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A recent visit to the Republic of Panama provided the opportunity to collect soil specimens for mycologic study. The results of the examination of these samples for the presence of *Histoplasma capsulatum* and other human pathogenic molds are presented in this report.

Over a two-week period, 100 soil samples were collected in several of the more accessible areas of the country. The majority of the specimens were gathered deliberately in areas frequented by chickens, as soil studies carried out previously in Williamson County, Tennessee, in collaboration with Zeidberg (1952, 1953) had demonstrated that a significantly higher percentage of soils gathered from chicken coops and chicken yards yielded *H. capsulatum* than soils obtained from other habitats. The approximate locations of the collecting sites are indicated on the accompanying map (Fig. 1).

All the specimens were scooped directly from the uppermost layer of the soil into 4 oz. collecting bottles, labeled, and flown to Georgia. There the soils were examined for *H. capsulatum* by injecting the supernate from soil suspensions intraperitoneally into mice and culturing small portions of their livers and spleens, and for *Microsporum gypseum* by baiting plates of moistened soil with keratinaceous substrata, viz. pieces of sterilized hair, feathers and cow horn shavings. The details of these procedures are outlined as follows:

A. *H. capsulatum* procedure[1], (Stewart and Meyer, 1932; Emmons, 1949; Ajello and Runyon, 1953):
   1. Approximately 10 ml. of a thoroughly mixed soil sample are suspended in 30 ml. of physiological saline.
   2. The suspension is stirred vigorously and allowed to stand for at least one hour.
   3. Five ml. of the supernatant fluid are pipetted off and one ml. aliquots injected intraperitoneally into each of 4 mice.
   4. The mice are treated once daily for 5 days with 1,000 units of streptomycin and 12,500 units of penicillin to prevent death by any pathogenic bacteria that may be present in the soil.
   5. After 8 weeks the mice are sacrificed and portions of their liver and spleen inoculated into tubes of a neutral dextrose-peptone agar.
   6. The isolation tubes are incubated at 25°C. and examined over a period of 6 weeks for the presence of pathogenic molds.

B. *M. gypseum* procedure (Vanbreuseghem, 1952; Ajello, 1953):
   1. Sterile petri dishes are half filled with soil.

[1] With this method other pathogens such as *Allescheria boydii*, *Coccidioides immitis* and *Cryptococcus neoformans* can be recovered from soil.

TABLE 1

Location and nature of soils yielding pathogenic fungi

Code No. of Positive Soil Samples	Isolate	Nature of Collecting Site	Locality
2	*M. gypseum*	Under tree used as chicken roost	Road between Chorrera and Capira
4	*M. gypseum*	Under wild plum tree used as chicken roost	La Campana
10	*M. gypseum*	Under wild plum tree used as chicken roost	La Campana
11	*H. capsulatum*	Under wild plum tree used as chicken roost	La Campana
14	*M. gypseum*	Soil along fence of chicken yard	Chilibri
15	*M. gypseum*	Soil gathered in front of chicken pen	Chilibri
16	*M. gypseum*	Soil from right side of chicken pen	Chilibri
18	*M. gypseum*	Soil in rear of chicken house	Chilibri
19	*M. gypseum*	Soil around chicken house	Chilibri
20	*M. gypseum*	Soil in rear of chicken house	Chilibri
25	*M. gypseum*	Soil in rear of chicken house	Chilibri
26	*M. gypseum*	Soil in rear of chicken house	Chilibri
27	*M. gypseum*	Soil in rear of chicken house	Chilibri
29	*M. gypseum*	Inside chicken coop	Ciricito
31	*M. gypseum*	Soil under guava tree used as chicken roost	Ciricito
32	*M. gypseum*	Soil under guava tree used as chicken roost	Ciricito
			Ciricito
37	*M. gypseum*	Under a tree roost	Ciricito
48	*M. gypseum*	Under house	Ciricito
49	*M. gypseum*	Soil in front of house	Ciricito
50	*M. gypseum*	Under house	Ciricito
51	*M. gypseum*	Chicken yard	Ciricito
52	*M. gypseum*	Under yucca tree	Ciricito
53	*M. gypseum*	Under papaya tree	Ciricito
54	*M. gypseum*	Under achiote bush	Ciricito
57	*M. gypseum*	Dry soil under termite study house	Barro Colorado Island
61	*M. gypseum*	Dry soil under termite study house	Barro Colorado Island
62	*M. gypseum*	Dry soil under termite study house	Barro Colorado Island
66	*M. gypseum*	Chicken yard in patio of hotel	Santiago
72	*M. gypseum*	Soil around El Rancho de Coca Cola cock fighting arena	Road between David and La Concepcion
73	*M. gypseum*	Soil around El Rancho de Coca Cola cock fighting arena	Road between David and La Concepcion
76	*M. gypseum*	Cock fighting pit	Road between David and La Concepcion
82	*M. gypseum*	Chicken house	Santa Rosa
83	*A. boydii*	Under nance tree used as chicken roost	Cuipo
85	*M. gypseum*	Under house	Cuipo
88	*M. gypseum*	Under house	Cuipo
89	*M. gypseum*	Under guava tree chicken roost	Cuipo
96	*M. gypseum*	Chicken pen under house	Cuipo
97	*M. gypseum*	Chicken pen under house	Cuipo

