THE USE OF CORTISONE-TREATED MICE IN THE
SCREENING OF SOIL FOR PATHOGENIC FUNGI

by

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To Dr. A. E. Evenson with my deep appreciation for her guidance
and encouragement throughout this investigation.

TABLE OF CONTENTS

INTRODUCTION	1
HISTORICAL REVIEW OF THE LITERATURE.	2
EXPERIMENTAL METHODS	15
Table I	16
RESULTS	20
Table II	21
Table III	27
DISCUSSION	28
SUMMARY	32
BIBLIOGRAPHY	33

INTRODUCTION

Sabouraud (1893) suggested that the dermatophytes were probably soil saprophytes and, a few years later, he predicted that this might also be true of the fungi producing systemic disease in man.

It has now been established that many of the fungi capable of producing human disease do lead a saprophytic existence. Ajello (1956), in reviewing the literature, listed twenty-one pathogenic, or potentially pathogenic, fungi that had been isolated from non-living sources. He therefore concludes that "In time, most, if not all, of the fungi that cause human disease will probably be found to be soil saprophytes." He emphasizes also the importance of soil surveys for pathogenic fungi in order to study their ecological requirements and thus to provide a "basis for the development of rational control measures."

There is considerable evidence that the administration of cortisone enhances experimental infections due to a variety of microbial agents, including fungi. It seemed reasonable therefore that cortisone-treated animals might be used effectively in carrying out soil screening experiments for pathogenic fungi and, that a more accurate picture of the pathogenic fungus flora of local soils might thus be obtained. This investigation was undertaken to test this hypothesis.

HISTORICAL REVIEW OF THE LITERATURE

Ajello (1956) reviewed the literature concerned with the isolation of pathogenic fungi from non-living sources and listed twenty-one pathogenic, or potentially pathogenic fungi, that had been shown to have a saprophytic existence. The names of these fungi together with the names of the investigators and the dates of their publications are listed below:

<u>Fungus</u>	<u>Investigator</u>	<u>Date</u>
<u>Absidia corymbifera</u>	Lichtheim	1884
<u>Absidia ramosa</u>	Lindt	1886
<u>Allescheria boydii</u>	Emmons	1950
	Ajello	1952
<u>Aspergillus fumigatus</u>	Renon	1897
<u>Candida albicans</u>	Negrone <u>et. al.</u>	1941
	Di Menna	1955
<u>Candida guilliermondii</u>	Lodder <u>et. al.</u>	1952
<u>Candida krusei</u>	Lodder <u>et. al.</u>	1952
<u>Coccidioides immitis</u>	Stewart <u>et. al.</u>	1932
<u>Cryptococcus neoformans</u>	Sanfelice	1894
<u>Epidermophyton floccosum</u>	Ajello, <u>et. al.</u>	1954
<u>Histoplasma capsulatum</u>	Emmons	1949
<u>Phialophora pedrosoi</u>	Trejos	1954
<u>Microsporium gypseum</u>	Mandels <u>et. al.</u>	1948

<u>Fungus</u>	<u>Investigator</u>	<u>Date</u>
<u>Nocardia asteroides</u>	Gordon <u>et. al.</u>	1946
<u>Phialophora jeanselmei</u>	Trejos <u>et. al.</u>	1954
<u>Phialophora verrucosa</u>	Melin <u>et. al.</u>	1934
	Conant	1937
<u>Rhizopus arrhizus</u>	Rabenhorst	1892
<u>Rhizopus oryzae</u>	Went <u>et. al.</u>	1895
<u>Sporotrichum schenckii</u>	De Beurmann <u>et. al.</u>	1908
<u>Trichophyton mentagrophytes</u>	Muende <u>et. al.</u>	1937
	Lurie <u>et. al.</u>	1955
<u>Trichophyton rubrum</u>	Ajello <u>et. al.</u>	1954

The results of his studies of 1215 soil samples collected from several areas in the United States and from other parts of the world were also included in this paper. Using the mouse inoculation technique initiated by Emmons (1942) and the hair plate technique of Vanbreuseghem (1952), he obtained the results summarized on the following page.

