capsulatum*. Purely by chance, macroconidia of *Microsporum gypseum* were isolated from a sample of soil that also had harbored the other fungus (Gordon *et al.*, 1952). Later on, the desire to test a procedure developed by Vanbreuseghem (1952) for the selective isolation of dermatophytes from soil led to the survey of some of the collected Williamson County soils for the presence of that group of fungus parasites. This resulted in the recovery of such a large number of isolates of *M. gypseum* (Ajello, 1953) as to permit an analysis of the comparative ecology of the two fungi. It is the purpose of this paper to report these observations.

MATERIALS AND METHODS

The mycological study of soil was begun on July 5, 1950. Soil was collected from all of the 23 civil districts in the County in order to obtain a wide geographical distribution of sampling. Other conditions for the selection of sites were: (1) at least two residents of the premises had had histoplasmin sensitivity tests, and those tested were predominantly either reactors to histoplasmin or nonreactors; or (2) a proved or suspected case of active histoplasmosis resided on the premises (there were two proved cases and one suspected case).

An average of about 5 samples of soil was obtained from each of the sites visited. In general, soil was collected from places where the activities of the residents were most apt to be concentrated, such as around the dwelling, in or near barns, chicken houses, springs, wells, etc. Samples were identified by number, and a record was kept of the source of the sample. Notation was made of possibly significant observations,

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such as the dampness of the soil, its contamination with animal excreta, and the presence of moss or fungi on the surface. About 4 ounces of surface soil were collected for each sample and placed in a sterilized, screw-cap bottle. These were shipped to the Mycology Laboratory of the Communicable Disease Center where they were studied according to the methods described below. The mycologist had no knowledge of the source of the soil samples. They were identified by number only, and only after his results had been reported were the sources made known to him. Any possibility of bias was thereby eliminated.

Separate procedures were employed in the search for *H. capsulatum* and *M. gypseum* in soil.

Procedure for H. capsulatum. Approximately 10 ml of each soil sample were added to 30 ml of physiological saline contained in 25 by 150 mm test tubes. The suspension was stirred and permitted to stand for at least one hour. Five ml of the supernatant were pipetted off, and one ml aliquots were injected intraperitoneally into each of 4 mice. The mice were treated for 5 days once daily with 1,000 units of streptomycin and 12,500 units of penicillin. The mice were sacrificed at the end of 8 weeks, and two tubes of neutral glucose peptone agar were inoculated with portions of the liver and two tubes with portions of the spleen. The tubes were incubated at 25 C and examined at regular intervals over a period of 6 weeks for the presence of pathogenic fungi.

Although the technique used to isolate *H. capsulatum* is basically similar to that employed by Emmons (1949), it has undergone several modifications during the course of the study.

At first the supernatant from a suspension of soil in physiological saline was injected directly into the peritoneal cavity of mice. Following the observations of Strauss and Kligman (1951) that gastric mucin lowered the resistance of mice to mycotic infections, the supernatant subsequently was mixed with equal parts of 5 per cent gastric mucin prior to injection into mice. This change necessitated treating the mice with penicillin and streptomycin over a period of 5 days to prevent the occurrence of death due to the presence of virulent bacteria in the soil inocula.

Despite the fact that 11 isolates of *H. capsulatum* were obtained from 299 soil samples using gastric mucin (Zeidberg *et al.*, 1952) questions arose regarding its effectiveness and the over-all efficiency of the mouse in recovering *H. capsulatum*. Accordingly, a series of controlled experiments was initiated which revealed that the mouse was extremely susceptible to infection by *H. capsulatum* (Ajello and Runyon, 1953). Following insertion of single tuberculate spores into the peritoneal cavity, the fungus was recovered in a high proportion of the mice. It was found that the rate of recovery of the organism was correlated directly with the length of time elapsing between inoculation and sacrifice of the mouse. Recovery approached 100 per cent at the end of 8 weeks. Surprisingly enough, gastric mucin did not permit earlier recovery of the fungus.

On the basis of these observations the procedure just described was developed.

Procedure for M. gypseum. A procedure developed by Vanbreuseghem (1952) for the selective isolation of keratinophilic fungi from soil was employed in the search for dermatophytes. Sterile petri dishes were half filled with soil, moistened with sterile distilled water, and baited by placing short strands of autoclaved human hair upon the surface of the soil. These preparations then were incubated at room temperature in a dark cupboard and examined periodically for the development of mycelium on the hair filaments. Hairs overgrown with mycelium were examined under the microscope and cultured on a medium containing cycloheximide, penicillin, and streptomycin. This medium had been found to permit the selective isolation of most human pathogenic fungi from a wide variety of sources that were contaminated heavily with bacteria and saprophytic molds (Georg, 1953; Ajello and Getz, 1954; Georg *et al.*, 1954).

RESULTS

Between July 5, 1950, and March 28, 1952, 493 samples of soil were collected from 112 premises in the County. All of these samples were cultured for *H. capsulatum* by the method described above. Seventy-one soil specimens selected at random from the above group were surveyed for the presence of dermatophytes (Ajello, 1953) using Vanbreuseghem's procedure. The results of these investigations are shown in table 1.

