Direct Isolation of *Histoplasma capsulatum* from Soil: Probable Etiological Relationship to Camp Gruber Pneumonitis. (19584)

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No reports of the direct isolation of *Histoplasma capsulatum* from the soil have appeared in the literature. Emmons(1), and Ajello and Zeidberg(2), and the authors(3) have been successful in isolating it indirectly from the soil by modification of the flotation method of Stewart and Meyer(4) combined with animal inoculation. Grayston, Loosli, and Alexander(5) similarly isolated it from mixed sawdust and soil from an unused silo. Attempts by these authors to isolate the organism directly by culture of these materials were unsuccessful. Streaking the dry material or using a portion of the sample prepared for animal inoculation on culture plates always resulted in the plates being overgrown with saprophytic fungi and bacteria. The present paper reports the first direct isolation from the soil.

Both the direct and indirect isolations of *H. capsulatum* reported in this paper were made from soil collected from an abandoned storm cellar at Camp Gruber, Okla. This storm cellar was entered on Mar. 17, 1944 by some 26 soldiers, all of whom subsequently developed a severe pneumonitis. The characteristics of this epidemic have been reported by Cain, Devins, and Downing(6), and Mickle (7). Epidemiological and case history studies revealed that the time of exposure of these men ranged from 3 minutes to 6 hours. Due to the cold weather prevailing at the time a fire had been built in the cellar by the men who chipped bark and wood chips from the elm tree posts supporting the roof and prying loose rotten boards from the ceiling. The widespread lung lesions which developed in the men exposed suggest that the infection was inhaled in the storm cellar.

Soil samples were collected on 4 different occasions from various locations in the interior of the storm cellar. These collections were made in July 1950, and in February, May and June 1951. The soil was placed in sterile quart Mason jars and transported to the laboratory for study. The lids were screwed lightly to permit at least partial aeration. The samples were stored in a room in which no cultures of *H. capsulatum* were maintained.

The direct isolation was made from soil collected in Feb. 1951. A modification of the Emmons' flotation method(1) was used. Twenty cc of soil were added to a sterile 100 ml glass-stoppered graduated cylinder. Thirty cc of sterile saline were added to the soil and the mixture was shaken vigorously for 2 or 3 minutes and then allowed to stand undisturbed. Samples were removed from these graduated cylinders from different depths of the supernate after 30 minutes, 1 hour, and 1½ hours. Varying amounts of penicillin, streptomycin and actidione were added to the supernate before plating on various media. Combinations of 2000, 4000 and 8000 units of penicillin and streptomycin and 0.1 mg to 1.6 mg of actidione per ml of the supernate were used. These antibiotics were also incorporated into the numerous selective media which were utilized. These media and antibiotics were tested previously to determine that they had no inhibitory or retarding action on 21 isolates of *H. capsulatum*.

A medium developed in our laboratory was the substratum on which the primary isolation of the fungus was made. This medium was made from an extract of fresh elm bark (*Ulmus americana*) obtained from recently felled American elms. The bark was cut into strips and separated into inner and outer portions. The inner portion, which consisted of both functional and nonfunctional phloem was the only material used in the preparation. One thousand g of the stripped and shredded bark were immersed in distilled water and brought to a boil for a period of 3 hours. The elm bark extract was then made up to a vol-

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ume of 5 liters by the addition of distilled water and filtered through coarse filter paper (E & D 615). Since the pH of the water extract was usually near 3.5, it was adjusted to 6.8 with a buffered solution (phosphate buffer) before adding agar. Twenty-five cc portions of the medium were dispensed into 160 ml medicine bottles and autoclaved at 121°C and 15 lb pressure for 15 minutes. It was then slanted in the bottles and cooled.

Nearly 400 attempts were made before the fungus was finally isolated from a single culture on the elm bark medium. This positive culture was obtained from the interfacial area between the supernate and the sediment of the soil sample. In nearly every case when preparations of the supernate from this region were examined microscopically, spores characteristic of *H. capsulatum* could be observed. The isolate was inoculated into mice and into 2 white rats. When the organs of these animals were aseptically macerated and streaked on various media the fungus was recovered. The blood sera of both rats showed a positive complement fixation test for histoplasmosis 99 days after infection. The isolate was also converted to the yeast phase on brain heart infusion blood agar at 37°C.