Locality	Number of Samples	<u>Allescheria</u> <u>boydii</u>	<u>Candida</u> <u>albicans</u>	<u>Coccidioides</u> <u>immitis</u>	<u>Cryptococcus</u> <u>neoformans</u>	<u>Histoplasma</u> <u>capsulatum</u>	<u>Microsporium</u> <u>gypseum</u>
Tennessee	710	15	1	--	5	67	30
Hawaii	100	1	--	--	1	--	23
Panama	100	1	--	--	--	1	36
Georgia	79	2	--	--	2	--	13
Arizona	48	--	--	1	--	--	*--
Michigan	44	--	--	--	--	*--	5
Nigeria	76	--	--	--	1	--	6
West Virginia	16	--	--	--	--	*--	2
Canada	12	--	--	--	--	*--	2
Maryland	11	--	--	--	1	--	*--
Alabama	10	--	--	--	2	--	3
Venezuela	5	--	--	--	--	4	*--
Peru	4	--	--	--	--	1	*--
TOTAL	1215	19	1	1	12	73	120

*Not tested

The year previous to this, Durie and Frey (1955), using the Van-breuseghem technique, reported the isolation of the dermatophyte, Microsporum gypseum, from Australian soils.

Egeberg and Ely (1956) found that Coccidioides immitis could be obtained from soil by direct culture on Sabouraud-glucose agar plates. Thirty-five of the 500 soil samples collected from a 12 to 14 acre area in the San Joaquin Valley, California, including 24 of 177 samples from animal burrows, yielded this pathogen. The investigators noted that samples collected at the end of the wet season, mid-April, yielded 19 positive samples out of the 120 tested, while samples collected at the end of the dry season, mid-January, yielded 16 positive samples out of the 380 tested.

A year later, Elconin, Egeberg and Lubarsky (1957) employing the same technique isolated Coccidioides immitis from 31 out of 428 soil samples studied. The soil was collected from the San Joaquin Valley area, California, and included 115 samples from animal burrows which yielded 18 isolates of the organism. These investigators also noted that the season of the year affected the results of their investigation as summarized below:

<u>Season</u>	<u>Number of Samples Studied</u>	<u>Number of Isolates</u>
Aug. 1955; 4 months after last rain	143	0
Dec. 1955; toward end of dry season, but after rain	132	0
May 1956; following rainy season, after 2 weeks of warm weather	153	31

Plunkett and Swatek (1957) reported their findings in a study of soil in the area of an Indian camp site near Inyokern, California, where some students developed coccidioidomycosis after excavating a cave. Using 0.8 ml of a 1:10 dilution of a soil suspension that had been heated to 40 C as an inoculum and Sabouraud-glucose agar containing penicillin, streptomycin and cycloheximide as a culture medium, the investigators isolated Coccidioides immitis from a limited area measuring approximately 6 x 10 ft. and located directly in front of the cave. They concluded that "Although the fungus is known to be air borne and high winds are frequent in the area studied, Coccidioides immitis infected soil was found only in small packets It appears that there is some unknown factor in the environment which favors the growth of this fungus in certain definite habitats."

Larsh, Hinton and Cozard (1956) reported the results of their investigation of the natural reservoirs of Histoplasma capsulatum. They utilized mice as the culture medium and a soil-saline suspension treated with 8000 units each of streptomycin and penicillin per ml for the inoculum. Each mouse, by subcutaneous inoculation, received 20,000 units of

penicillin 2 hours prior to infection. Aureomycin was added to their drinking water 24 to 48 hours prior to infection and for several days after it. Histoplasma capsulatum was isolated from 47 of the 1024 soil samples studied. Twenty-two of these isolates were from soil and litter collected from the floor of chicken houses and in an area of high skin sensitivity to histoplasmin. The investigators pointed out that soil from chicken houses not in use for months or years proved to be positive for Histoplasma capsulatum more often than houses in use."

Ajello (1959) reported the isolation of a new dermatophyte, Microsporum cookii, from soil and from animals. This dermatophyte is epizootic on rodents in the United States but has not yet been proven to be pathogenic for man or for other animals.

Frey (1959), employing the Vanbreuseghem technique, found a new species, Aleurisma keratinophilum, in 9 soil samples collected in Australia and New Guinea. When tested for pathogenicity this organism proved to be keratolytic in guinea pigs.

Marples (1960) attempted to demonstrate Microsporum distortum in New Zealand soil in an area where children had acquired an infection caused by this organism. She reported that Keratinomyces ajelloi grew on all the plates and may have inhibited the growth of Microsporum distortum.