2. Sufficient sterile distilled water is added to moisten the soil thoroughly.
3. Short strands of autoclaved hair or pieces of other keratinaceous materials are placed upon the surface of the moist soil.
4. The baited dishes are incubated at room temperature and observed over a period of 8 weeks.
5. Those pieces of bait that become covered with mycelium are examined microscopically and cultured on a medium containing cycloheximide, penicillin and streptomycin. This medium permits the isolation of many human pathogenic fungi from sources heavily contaminated with bacteria and saprophytic molds (Georg, 1953; Ajello and Getz, 1953).

RESULTS

From the 100 soil samples, through use of the mouse technique, one isolate of *H. capsulatum* and one of *A. boydii* were obtained while 36 of the soils, when baited with keratin, yielded the dermatophyte *M. gypseum*. In Table 1 the nature of the collecting sites and geographic location of the positive soils are presented.

Culturally and morphologically, the Panamanian isolate of *H. capsulatum* was basically similar to the human isolates maintained in the culture collection of the Communicable Disease Center's mycology laboratory and to the soil isolates that have been recovered from Williamson and Shelby Counties, Tennessee. The appearance of the La Campana isolate, in a set of primary isolation tubes inoculated with pieces of liver and spleen is seen in Figure 2. It produced tuberculate spores in abundance (Fig. 3) and was readily converted to the yeast-phase by

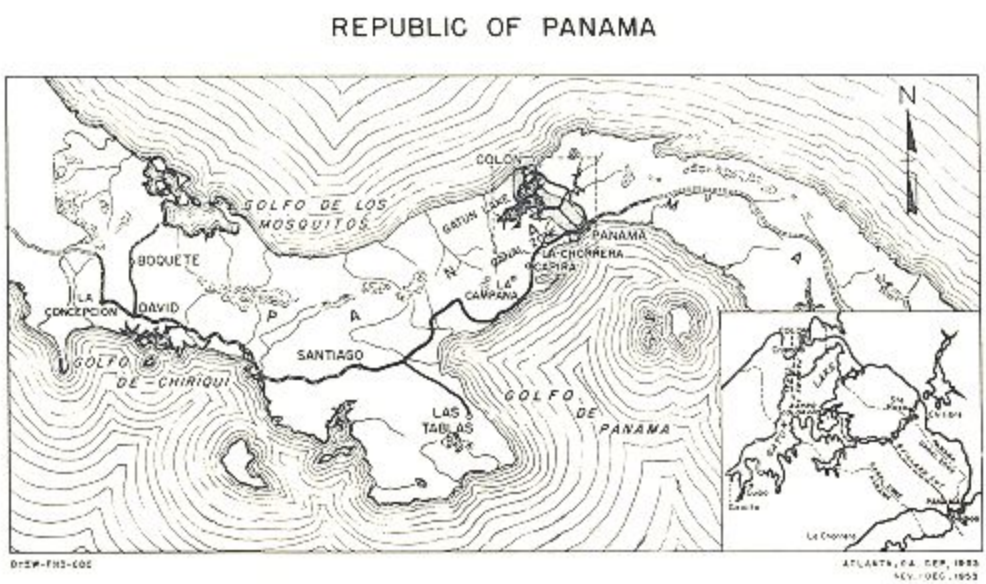
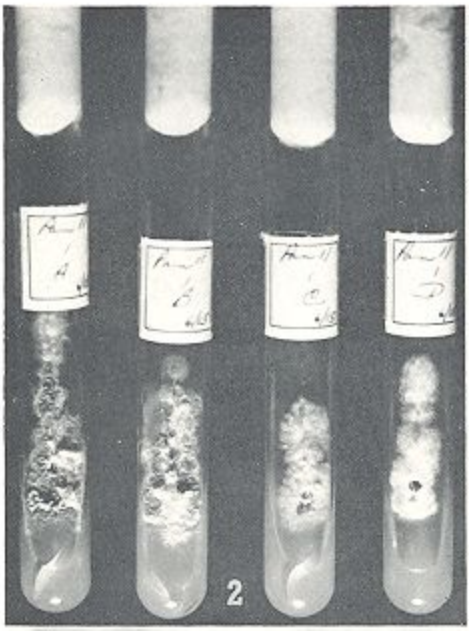
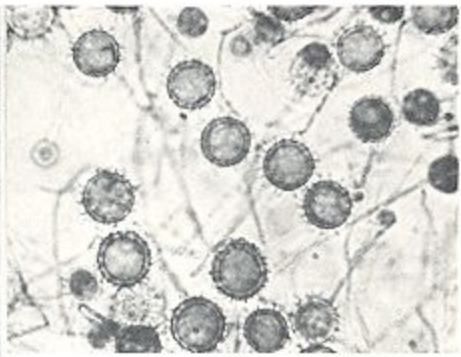
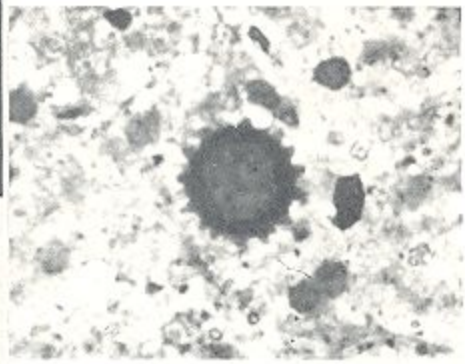
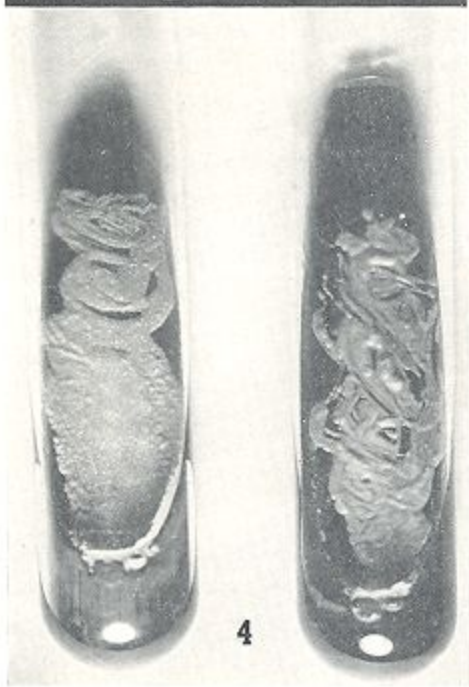