H. capsulatum was isolated following mouse inoculation from the samples collected in June 1950, and in Feb. and June 1951. The following method was employed: 10 cc soil were placed in sterile 100 cc graduate and saline added to 100 cc. After 5 minutes of shaking, the sample was allowed to stand 30 minutes. All the clearest supernatant fluid (usually about 75 to 80 cc) was drawn off with a sterile pipette and put in sterile tubes. These tubes were centrifuged at 2500 r.p.m. for 30 minutes. The supernate was discarded and the lower 2 or 3 cc in each tube were combined to make a volume of 15 cc. Eight thousand units of streptomycin and penicillin were added to each cc. One cc was inoculated intraperitoneallv into white mice.

The organism was isolated from one of 9 mice inoculated with the June 1950 soil, 2 of 14 mice inoculated with the February 1951 soil. and one of 2 mice inoculated with the June 1951 soil.

In addition to the one direct isolation of *H. capsulatum* and 3 isolations following animal inoculation with the soil of the cellar in which the pneumonitis was contracted, other evidence that the organism is etiologically related to the pneumonitis is available from follow-up of the soldiers involved in the epidemic. While these results will be reported in full later, it appears worth while to summarize them briefly here. Follow-up studies were obtained in 1951 of 23 of the 26 men involved in the 1944 epidemic at Camp Gruber, and of all of 8 men who were in the same organization as the men involved in the epidemic, but who remained well. These men served as controls. The studies were undertaken with the cooperation of the Commission on Acute Respiratory Diseases of the Armed Forces Epidemiological Board.

The results of the follow-up studies show that all of the 22 men tested who were involved in the 1944 pneumonitis epidemic reacted to histoplasmin, while only 6 reacted to tuberculin, 3 to blastomycin, and one to coccidioidin. Among 8 control persons of the same organization who did not become ill, 4 of 8 reacted to histoplasmin. Three of these gave a history of brief exposure in the suspected storm cellar. Four also reacted to tuberculin, one to coccidioidin, and none to blastomycin. Since all of the men involved had spent all of their non-Army residence in areas where the skin test sensitivity to histoplasmin is low, it appears that their skin reaction to this antigen is probably connected with their Army service.

In addition to skin tests, follow-up blood complement fixation tests for histoplasmosis were performed. Four of the 20 pneumonitis cases showed a positive test, whereas none of the 8 controls showed such a result.

The most dramatic evidence is shown in the x-ray picture. Disseminated calcifications were seen in the chest x-rays of 16 of the 22 men who had pneumonitis at Camp Gruber while such calcifications were found in none of the 8 controls. Since White and Hill(7), and Furcolow(8) have reported that over 95% of 298 cases of disseminated calcification showed a positive histoplasmin skin test, and since Furcolow(9,10) has followed 2 cases of disseminated lung lesions due to proved histo-

plasmosis from the acute lesions to final disseminated calcification, this furnishes strong supporting evidence of the etiological relationship.

Summary. We report the first direct isolation of *H. capsulatum* from natural sources; *i.e.*, soil from a storm cellar in which severe pneumonitis was contracted by soldiers in 1944. This organism was also isolated from soil from the storm cellar following animal inoculation on three occasions. This evidence and that obtained by studies of the men involved in the pneumonitis epidemic indicates that the epidemic was caused by H. *capsulatum.*

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Effect of Deficiencies of Sodium and Potassium on Motility of the Intestine of the Rat.* (19585)

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Workers in this laboratory frequently have observed that animals receiving potassium deficient diets had distention of the stomach and of the small intestine. Also, many rats which were fed a potassium deficient diet containing 19% cellulose died of intestinal obstruction. These are evidences of decreased intestinal tone and motility.

Gazes *et al.*(1) reported that isotonic potassium chloride administered intravenously stimulated intestinal motility in human beings and dogs with adynamic ileus. Webster *et al.* (2) observed that rats fed a diet containing 0.01% potassium exhibited decreased intestinal tone and finally died of a condition resembling paralytic ileus. Since the observations of these workers were essentially qualitative, quantitative observations of the effect of dietary potassium deficiency on intestinal motility are of interest. The possible interrelationship of sodium with potassium in this regard also was investigated since increased levels of sodium have been shown to increase the mortality and the severity of the heart lesions of potassium deficient rats(3).

Experimental procedure. Groups of 5 adult female albino rats weighing from 180 to 220 g each were placed on each of the 4 experimental diets employed. The diets were essentially the same as the low sodium and potassium diets of Grunert and Phillips(4). The deficient diets contained 0.005% sodium when sodium was deficient and 0.005% potassium when potassium was deficient. The control diet contained 0.25% sodium and 0.5% potassium. After 6 weeks on the experimental

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