Direct Exposure of Mice to Soils Known to Contain *Histoplasma capsulatum.* (22890)

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No report of natural infection of laboratory animals by direct exposure to soil known to contain *Histoplasma capsulatum* has appeared in the literature. However, animals have been successfully used to recover the fungus indirectly from soil(1) and from air samples(2) collected at point sources of human cases of histoplasmosis. Failure to isolate the etiological agent from soil and other material from sites of epidemics of histoplasmosis in humans has led to direct exposure of animals to these areas. In one reported epidemiologic study of an outbreak of histoplasmosis, a healthy dog, negative to histoplasmin skin test, was exposed to premises where humans and animals had become infected. This procedure resulted in disseminated histoplasmosis in the exposed animal(3).

It would be of interest to determine the effect of exposing animals directly on soils containing the mycelial phase of *H. capsulatum.* Through such studies the efficiency of the method to produce experimental histoplasmosis could be determined. Further evidence as to the natural method of contracting the disease might be obtained. In addition, a comparison of this method to that of usual technics for isolation of the fungus from soil could be made.

Methods. White Swiss mice, 4 to 5 weeks old, were exposed to 2 types of soil samples: one naturally contaminated; the other artificially inoculated with the mycelial phase of *H. capsulatum.* Naturally contaminated soil was collected 5 months previously from a farm where an epidemic of histoplasmosis had occurred. The fungus had been successfully isolated from this sample during routine epidemiologic studies. From the time of original collection, the sample was maintained at room temperature in the pasteboard carton in which it had been collected. During this period the soil had become quite dry. The artificial soil culture was prepared 16 months prior to its use by inoculating 100 ml of sterile soil contained in 250 ml Erlenmeyer flask, with a suspension of the mycelial phase of *H. capsulatum.* The soil culture was incubated at room temperature in a jar in which relative humidity of 100% was maintained. One week prior to the experiment, the flask was removed from the humidity jar and the soil allowed to dry. Approximately 100 ml of each type of soil was placed in the bottom of 2 clean, stainless steel mouse cages. Ten White Swiss mice were placed in each cage. Five control mice were placed in a third cage on clean sawdust. The 3 cages were placed in close proximity in a safety hood. Ultraviolet lights and blower to filter remained on. Mice were protected from exposure to ultraviolet rays by a layer of heavy wrapping paper between the light source and top of cages. After animals had been exposed to the soil for one week, they were transferred to clean cages and retained in the safety hood. Four weeks after removal from the soil the mice were sacrificed and autopsied. The liver, spleen and lungs of each mouse were cultured separately on Sabouraud's agar containing 20 units of penicillin and 40 units of streptomycin per ml. The plates were incubated at room temperature for one month, and observed periodically to determine the presence of *H. capsulatum.* By similar procedure, an attempt was made to determine the period of exposure necessary for mice to contract histoplasmosis. The naturally infected soil, proved positive for *H. capsulatum,* was obtained from a different epidemic site and maintained 10 months prior to its use. Identical artificially inoculated soil cultures were used. Twenty-one mice were exposed to each type of soil and 7 mice served as controls. At intervals of 1, 2, and 4 days, 7 mice were removed from each experimental soil and placed in clean cages. Four weeks after their removal from the soil, each group of mice was

TABLE I. Exposure of Mice for 7 Days to Soils Seeded with *Histoplasma capsulatum.*

Infected soil	No. days exposed	No. mice pos./No. autopsied	% positive	% tissue positive		
				Liver	Spleen	Lung
Natural	7	8/8	100	100	100	100
Artificial	7	9/9	100	100	100	100
Controls	*	0/5	0	0	0	0

* Not exposed.

sacrificed and autopsied. Tissues were cultured and observed in the same manner as the previous series.

Results. Table I presents the results of experimental histoplasmosis produced in mice exposed for 7 days to natural and artificially seeded soils with *H. capsulatum.* It is of interest that disseminated histoplasmosis occurred in all mice exposed to these soils for 7 days. The fungus was isolated from liver, spleen, and lungs of all experimental mice. No intestinal lesions or lymph node enlargements were observed. All tissues from control mice were negative for the fungus. Two animals from naturally contaminated and one from artificially seeded soils were destroyed by cannibalism during the 4 weeks following their removal from soils.