Emmons (1954)(1955) reported his observations concerning the prevalence of Cryptococcus neoformans in soil and in pigeon habitats and expressed the view that the association with pigeon habitats was purely a saprophytic relationship.

In further studies (1960) he described the isolation of virulent

strains of this organism from 63 of 91 samples of soil and pigeon habitats. These isolates were obtained by direct inoculation on agar slants which were then incubated at 37 C. The soil samples were also tested by animal passage for the presence of Histoplasma capsulatum. The results were negative.

In a study undertaken to search for the natural habitat of Blastomyces dermatitidis in an area endemic for North American Blastomycosis, McDonough, Ajello, Aushermann, Brinkman, Balows and McClellan (1961) reported the isolation of several pathogenic fungi but not Blastomyces dermatitidis. Their methods included: (1) intravenous mouse inoculation; (2) direct culture on (a) brain-heart-infusion-dextrose agar (B.H.I.D.) containing 0.05 mg of chloromphenical per ml, (b) B.H.I.D. agar with 6% human blood added as well as 20 units of penicillin and 40 units of streptomycin per ml, and (c) Sabouraud-dextrose agar containing 0.05 g per ml of chloramphenical and 0.5 g per ml of cycloheximide; and (3) the hair plate technique of Vanbreuseghem. They believe that the comparative value of their techniques are obvious from the following summary of their results:

<u>Fungus</u>	<u>Number of Positive Samples</u>	<u>Method of Isolation and Number of Positive Samples</u>		
		<u>Mice</u>	<u>37C</u>	<u>Hair-Plate</u>
<u>Allescheria boydii</u>	46	12	36	0
<u>Candida albicans</u>	1	0	1	0
<u>Cryptococcus neoformans</u>	10	10	0	0
<u>Histoplasma capsulatum</u>	36	36	0	0
<u>Keratinomyces ajelloi</u>	55	0	0	55
<u>Microsporum cookii</u>	5	0	0	5
<u>Microsporum gypseum</u>	163	0	4	163

A few months later, Denton, McDonough, Ajello and Aushermann (1961) reported the isolation of Blastomyces dermatitidis from a soil sample collected from the earth floor of a stripping shed attached to a tobacco barn in Lexington County, Kentucky. Two years previous to the date of collection, this shed sheltered a dog that died of North American Blastomycosis. The method employed differed from that usually followed in that it utilized Mycobacterium fortuitum as an adjuvant and intravenous, rather than intraperitoneal, inoculation of the sample into adult white Swiss mice.

Investigations for pathogenic fungi in Arizona soils have not been extensive. Emmons (1942) carried out an investigation in the San Carlos area, where 5 of the 150 soil samples studied yielded Coccidioides immitis.

Ajello (1956) isolated the same organism from 1 out of 48 soil samples. In the same year, Ajello, Reed, Maddy, Budurin and Moore (1956), using the mouse inoculation method, reported the isolation of Coccidioides immitis from 1 of 24 soil samples collected from an area where 5 dogs developed coccidioidomycosis. This soil sample came from a rodent burrow at the base of a mesquite bush.

Justice and Evenson (1958), using the Vanbreuseghem technique, noted the presence of Microsporium gypseum in soil samples collected in the Tucson area.

It is obvious from the preceding summary that the methods used in soil sampling for fungi causing systemic disease have deviated very little from the original mouse inoculation technique used by Emmons for the isolation of Coccidioides immitis and applied later to other fungi. However,

the hair-plate technique of Vanbreuseghem for the Keratinolytic fungi and direct culture on media inhibitory to bacteria have been used to some extent.

In the planning of this investigation, it was felt that the accumulated information concerning the enhancement of infection in cortisone-treated animals might be applied practically to soil surveying for pathogenic fungi.

Kass and Finland (1953) after carefully reviewing the literature concluded that ACTH and cortisone reduced the resistance of the laboratory animal to microbial infections.

