many of which are intracellular, those in tissue culture fluid are almost completely extracellular.

The demonstrated ability of *Toxoplasma* to multiply *in vitro* in mammalian cells also suggests that this organism may be encountered on occasion by those utilizing tissue culture procedures for the isolation of viruses from man. In such cultures, the phenomena associated with the multiplication of *Toxoplasma* might be erroneously ascribed to bacterial contamination or to viral cytopathogenicity.

Summary. The RH strain of *Toxoplasma gondii* has been propagated for 15 serial passages and a second strain for 7 passages in roller tube cultures of mouse embryonic tissues. Multiplication of the RH strain was also observed in roller cultures of human foreskin, uterus and embryonic skin-muscle tissues. In each instance, the appearance of organisms in the culture was associated with extensive degenerative changes and ultimate destruction of the tissue. At the point of apparent maximum release, the culture fluid contained large numbers of the protozoa.

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Epidemic Histoplasmosis and Airborne *Histoplasma capsulatum.* (20789)

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Several authors(1.2) have suggested that infection with *Histoplasma capsulatum* is acquired by the airborne route. This route of infection is supported by the primary localization in the lungs, the frequency of pulmonary involvement, the small size of the majority of the spores(3). and the occurrence of laboratory infections(4). This evidence is further supported by the recent report of a number of epidemics of histoplasmosis(5.6) where infections appeared to have been acquired from the air. *H. capsulatum* has been isolated from man, as well as from wild and domesticated animals, and from soils collected in various areas of the United States(6,7). The study reported in this paper represents the first isolation of the fungus from the atmosphere. An epidemic of histoplasmosis in a farm family near Atchison, Kansas, afforded an excellent opportunity to culture the air for the presence of *H. capsulatum* in a specific locality. The entire family of 5 became ill with an influenza-like illness about 10 days after cleaning an unused chicken house. One patient required hospitalization for 62 days. *H. capsulatum* was isolated from farm animals and the soil both inside and immediately outside of the chicken house. All 5 persons involved in the epidemic had pulmonary lesions demonstrable by X-ray and all of them had initially positive serological test for histoplasmosis, followed by falling titers at later examinations. A similar epidemiological picture was found near Beverly, Mo. A tenant farmer had cleaned an abandoned chicken house and contracted histoplasmosis as shown

by X-ray and serological changes. The etiological agent was isolated from the debris in the chicken house.

Material and methods. The air in the chicken houses was sampled using the Venturi Scrubber air sampler. In this sampler, particles are removed from the air by the scrubbing action of the fluid spray produced by Venturi action. The fluid is recirculated by suction created at the Venturi constriction. The principles of the Venturi Scrubber have been adequately described(8). The apparatus is used industrially for the cleansing of air(9). Two sizes of scrubber samplers were used in these studies. The larger sampler has a Venturi tube with the following approximate dimensions: Length 13½ inches, diameter 2 inches, and the throat constriction ¾ of an inch. When the scrubber is in operation, the working volume of the liquor in the bowl is approximately 1270 ml. The smaller sampler has the following approximate dimensions: Length 4¼ inches, diameter ⅝ of an inch, and throat constriction ¼ of an inch. The scrubbing liquor in the bowl at working volume is approximately 36 ml. The larger sampler was used with a tank-type vacuum cleaner which pulled 16 cfm. of air through the sampler. The smaller sampler was used with a vacuum pump which pulled 2.5 cfm of air through the scrubber. The samplers were sterilized in the autoclave prior to use. Sterile distilled water was used in the samplers as the scrubbing liquor. At the completion of the sampling period, the scrubbing fluid from the air sampler was filtered through a sterile Goetz millipore filter to concentrate the solid material captured from the air. The sediment when scraped from the filter pads and resuspended in sterile saline. The suspension was treated with 8000 units each of penicillin and streptomycin per ml. The treated sample was stirred and permitted to stand undisturbed for 30 minutes. After shaking the sample, white Swiss mice were inoculated with one ml of the suspension intraperitoneally. The following 3 consecutive days each mouse received 10000 units of penicillin subcutaneously. Four weeks after inoculation the mice were sacrificed. The liver, spleen, and adrenals of each mouse were placed in a sterile mortar and macerated, using sterile saline as the diluent. The macerated tissues were cultured on one brain heart infusion plate with 10% blood and 2 Sabouraud's plates. These plates contained 40 units of streptomycin and 20 units of penicillin per ml of media. One ml of the tissue-saline suspension was inoculated onto each plate. The plates were incubated at room temperature for 4 weeks. Colonies were examined using lacto-phenol blue preparations. On all but one occasion the air sampling on the farms was limited to the interior of the chicken houses, and only the large Venturi Scrubbers were employed. These scrubbers were supported 2 or 5 feet off the ground floor. On the one occasion when the small Venturi Scrubber was used, it was supported 6 inches off the ground on the outside of the chicken house.