FIG. 1. Map of the Republic of Panama and the Canal Zone indicating soil collecting areas









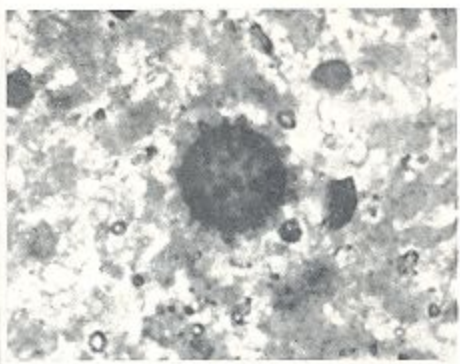


FIG. 2. Appearance of *H. capsulatum* in a group of primary isolation tubes inoculated with pieces of liver (A, B) and spleen (C, D) from mouse No. 1 injected with 1 ml. of soil suspension (Specimen No. 11).

FIG. 3. Tuberculate spores produced by the Panamanian isolate of *H. capsulatum* on Sabouraud dextrose agar. (Original magnification 475×).

FIG. 4. Yeast phase of this isolate obtained by culturing on Francis' glucose cystine blood agar (L) and on Thompson's medium (R) at 37°C.

FIG. 5. Median optical view of a tuberculate spore of *H. capsulatum* filtered from soil specimen No. 11 with a membrane filter (Original magnification 980×).

FIG. 6. Surface view of same spore showing thick wall and tubercles (Original magnification 980×).

FIG. 7. View of site in the village of La Campana, where soil sample that yielded *H. capsulatum* was collected.

subculturing on tubes of Francis' glucose cystine agar (Campbell, 1945) and Thompson's (1945) medium incubated at 37°C. (Fig. 4).

With membrane filters, according to a procedure developed by Gordon and Cupp (1953), it was possible to observe directly the tuberculate spores of *H. capsulatum* that were present in soil specimen No. 11 (Figs. 5, 6).

The demonstration of these spores in soil reconfirms the fact, first established by Emmons (1949), that *H. capsulatum* exists in nature as an actively growing saprophyte since tuberculate spores are never produced *in vivo*[2]. A view of the site in the village of La Campana, where soil sample No. 11 was collected, is presented in Fig. 7.

The isolate of *A. baydii*, a mold capable of causing mycetomas, was in its perfect stage producing both asexual conidia borne on simple conidiophores and ascospores in cleistothecia. Spore suspensions treated with gastric mucin and injected intraperitoneally into mice induced death within 10 days, a finding which had been demonstrated previously with a large series of human and soil isolates of this fungus (Ajello, 1952; Ajello and Anthony, 1953).