The first report on the effect of cortisone in experimental fungus disease was that of Kligman, Baldrige, Rebell and Pillsbury (1951). They were concerned with Trichophyton mentagrophytes infection in cortisone-treated and non-treated guinea pigs. The guinea pigs in Group A received 5 mg of cortisone daily beginning 10 days prior to infection, those in Group B received 5 mg of cortisone daily beginning 1 day after infection, the animals in Group C were treated with 5 mg of cortisone beginning 16 days after infection and those in Group D were the controls and received no cortisone. The differences in the number of days required for the infection to reach the climax and to heal are summarized below:

Group	Primary Infection		Reinfection--2 months after healing of primary infection	
	Reached Climax (in days)	Healing time (in days)	Reached Climax (in days)	Healing time (in days)
A	23	52	25	35
B	—	55	—	—
C	15	45	—	—
D	16	32	8	20

Cavallero and Sala (1951) in their study of the effect of cortisone on experimental granulomatous infection with Coccidioides immitis, noted that, on post mortem examination 3 of 5 cortisone-treated rats showed a diffused infection of the spleens and lungs while the control rats showed only local tubercle-like granuloma.

Syverton, Werder, Friedman, Roth, Graham and Mira (1952) found that a fulminant infection due to Candida albicans resulted in mice that had received, prior to infection, a single subcutaneous dose of 4.0 mg cortisone and 400 r of x-ray radiation. Twenty out of 24 mice died within 6 days after the infection, and all were dead within 9 days. In the control groups only 4 of 24 mice died. Similar results were obtained when Blastomyces dermatitidis was the infecting agent.

Seligman (1953) investigated the effect of antibiotics and cortisone on the enhancement of the virulence of Candida albicans. He reported that an intraperitoneal injection into mice of Candida albicans and cortisone (2.5 mg) in an emulsion consisting of propyleneglycol, 50% N.N-dimethylacetimide proved to be harmless. However, when 5 mg of cortisone were injected subcutaneously at the time the fungus was introduced intraperitoneally 3 mice out of 10 died and Candida albicans was recovered from the kidneys of each mouse. When 2.5 mg of cortisone were injected subcutaneously 2 hours prior to and again 2 hours after the intraperitoneal introduction of the fungus, 21 of a group of 22 mice died within 2 weeks and Candida albicans was recovered from the kidneys of 19 mice. Candida albicans was not recovered from the kidneys of the controls that received no cortisone. The author therefore concluded that cortisone has a definite enhancing effect on infection due to this fungus.

Newcomer, Wright, Tarbet, Winer and Sternberg (1953) reported that cortisone had, in vitro, essentially no effect upon the growth of Coccidioides immitis. In in vivo studies, using mice, they noted that the results obtained on basis of mortality rate showed no significant difference between the controls and the cortisone-treated mice. However, the histopathological studies showed that though there was no difference during the first 8 hours after infection, differences were noted after that time. By the 8th day the cortisone-treated mice had a more extensive necrosis of the granulomas as well as an increase in the size of the spherules of Coccidioides immitis. Mature spherules were observed by the 8th day in the groups that received 0.56 mg and 2.24 mg of cortisone, and by the 10th and 11th days in the controls. Reproduction was observed by the 10th and 11th days only in the cortisone-treated mice and not in the controls.

Mankowski and Littleton (1954) reported that mice infected with either Cryptococcus neoformans or Histoplasma capsulatum and receiving large doses of intraperitoneal cortisone injections (0.95 mg - 3.75 mg - per injection) for 3 days prior to infection showed an increased mortality rate and a reduction in weight as compared with the controls.

Vogel, Michael and Timpe (1955), using guinea pigs as the experimental animal and Histoplasma capsulatum as the infective agent, studied the effect of cortisone in experimental histoplasmosis. They used animals receiving intraperitoneally 5 mg of cortisone daily beginning at the time of infection and continuing until 2 weeks prior to autopsy as well as a second group receiving the same amount of cortisone but starting 2 weeks after the infection and continuing till autopsy. A third

group received no cortisone. Their results showed that there was no adverse effect such as weight loss, increased mortality rate or morbidity. However, when skin testing with histoplasmin was performed on the 5th week, the animals in the control group had a stronger positive reaction than the animals in either of the cortisone-treated groups. Complement fixation titers were about the same in the 3 groups. The histopathological studies of the liver, spleen, adrenal gland and lungs showed a greater degree of dissemination of the organism in the cortisone-treated animals than in the controls. The culture findings from the impression smears of the spleen showed similar results.

