

TABLE 1

H 13	<i>Helianthus</i> tumor	Isolated from secondary sunflower tumor. July 1941. P. R. White
K I	" "	From a secondary tumor on an <i>in vitro</i> graft. Subcultured June 28, 1946. R. S. de Ropp.
K II	" "	Isolated from induced tumor. R. S. de Ropp.
K III	" "	Isolated from induced tumor. R. S. de Ropp.
P I	" "	Isolated from primary tumor 14/12/45. R. S. de Ropp.
P II	" "	Isolated from a different segment of same primary tumor as P I (14/12/45). R. S. de Ropp.
P III	" "	Isolated from primary tumor Feb. 1946. R. S. de Ropp.
V 60	<i>Vinca rosea</i> tumor	Isolated from heat-treated plants of <i>Vinca rosea</i> . Apr. 1943. P. R. White.
PN	<i>Parthenocissus</i> normal	G. Morel
PT	" tumor	" "
PA	" accustomed	" "
NT	<i>Nicotiana</i> tumor	" "

agar contained, per liter, 20 g proteose-peptone #3, 3 g yeast extract, 3 g malt extract, 5 g dextrose, 3 g beef extract, and 15 g Difco agar. All the tumor tissues showed the presence of bacteria except PN and PA.

The bacteria isolated from our cultures of plant tumor tissues are more fastidious in their nutritional requirements than *Agrobacterium tumefaciens*, which grows readily on the tissue culture medium at room temperature. The contaminating bacteria are gram-negative rods. They grow on AC agar but not on the tissue culture medium, and they grow more rapidly at 36° C than at room temperature. The bacteria isolated from each tumor were placed on disks from carrots that had been tested for ability to form tumors when inoculated with virulent *A. tumefaciens*. No tumors were produced.

The plant tumors were retested for bacteria by fragmenting bits on slopes of AC agar or on the medium supplemented with yeast extract described by Theis, Riker, and Allen (1). Again bacteria grew from all but PN and PA.

Thirteen different plant tumor tissues were obtained from another laboratory. Bacteria were demonstrated in four of these. This group of tumor tissues included duplicates of five of the strains of tumor tissues in our collection—namely, K I, NT, P I, P II and H 13. The P I, P II, and H 13 were sterile, the K I and NT were contaminated.

The contaminating bacteria would not seem to be causally associated with the tumorous condition of the tissue, since our P I, P II, and H 13 contained bacteria, whereas the same strains of tumors from another laboratory did not. However, plant tumor tissues that appear to be bacteria-free when grown on a medium suitable for the cultivation of tumor tissues

may contain bacteria. This possibility must be considered and eliminated by the use of suitable test media and incubation temperatures when sterile plant tumor tissues are essential for the purposes of a particular investigation.

Reference

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The Isolation of *Histoplasma capsulatum* from Soil in an Unused Silo¹

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The epidemiology of histoplasmosis in man is largely unknown. The fungus *Histoplasma capsulatum* has been isolated by Emmons from a variety of wild and domestic animals and from 6 of several hundred samples of soil collected from farms on which animals harboring the fungus had been trapped (1, 3). There is no direct evidence, however, that infected animals serve as vectors in the case of histoplasmosis in man or that contaminated soil is important in the epidemiology of the disease.

This report concerns the study of the possible source of the fungus (*H. capsulatum*) responsible for extensive pulmonary infections in 3 male members of a farm family living near Lowell, Ind. The father (A. E., age 53) and his two sons (E. E., age 20, and A. E., Jr., age 5), had been well until about Sept. 20, 1950, when they became ill at the same time with fever, chills, non-productive cough, and weakness. X-rays of the chest revealed widespread noncalcified pulmonary infiltrates in all three. E. E. and A. E., Jr., were clinically ill for only 2 weeks, but the father failed to recover immediately and was admitted to Billings Hospital on Oct. 28, 1950. He had a stormy course but gradually improved and was discharged after 2 months' hospitalization. Sputum specimens were examined for the presence of fungi and acid-fast bacilli by culture and animal inoculation. Specimens collected on the fifth and twelfth hospital day yielded *H. capsulatum*, as did a bone marrow culture taken on the tenth hospital day. In all three, skin tests were positive to histoplasmin 1:100 dilution but negative to tuberculin (PPD 0.0001 mg) and coccidioidin 1:100. Sera from all three yielded highly positive complement fixation titers (A. E., 1:160; E. E., 1:320; and A. E., Jr., 1:160) in a test employing yeast phase histoplasma as antigen (4).