In the second series of experiments, periods of exposure to naturally and artificially seeded soil samples were varied. The results are summarized in Table II. All mice placed on artificially seeded soil developed disseminated disease after 1, 2 or 4 days of exposure. The number of colonies of *H. capsulatum* that developed on Sabouraud plates from various tissues, suggests little or no difference in degree of infection after 1, 2 or 4 days exposure on soils.

There was a difference in susceptibility of mice on contaminated soil in that one mouse exposed for one day and another exposed for 4 days did not yield positive cultures from any tissue. However, all 7 mice exposed for 2 days developed disseminated histoplasmosis. The fungus was isolated from liver and spleen of all 7 mice and from 6 of the 7 lungs cultured on Sabouraud's medium. The plates of lung tissue from one mouse were contaminated and overgrown within the first week of incubation. This contaminating fungus made it impossible to detect the presence or absence of *H. capsulatum* in lungs of this mouse. Examination of the intestines and lymph nodes of all animals revealed no gross pathology. However, no cultures were attempted from these tissues.

The 7 control mice were sacrificed at termination of experiment. Although these mice were in the same hood with the experimental mice for 5 weeks, no isolation of fungus was made from individual tissues.

Discussion. There is much speculation as to the significance of the extremely high histoplasmin skin test sensitivity among humans in certain localities of the United States. Many epidemiologists consider this sensitivity to result only from actual association with *H.*

TABLE II. Exposure of Mice for One, 2, and 4 Days to Soils Seeded with *Histoplasma capsulatum.*

Infected soil	No. days exposed	No. mice pos./No. autopsied	% positive	% tissue positive		
				Liver	Spleen	Lung
Natural	1	6/7	86	86	86	86
	2	7/7	100	100	100	86*
	4	6/7	86	86	86	86
Artificial	1	7/7	100	100	100	100
	2	7/7	100	100	100	100
	4	7/7	100	100	100	100
Controls	†	0/7	0	0	0	0

* Plates containing lung tissue from one mouse were contaminated.
† Not exposed.

capsulatum, although it is not certain that infection precedes sensitization. Environmental and epidemiological studies would suggest that the fungus is endemic in these localities and infection can result from contact with it in nature. Nevertheless, there is still no unanimity among epidemiologists as to the mode of infection in histoplasmosis. The results of many researchers point to the respiratory route as portal of entry of the etiological agent. In recent years the ever increasing number of epidemics in humans associated with dusty activities and of accidental infections among susceptible laboratory personnel would seem to support this hypothesis. The conditions under which infections have occurred among laboratory personnel leave little doubt that the disease was contracted by inhalation of the mycelial phase of the fungus from contaminated atmosphere. However, no controlled experiments involving humans have been reported to establish unequivocally the natural route of infection of histoplasmosis in man.

The experimental methods used simulated the natural environment of wild rodents and led to the development of histoplasmosis in the white mouse. Under conditions of these experiments, only the natural motion of mice produced dust aerosols. The large number of mice showing disseminated histoplasmosis with no apparent gross intestinal pathology is suggestive that the disease was contracted by inhalation of the fungus from dust aerosols. However, the experimental procedure did not completely preclude the possibility that other routes of infection existed, although these are unlikely(4).

The greater percentage of infections in mice exposed to artificially seeded soils was to be expected. Several previous determinations showed an average of 180,000 infectious units per ml of soil. Exposure for a period of several days to 100 ml of soil containing such a large number of viable infectious units certainly should have produced disease in susceptible animals.