Levine, Zimmerman and Scorza (1957) noted that the inflammatory response in mice to subcutaneous inoculation of Cryptococcus neoformans was abolished when cortisone in an amount of 2.5 mg per injection was administered intraperitoneally $1\frac{1}{2}$ hours prior to infection and again a week later. They also noted that there was a more rapid dissemination of the fungus in the cortisone-treated mice than in the controls.

Roth, Friedman and Syverton (1957) studied the effect of roentgen radiation and cortisone on the susceptibility of mice to Candida albicans. They found that the single doses of cortisone (2 mg to 7.5 mg) administered intramuscularly 2 hours prior to the intraperitoneal introduction of Candida albicans, reduced the resistance of the 12 to 18 weeks old Swiss albino mice to a degree proportional to the amount given. They noted also that when cortisone and x-ray radiation were given in combination, an increased dosage of either enhancing agent with a constant dosage of the other resulted in an increased mortality rate and decreased the mean survival time. To study the duration of enhancement by cortisone,

the investigators injected 6 groups of animals with 4.0 mg of cortisone. Two, 24, 48, 72, 96 or 120 hours later, the animals were challenged with the organism. Basing their results on the accumulated percental mortality, they found that enhanced susceptibility was apparent as early as 2 hours and as late as 120 hours after treatment with cortisone. They noted also that enhancement was at a maximum at 48 hours after treatment.

Louria and Browne (1960) observed that mice receiving daily intraperitoneal cortisone injections (0.1 mg to 2.0 mg per injection) for 6 days prior to and 28 days after the intravenous infection with either Histoplasma capsulatum or Cryptococcus neoformans showed a mortality rate similar to that of the controls. However, when Candida albicans was the infecting agent, a mortality rate of 10% for the controls and 90% for the cortisone-treated mice was recorded when treatment was started 2 to 6 days prior to infection. When treatment was started the day after infection, the mortality rate was reduced to 70%.

Basing his results on gross observations of the spleen and on the histopathological studies of the tissues, Winner (1960) found no differences in cortisone-treated and control guinea pigs infected intradermally or intraperitoneally with Candida albicans. The treated guinea pigs received intramuscular cortisone injections in the amount of 5 mg per gram of body weight on alternate days for four weeks prior to infection.

EXPERIMENTAL METHODS

Fifty-seven soil samples from selected sites in and around Tucson, Arizona, were subjected to study for pathogenic fungi. These samples consisted of soil from the trunk and root zones of various plants (living and dead), from rodent burrows, from ant hills and from termite tunnels. One sample was selected because it was protected by a plank from the direct rays of the sun. Another sample protected from the sun by a cover of animal dung and possibly enriched thereby was also included. The specific sources of the samples are given in Table I together with the dates of collection.

In the case of samples collected near plants, collections were made 3 to 6 inches below the surface and immediately adjacent to the main trunk or root of the plant.

The soil samples were collected during the months of September, October and November, 1960. The dates of collection and the dates of the last rain preceding the collection (obtained from the Weather Bureau) are noted below:

<u>Date of Collection</u>	<u>Date of Last Rain Before Collection</u>
September 18, 1960	September 9, 1960
September 24, 1960	September 9, 1960
September 25, 1960	September 9, 1960
October 7, 1960	September 30, 1960
October 16, 1960	October 14, 1960
October 24, 1960	October 14, 1960
November 26, 1960	November 6, 1960

In the beginning, the soil treatment was essentially that of Emmons

TABLE I
Sources of Soil Samples

Sources	Total No. of Samples	No. of Samples	Date of Collection	Area of Collection
Trunk zone of living Palo Verde (<u>Cercidium microphyllum</u>)	14	24,25,26,27,28 31 72,74,75,77,78 73,76,79	Sept.18, 1960 Sept.25, 1960 Nov. 26, 1960 Nov. 26, 1960	Saguaro Nat'l Monument Tucson Mountain Park Duval Mines Road Area Twin Buttes Road Area
Root zone of living Creosote bush (<u>Larrea tridentata</u>)	2	61,65	Oct. 24, 1960	Pantana Ranch
Root zone of living Iron Wood (<u>Olneya tesota</u>)	3	32,33,34	Sept.25, 1960	Tucson Mountain Park
Root zone of living Barrel Cactus (<u>Echinocactus Wislizenii</u>)	3	21,22,23	Sept.18, 1960	Saguaro Nat'l Monument
Root zone of living Bur Sage (<u>Franseria sp.</u>)	1	69	Oct. 24, 1960	Pantana Ranch
Root zone of dead Century plant (<u>Agave sp.</u>)	1	71	Oct. 24, 1960	Pantana Ranch
Rodent Burrows	9	30,44,45,46,47,48 63,67,68	Sept.24, 1960 Oct. 24, 1960	Tucson Mountain Park Pantana Ranch