During the 2 months preceding their illness the two men had undertaken in addition to the usual farm chores (a) removal of "blow sand"—a fine shifting silt from driveways; (b) grinding feed composed of

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corneobs, oats, and straw in a closed shed; and (c) cleaning out a silo. The last activity was undertaken during the first week in September, 2-3 weeks before the onset of infection. It consisted of shoveling the silo floor clear of a foot of dried vegetable and sawdust material. The silo had been employed as an ice-house but had been unused for a number of years. A. E., Jr., sat in the truck into which his father and older brother shoveled the dust. Occasionally some would land on his head, and sometimes he threw handfuls in the air and back at the others.

It seemed important to survey the farm, and particularly the silo dust, as the most likely source of the fungus. The methods used for isolation of *H. capsulatum* were adapted after those suggested by Emmons (1). Briefly, these consisted of diluting the samples 5-10 times with normal saline in a cylindrical graduate. After thorough mixing the suspension was allowed to stand for an hour while the larger particles settled. One-ml portions of the supernatant were inoculated intraperitoneally into each of a group of 10 Swiss mice (CFW strain). Two to three mice from each group were then sacrificed at intervals of 10-30 days after inoculation, and their spleens and livers removed, ground in sterile sand, and cultured for the presence of fungi.

The culture media employed were corn-meal agar (5), Sabouraud's glucose agar (5), and blood agar plates which were enriched with glucose and cysteine and to which penicillin and streptomycin were added. The media were then streaked with the homogenized spleen and liver mixture, and all were incubated at room temperature. In addition, some duplicates of the blood agar plates were incubated at 37° C. The cultures were observed for 2 months. They were considered positive for *H. capsulatum* only after demonstration of the characteristic tuberculate chlamydospores.

On November 27, 1950, 70 samples of soil from about the farm buildings, yard sand, ground feed, and material from the silo were collected and pooled into 15 groups and examined for *H. capsulatum* as described above. The homogenized liver and spleens of mice inoculated with Groups 2 and 7 yielded *H. capsulatum* on culture with all three media. Both groups were from the silo. Group 2 consisted of one large sample from the floor of the silo. Group 7 was made up of 5 samples scraped from the walls of the silo. Examination of these 5 samples individually yielded histoplasma organisms from one.

Four months later, March 26, 1951, a second survey of the farm for the presence of the fungus was made. Sixty samples pooled into 6 groups were examined. Groups 2 and 6 containing material from the floor and from a ledge near the floor of the silo yielded *H. capsulatum* organisms.

It seems most probable that the father and two sons became infected from the inhalation of large quantities of infectious silo dust at the time the silo was cleaned. Monthly surveys of the silo environment are under way in an effort to learn more about the pres-

ence of this fungus in nature and the epidemiology of the disease in man and animals.

Addendum: Four additional monthly surveys have been made. Out of a total of 106 soil samples from the silo, 26 have yielded *H. capsulatum*.

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Differential Effects of Cerebellar Anterior Lobe Cortex and Fastigial Nuclei on Postural Tonus in the Cat¹

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The influence of the anterior lobe (cortex and nuclei) of the cerebellum upon postural tonus has classically been considered a unitary one, its ablation causing marked hypertonicity of the antigravity extensors, and its presence partially inhibiting the tonus of these muscles even in the decerebrate animal. Electrical stimulation of the medial part of the anterior lobe cortex itself, or its nuclei of projection, the fastigial nuclei, inhibits extensor and facilitates flexor tonus in the normal or decerebrate animal, the inhibition being followed, upon cessation of the stimulus, by a powerful extensor rebound which exceeds for a length of time the tonus present before the stimulus. When ablation or stimulus is confined to one side, the extensor release or its inhibition has been considered to be primarily on the ipsilateral side, although reciprocal effects have been noted in the contralateral limb. These effects, at least in the cat and the dog, are more pronounced in the fore than in the hind legs.

Since electrical stimulation of the medial anterior lobe cortex and of the fastigial nuclei give similar inhibitory and rebound phenomena, it has been assumed that these structures similarly affect tonus mechanisms. That such an assumption is erroneous is indicated by the following acute experiments on decerebrate cats. Fig. 1 shows schematically the relative position of the two forelegs following decerebration (A), the lesions indicated by crosshatch.

When the cortex only of the medial anterior lobe is removed by aspiration on one side (RMC), the response of the forelegs is the classical one—i.e., augmentation in extensor and decrease in flexor tonus in the leg of the same side (Fig. 1, B). Moreover the effect is reciprocal in that the opposite distribution

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