Results. Table I presents a summary of the results of air sampling at 2 sites.

Reference to Table I shows that positive samples were obtained from the Kansas site on only one day. Perhaps this is explained by the fact that on the previous day the chicken house was raised approximately 6 inches off the ground in preparation for moving it to another location on the farm. The chickens had been removed 4 months previously. Since the chicken house had no flooring, the dry straw and droppings covering the ground remained undisturbed. The intakes of the large Venturi samplers were faced east 2 feet off the ground floor. These samplers were in operation for 3 hours. The smaller sampler was assembled outide the chicken house on the north side 6 inches from the building. The intake of the sampler was faced south. It was 6 inches off the surface of the soil. A 30-minute sample of the air was taken at this point. The wind velocity was estimated at 10 to 12 miles per hour, but the air was not visibly dusty. The soil surface and debris on the floor of the chicken house were not disturbed during the sampling except by the southerly wind. Air sampling on this day, resulted in isolation of *H. capsulatum* from the air with both the large and small Venturi scrubbers.

The air sampling results obtained at the Missouri site are particularly significant be-

TABLE I. Summary of Results of Air Sampling.

Location	Date	Type Venturi used	Sampling time, hr	Ht of samples from ground, ft	No. of mice inoc.	No. mice died 48 hr and not cultured	No. pos. mice	% pos. mice
			(each sampler)					
Kans. site	12/11/52	Large	1.5	5	7	6	0	0
	1/27/53	"	1.5	5	8	0	0	0
	2/18	"	.5	5	9	0	0	0
	3/13	"	1.5	5	7	0	0	0
	4/14	"	3.0*	2	35	5	11	37
	4/14	Small	.5	½	4	0	1	25
Mo. site	10/31/52	Large	1.5	2	7	4	0	0
	7/ 8/53	"	4.5*	2	5	0	0	0
	7/ 8	"	4.5	5	5	0	0	0
	7/17	"	4.0*	2	8	4	2	50
	7/17	"	4.0	5	5	1	2	50
	7/23	"	4.0†	2	7	0	0	0
	7/29	"	4.5†	2	7	0	7	100
	8/ 6	"	4.5*	2	7	0	3	43
Total					121	20	26	26

* Two samples were run simultaneously and fluids combined.
† Three " " " " " " "

cause *H. capsulatum* could be repeatedly isolated from the air in the chicken house. Considering the possibility of respiratory infection, it is especially important to note that on one occasion the intake of the sampler was 5 feet above the floor. The tenants cleaned the chicken house on July 26, 1953, removing planks that had been laid loosely on the ground. This cleaning did not alter the presence of *H. capsulatum* since the fungus was recovered before and after the cleaning of the building. During the 3 sampling periods when *H. capsulatum* was isolated the wind was calm and the debris on the flooring was not disturbed.

Summary. It is believed that these 5 isolations represent the first time that *H. capsulatum* has been obtained from naturally contaminated air. These results and the presence of an epidemic pulmonary disease in the residents on these farms provide evidence that *H. capsulatum* can be airborne. These results also show that the large and small Venturi scrubbers of the type employed are capable of removing the spores of *H. capsulatum* from air.

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