TABLE I (Continued)
Sources of the Soil Samples

Source	Total No. of Samples	No. of Samples	Date of Collection	Area of Collection
Termite tunnels	17	35,36,37,38,39) 40,41,42,43)	Oct. 7, 1960	Ina Road, about 1 mile west of Camp- bell Avenue
		50,51,52,53,54	Oct. 16, 1960	Sonora Arizona Desert Museum
		55,56,57	Oct. 24, 1960	Pantana Ranch
Ant hills	3	58,59,60	Oct. 24, 1960	Pantana Ranch
Soil under decaying Cholla (<u>Opuntia fulgida</u>)	2	62,64	Oct. 24, 1960	Pantana Ranch
Soil underneath plank	1	66	Oct. 24, 1960	Pantana Ranch
Soil under animal dung	1	70	Oct. 24, 1960	Pantana Ranch

(1942) as modified by Ajello, Reed, Maddy, Budurin and Moore (1956).

20 g of each soil sample were suspended by vigorous shaking in 60 ml of sterile physiological saline. The suspension was allowed to settle for 5 minutes. Then with a sterile pipette, a 10 ml sample of the suspension was transferred to a sterile 20 ml tube and treated with 5000 units of penicillin (Crysticillin, Squibb) and 1000 units of streptomycin (Squibb) per ml to reduce the numbers of bacteria. The suspension was allowed to stand overnight at room temperature before it was used for animal inoculation. Then 1 ml of this suspension was inoculated intraperitoneally into each mouse. Because of the resulting high mortality rate, the procedure was changed by increasing the weight of the soil sample from 20 g to 30 g and decreasing the volume of the inoculation from 1.0 to 0.5 ml.

Three to four weeks old female white Swiss mice, Webster strain, were obtained from the Animal Supply Company, Napa, California. They were divided into groups of 8, and each of these was again divided into 2 subgroups (A and B) of 4 each. The animals in subgroup A were to receive cortisone and those in subgroup B were to be the controls. Five days after the animals arrived at the laboratory, they were weighed (in groups of 4) and the cortisone dosage for subgroup A was calculated according to the method of Hobson (1960) so that each mouse received 25 mg of cortisone (Cortone Acetate, Merck) per kg body weight suspended in 0.5 ml of sterile physiological saline. The 4 animals of subgroup A received one subcutaneous injection daily for 5 days preceding the inoculation of the soil suspension. The mice in subgroup B received none. The cortisone-treated animals continued to receive cortisone on alternate

days until the termination of the experiment which lasted 4 to 6 weeks. All animals were weighed weekly and all were observed closely for symptoms of illness. Animals dying before the termination of the experiment were subjected to autopsy and examined for lesions. The lungs, livers, spleens, and any diseased tissue were removed to a sterile petri dish, minced, and cultured on appropriate media. All mice surviving the experimental period were sacrificed and treated similarly.

The media used for the cultivation of fungi included (1) Mycosel agar (B.B.L.), (2) Mycological agar (Difco) and (3) Subouraud-glucose agar. The cultures were incubated at room temperature and were held for at least 4 weeks before they were discarded as negative.

Any molds growing on these media were identified by their macro- and micromorphology. In a few instances, verification was sought through animal inoculation.

The yeasts that appeared were studied using the methods of Wickerham (1951) (1957) and were identified according to the scheme of Lodder and Kreger-Van Rij (1952).

For the identification of Nocardia, the techniques of Gordon and Smith (1955) and Gordon and Mihm (1957) were followed.

RESULTS

Table II summarizes the results of this investigation and shows the source of each sample, the comparative weights of the cortisone-treated mice and the controls and the fungi or fungus-like organisms that were isolated.

Only 5 of the 57 soil samples yielded mold cultures 3 of which, #25 from the trunk zone of a palo verde tree and #30 and #68 from animal burrows, were diagnosed as Coccidioides immitis. Soil sample #54 from a termite tunnel yielded Aspergillus fumigatus. Positive cultures were obtained in these 4 instances only in the mice that received cortisone treatments and not in the controls.