In contrast to the favorable conditions under which artificially seeded soils were held, the 2 naturally contaminated soils, collected 5 and 10 months previously, were maintained at room temperature in the original pasteboard cartons. These soils had yielded *H. capsulatum* soon after their collection from the epidemic sites, using a modification of the flotation method followed by mouse inoculation. It was of interest to re-examine the results of these studies to ascertain the infectivity of soils at the time of the experiment. The naturally infected soil used in the first exposure study produced histoplasmosis in only 7 of 14 mice inoculated by the indirect method. It is significant that mice directly exposed for 7 days to this soil, after 5 months storage, resulted in 100% infectivity. All 7 mice inoculated indirectly with the second epidemic soil developed disseminated histoplasmosis. After storage for 10 months, this soil produced histoplasmosis in 90% of the animals directly exposed for 1, 2 and 4 days. No mice were exposed to this soil for 7 days. This factor, rather than the difference in storage time may account for the 90% infectivity by direct exposure of mice to this soil.

These preliminary studies indicate that the direct exposure method is more efficient than the indirect method of isolating *H. capsulatum* from contaminated soil. Since the mice do not have to combat bacteremia due to intraperitoneal inoculation by the indirect method, the mouse mortality rate is much lower by direct exposure. In addition, the direct method requires less time and equipment. However, further studies should be made to determine the efficiency of the direct method when only a few infectious units are present. Studies are now under investigation in an attempt to determine, on a large number of samples, the comparative efficiency of direct and indirect methods of isolating *H. capsulatum* from the same soil sample.

The universal infection acquired by mice under simulated natural conditions raises the question as to the fate of such naturally infected mice in nature. One wonders if such mice survive or die, since experimental evidence(4) suggests a high mortality after a rather long period of latency. On the other hand, no epidemiologic evidence of low mouse

populations or abnormal deaths has been reported in areas where the fungus has been known to exist in the soil. Further experiments on the ultimate fate of naturally infected mice are now in progress in an attempt to elucidate this problem.

Summary. 1. Susceptibility of white Swiss mice to infection with *H. capsulatum* by inhalation of dust aerosol was determined by direct exposure of animals to known positive soil samples. 2. Disseminated histoplasmosis occurred in a large percentage of mice. 3. Naturally contaminated soil samples held 5 and 10 months at room temperature remained highly infectious. 4. Artificially seeded soil cultures were 100% infective to the white mouse at exposures of 1, 2, 4, and 7 days. 5. These data add support to the hypothesis that man and animals become infected with *H. capsulatum* by the respiratory route. 6. The safety hood and associated safety procedures proved completely effective in preventing infection of the control mice although they were placed in close proximity to animals exposed to known positive soil samples.

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Human Amnion Cell Cultures; Susceptibility to Viruses and Use in Primary Virus Isolations.* (22891)

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Multiplication of the 3 types of poliomyelitis viruses in tissue cultures of human amnion cells has been reported by Zitcer, *et al.* (1). Because of abundance and availability of placental membranes, the studies reported here were initiated to determine the susceptibility of amnion cell cultures to other viruses and to investigate the possibility of large scale use of these cells for primary isolation of viral agents.

Materials and methods. Preparation of amnion cultures. Membranes were collected over a 14-hour period and placed in jar containing 200 ml sterile balanced salt solution to which penicillin and streptomycin had been added at a concentration of 100 units and 100 μg/ml respectively.† The jar containing the membranes was kept at room temperature until brought to the laboratory. After separation from the chorion, the amnion was washed in several changes of sterile balanced salt solution, cut into approximately 10 cm pieces and freed of blood clots by digestion in 0.25% trypsin solution for 30 to 45 minutes. The supernatant solution containing blood cells was then discarded and fresh trypsin solution added to the minced tissues. The cells were thereafter liberated by gentle agitation with a magnetic stirrer for 6 to 14 hours without decanting. This simplified the trypsinization process and cells obtained by this method did not appear to be damaged by the prolonged digestion. Cells were then centrifuged at 1,000 rpm for 10 minutes and washed twice in sterile balanced salt solution before being suspended at a 1/150 dilution in Eagle's synthetic medium supplemented with 10% horse serum. The yield of packed cells from one membrane varied from 0.5 to 1.5 ml. A small percentage of membranes yielded

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