Best results in the recovery of *M. gypseum* were obtained with hair. Although in some instances the pieces of feather revealed *M. gypseum* before the other baits, it tended to disappear quickly from the soil plates as the result of the keratinolytic action of various microorganisms. Cow horn shavings were invaded

[2] The observation by Haley (1952) of mycelium and tuberculate spores of *H. capsulatum* in a mouse injected with yeast-phase cells may be accounted for on the basis of the influence of necrotic tissue on diphasic fungi.

by bacteria in a relatively short time and thus they too did not prove to be as satisfactory as hair for isolating *M. gypseum*.

All the isolates of *M. gypseum* sporulated heavily while growing on the baits in the soil plates. Pure cultures were obtained with little difficulty by using the cycloheximide medium previously mentioned.

Subcultures of the Panamanian isolates of *H. capsulatum*, *A. boydii* and three of the *M. gypseums* have been deposited in the American Type Culture Collection, Washington, D. C. and in the Centraalbureau voor Schimmelcultures, Baarn, Netherlands.

DISCUSSION

An anomalous situation has long existed relative to the status of histoplasmosis on the Isthmus of Panama. Despite the fact that the first three known cases of that disease were discovered there by Darling (1906, 1907, 1908) within a period of 8 months (Dec. 7, 1905 to Aug. 6, 1906), only two other cases, one involving a dog (Tomlinson and Grocott, 1945), the other a child (Draheim *et al.*, 1951), have been subsequently encountered in that country.

That histoplasmosis is not a rare disease in Panama has been proven recently by skin-testing surveys carried out by Tucker (1952) and de Pinzón (1952). Their surveys indicate that a benign and not uncommon form of histoplasmosis exists in this region of the world. Of 1,000 individuals entering the Gorgas Hospital in Ancon, the Canal Zone, 39 per cent were found by Tucker to give positive skin test reactions to histoplasmin; while sensitivity studies carried out in the Gatun Lake villages of Cuipo and Ciricito by de Pinzón revealed that 25 per cent were reactors.

Despite this immunologic evidence of the existence of a prevalent but benign form of histoplasmosis and records of 5 fatal cases of that disease, the etiologic agent, *H. capsulatum*, up to the present instance, has never been isolated from any source in Panama. The recovery of *H. capsulatum* from a soil specimen gathered in the village of La Campana, not only represents the first isolate of that organism ever obtained in Panama, but also unequivocally establishes this parasite as being indigenous to this area, thus dispelling any doubts relative to the endemicity of histoplasmosis in Panama.

The recovery of *A. boydii* from soil gathered in the village of Cuipo confirms the fact previously established by Emmons (1952) and Ajello (1952) that this mold exists in nature as a saprophyte. It is interesting to note that as yet no instances of human infection by *A. boydii* have been recorded in Panama. Undoubtedly this is a reflection of the basically saprophytic nature of *A. boydii* and perhaps, to a lesser extent, the general lack of available medical mycological diagnostic services in many parts of the Isthmus.

Despite the demonstrated prevalence of *M. gypseum* in soil throughout the Republic, few instances of infection by that parasite have been observed in Panama. Shrager (1952) has noted the only two known Panamanian cases of ringworm caused by this interesting organism. These were found among several hundred cases of ringworm examined at the Gorgas Hospital.

The percentage of recoveries of *M. gypseum* from Panamanian soils (36%) is in close agreement with that obtained from soils of Tennessee (35.1%) and Georgia (26.2%) (Ajello, 1953). A small number of specimens gathered in the Province of Ontario, Canada[3] and W. Virginia[3] were found to be positive for *M. gypseum*, 16.6 per cent and 12.5 per cent, respectively. All these findings suggest that this dermatophyte is remarkably abundant and widespread in soil.

The Panamanian findings are in no sense unique, since soils gathered in many areas of the United States and Canada by several investigators have yielded a remarkable variety of human pathogenic fungi (Stewart and Meyer, 1922; Emmons, 1949, 1951). It is to be expected that when similar studies are carried out elsewhere in the world a similar pattern will unfold.

ACKNOWLEDGMENTS

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Credit is due to Dr. L. C. Runyon for technical assistance in carrying out the mouse isolation procedures and to my wife, Gloria Ajello, for recording all pertinent field data.

SUMMARY

Soil studies carried out with specimens collected on the Isthmus of Panama yielded isolates of *Histoplasma capsulatum*, *Allescheria boydii* and *Microsporum gypseum*. This constitutes the first record of the occurrence of these three human pathogenic molds in the soil of tropical America. The techniques used in the recovery of these fungi are outlined, and the implication of the findings are discussed.

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