Analysis by source of the samples indicated a clear-cut difference in the nature of the soils

TABLE 1

Results of examinations of soil samples collected from 112 premises by source of sample, Williamson County, Tennessee, July, 1950, to March, 1952

SOURCE OF SAMPLE	HISTOPLASMA CAPSULATUM			MICROSPORUM GYPSEUM		
	Samples examined	Isolations		Samples examined	Isolations	
		Number	Per cent		Number	Per cent
Total	493	28	5.7	71	27	38.0
Under dwelling	83	6	7.2	14	4	28.6
Near dwelling	136	1*	0.7	15	9	60.0
Inside chicken house	71	13	18.3	12	0	—
Chicken yard	32	8	25.0	7	1	14.3
Barnyard	44	0	—	12	8	66.7
Inside barn	14	0	—	5	4	80.0
Bank of water course	64	0	—	1	0	—
In open	33	0	—	2	1	50.0
Other	16	0	—	3	0	—

* Grossly contaminated with chicken manure.

that harbored the two fungi. Thus, the largest percentage of isolations of *H. capsulatum* was from soils collected inside chicken houses and chicken yards, whereas the greatest proportionate yield of *M. gypseum* was from soils obtained inside barns and in barnyards.

H. capsulatum was found with significantly greater frequency in soils from sheltered sites as compared to other soils. The fungus was isolated from 11.3 per cent of 168 samples of sheltered soils compared to only 2.8 per cent of 325 specimens from unsheltered sites. On the other hand, *M. gypseum* was cultured from 25.8 per cent of 31 sheltered soil specimens and from 47.5 per cent of 40 other soil samples. Although these latter findings were within the probability of chance variation, the fact that they were in the opposite direction to those observed for *H. capsulatum* may be of some significance in comparing the two fungi ecologically.

DISCUSSION

Since *H. capsulatum* is a systemic pathogen, whereas *M. gypseum* is known to attack only ectodermal tissue, inherent differences in their physiology are to be expected. It is indeed surprising that both fungi should have been found living freely in soil as demonstrated by the finding therein of the tuberculate spores of *H. capsulatum* (Emmons, 1949) and the macroconidia of *M. gypseum* (Gordon *et al.*, 1952), structures produced only on inanimate substrata. It would be more surprising, however, if both could find optimal conditions for survival under identical environmental conditions. The data presented indicate rather that these two organisms have distinct ecological requirements.

H. capsulatum appears to prefer soils upon which chickens have congregated. There is no ready explanation for the frequent association of chickens and soils harboring *H. capsulatum*. On the other hand, *M. gypseum* is a keratinophilic fungus and is not unexpected, therefore, to be found in soils upon which keratin is apt to be shed. Thus, the highest proportion of isolations of this fungus was obtained from soils collected in barnyards, inside barns, and under and around dwellings where wild and domestic animals tend to concentrate in their search for food and shelter. As indicated elsewhere (Ajello, 1953), knowledge that *M. gypseum* commonly occurs in soil may well alter heretofore accepted concepts of the epidemiology of ringworm infections caused by this agent.

In a previous report (Zeidberg *et al.*, 1952) reference was made to the occurrence of acute pulmonary infections resembling histoplasmosis in individuals who had been exposed to high concentrations of pigeon or chicken excreta. Recently additional suggestive evidence of the implication of fowl excreta in epidemics of histoplasmosis has appeared in the literature (Grayston and Furcolow, 1953). There is, however, only empiric observation of this association, but no explanation. Histoplasmin testing of chickens in Williamson County (Zeidberg *et al.*, 1952) and in Central Missouri (Menges, 1951) indicates that in areas where the prevalence of sensitivity in humans is quite high, in chickens it is negligible. Studies conducted by one of us (L. A.) indicate that *H. capsulatum* will not grow *in vitro* at 42 C, the normal body temperature of fowl. Similar observations have been made by other investigators (Menges *et al.*, 1952). Efforts to infect chickens by the intraperitoneal inoculation of either the tuberculate spores or yeast cells of *H. capsulatum* resulted in failure. A similar unsuccessful effort to establish the fungus in chicks was experienced by Thomison[3]

[3] Department of Pathology, Vanderbilt University Medical School.

(personal communication, 1953) who tried intracerebral, intravenous, and intraperitoneal inoculations of yeast phase cells. Cultures of 25 samples of chicken manure collected in Williamson County were negative for *H. capsulatum*. The evidence is strongly against implicating fowl either as reservoirs or as carriers of the disease. Yet, they appear in some way to be associated with the occurrence of the fungus in nature. It is suggested that some components or properties of fowl excreta, along with other factors, may be of importance in determining the presence of this fungus in the soil. Since chickens are distributed throughout the world, while endemic histoplasmosis has a limited geographic distribution, it is obvious that other factors, perhaps inherent in soil itself, must play an important role in the occurrence of the fungus in a particular area.

M. gypseum, in contrast to *H. capsulatum*, appears to have a more general distribution in nature. It is not confined in the main to one type of habitat but apparently occurs wherever keratinaceous material is deposited.

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SUMMARY

Histoplasma capsulatum was cultured from 5.7 per cent of 493 soil samples collected in Williamson County, Tennessee, and *Microsporum gypseum* was isolated from 38.0 per cent of 71 of these samples.

H. capsulatum was found predominantly in soils obtained from chicken houses and chicken yards. Investigations indicate that chickens are not a reservoir of histoplasmosis, and their association with the fungus remains unexplained.

M. gypseum was found chiefly in soils from barns, barnyards, and around dwellings where animals are apt to be concentrated. This finding may be explained by the keratinophilic character of the dermatophyte.

The comparative ecology of *H. capsulatum* and *M. gypseum* is discussed, and the importance of studying the occurrence of microorganisms in their natural habitat is emphasized.

Methods for isolation of both fungi from soil are described.

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