Soil sample #71 came from the root zone of a century plant which was said to have died recently as a result of beetle attack. The animals inoculated with this soil showed symptoms of severe illness within 2 days. One animal from the cortisone-treated group died on the 4th day, and one from the controls died on the 3rd day after infection. Both animals were subjected to autopsy. On the 12th day after soil inoculation, when it appeared that the remainder of the animals would not survive another 24 hours period, they were sacrificed. All the cultures from the cortisone-treated mice and from the controls yielded a mixture of fungi but the largest number of fungi came from the cortisone-treated mice. Separation of the mixture was difficult and possibly incomplete. However, the following fungi were identified:

TABLE II

The Results Observed in Cortisone-Treated and Untreated
Animals Injected with Soil Suspensions

Soil Sample	Source	Cortisone-Treated Mice				Controls			
		Initial wt.(g)	Final wt.(g)	% wt. gain	Mycological Findings	Initial wt.(g)	Final wt.(g)	% wt. gain	Mycological Findings
24	Palo Verde	13.5	19.5	44.4	<u>T. glabrata</u>	10.0	23.0	130.0	<u>T. glabrata</u>
25	Palo Verde	13.5	22.0	62.9	<u>C. immitis</u> <u>T. glabrata</u>	13.0	28.3	117.7	
26	Palo Verde	16.0	22.8	42.5	<u>T. glabrata</u>	15.0	28.5	90.0	
27	Palo Verde	12.3	24.0	95.1	<u>T. glabrata</u>	17.3	24.0	38.7	<u>T. glabrata</u>
28	Palo Verde	15.0	23.3	55.3		11.8	24.3	105.9	
31	Palo Verde	14.5	24.3	67.6	<u>T. glabrata</u>	14.8	27.8	87.8	
72	Palo Verde	15.4	26.2	70.1	<u>T. glabrata</u>	14.0	25.7	83.6	<u>T. glabrata</u>
73	Palo Verde	15.9	24.0	50.9	<u>T. glabrata</u>	17.2	28.0	62.8	<u>T. glabrata</u>
74	Palo Verde	19.7	22.2	12.7	<u>T. glabrata</u>	18.7	25.0	33.6	
75	Palo Verde	16.5	34.3	107.9		16.7	23.8	42.5	
76	Palo Verde	14.2	22.2	56.3		20.2	23.7	17.3	
77	Palo Verde	21.0	24.0	14.3		21.7	32.7	50.6	

*Weight of individual mouse calculated from weight of group.

TABLE II (continued)

The Results Observed in Cortisone-Treated and Untreated
Animals Injected with Soil Suspensions

Soil Sample	Source	Cortisone-Treated Mice				Controls			
		Initial wt.(g)	Final wt.(g)	% wt. gain	Mycological Findings	Initial wt.(g)	Final wt.(g)	% wt. gain	Mycological Findings
78	Palo Verde	18.6	22.8	22.6	<u>T. glabrata</u>	20.4	22.4	9.8	<u>T. glabrata</u>
79	Palo Verde	18.4	22.8	23.9	<u>N. asteroides</u> <u>T. glabrata</u>	20.4	26.8	31.4	<u>N. asteroides</u>
61	Creosote bush	15.4	24.8	61.0	<u>T. glabrata</u>	16.4	24.7	50.6	<u>T. glabrata</u>
65	Creosote bush	13.8	27.0	95.6	<u>T. glabrata</u>	13.2	26.2	98.5	<u>T. glabrata</u>
32	Iron Wood	14.8	24.0	62.2	<u>T. glabrata</u>	14.0	28.0	100.0	<u>T. glabrata</u>
33	Iron Wood	13.0	23.0	76.9	<u>T. glabrata</u>	13.7	26.3	91.9	<u>T. glabrata</u>
34	Iron Wood	16.0	27.3	70.6		15.7	30.0	91.1	
21	Barrel Cactus	10.5	24.3	131.4		14.2	25.5	79.6	
22	Barrel Cactus	15.0	22.5	50.0	<u>T. glabrata</u>	15.2	23.6	55.3	<u>T. glabrata</u>
23	Barrel Cactus	14.0	22.7	62.2	<u>T. glabrata</u>	10.7	22.2	107.5	
69	Bur Sage	16.0	28.8	80.0	<u>T. glabrata</u>	16.0	26.5	65.6	<u>T. glabrata</u>
30	Rodent Burrow	15.0	21.5	43.3	<u>C. immitis</u> <u>T. glabrata</u>	13.3	29.5	121.8	

TABLE II (Continued)

The Results Observed in Cortisone-Treated and Untreated
Animals Injected with Soil Suspensions

Soil Sample	Source	Cortisone-Treated Mice				Controls			
		Initial wt.(g)	Final wt.(g)	% wt. gain	Mycological Findings	Initial wt.(g)	Final wt.(g)	% wt. gain	Mycological Findings
44	Rodent Burrow	11.5	25.2	119.1	<u>T. glabrata</u>	11.0	25.2	129.1	<u>T. glabrata</u>
45	Rodent Burrow	11.0	23.7	115.4	<u>T. glabrata</u>	10.5	24.5	133.1	
46	Rodent Burrow	10.5	25.5	142.9		9.6	27.0	181.3	
47	Rodent Burrow	11.0	24.7	124.5	<u>T. glabrata</u>	11.0	23.0	109.1	
48	Rodent Burrow	8.0	21.5	168.8	<u>T. glabrata</u>	12.7	31.0	144.0	
63	Rodent Burrow	10.7	26.0	142.9	<u>T. glabrata</u>	14.7	28.0	90.5	<u>T. glabrata</u>
67	Rodent Burrow	14.8	26.8	81.1		11.8	25.0	111.9	
68	Rodent Burrow	16.8	24.0	42.8	<u>C. immitis</u> <u>T. glabrata</u>	15.8	30.5	93.0	
35	Termite Tunnel	12.8	23.8	85.9		16.3	26.8	64.4	<u>T. glabrata</u>
36	Termite Tunnel	17.0	25.0	47.1	<u>T. glabrata</u>	15.7	25.0	59.2	
37	Termite Tunnel	17.3	23.0	32.9		16.5	27.5	66.6	
38	Termite Tunnel	16.3	22.8	39.8	<u>T. glabrata</u>	14.8	27.0	82.4	<u>T. glabrata</u>
39	Termite Tunnel	15.8	24.0	51.9		17.0	26.8	57.6	<u>T. glabrata</u>

TABLE II (Continued)

The Results Observed in Cortisone-Treated and Untreated
Animals Injected with Soil Suspensions

Soil Sample	Source	Cortisone-Treated Mice				Controls			
		Initial wt.(g)	Final wt.(g)	% wt. gain	Mycological Findings	Initial wt.(g)	Final wt.(g)	% wt. gain	Mycological Findings
40	Termite Tunnel	15.0	25.2	68.0	<u>T. glabrata</u>	14.0	22.6	61.5	<u>T. glabrata</u>
41	Termite Tunnel	16.2	25.3	56.1		16.2	28.2	74.0	<u>T. glabrata</u>
42	Termite Tunnel	13.2	20.6	56.1	<u>T. glabrata</u>	11.0	22.0	100.0	<u>T. glabrata</u>
43	Termite Tunnel	12.0	23.2	93.3	<u>T. glabrata</u>	12.7	25.5	100.7	
50	Termite Tunnel	13.7	25.7	87.5	<u>T. glabrata</u>	15.7	31.5	100.6	
51	Termite Tunnel	16.2	30.7	89.5	<u>T. glabrata</u>	12.2	24.7	107.5	<u>T. glabrata</u>
52	Termite Tunnel	14.7	24.0	63.3		12.5	29.7	137.6	
53	Termite Tunnel	12.5	27.7	121.6	<u>T. glabrata</u>	14.7	29.5	100.5	
54	Termite Tunnel	9.6	18.0	87.5	<u>Asp. fumigatus</u>	14.2	31.5	121.9	
55	Termite Tunnel	13.2	23.0	74.2		13.7	28.2	105.8	
56	Termite Tunnel	15.0	27.0	80.0		16.7	29.7	77.8	
57	Termite Tunnel	13.5	28.5	111.1	<u>T. glabrata</u>	13.8	29.0	110.2	
58	Ant Hill	17.2	26.7	55.2		17.2	26.3	52.9	

TABLE II (Continued)

The Results Observed in Cortisone-Treated and Untreated
Animals Injected with Soil Suspensions

Soil Sample	Source	Cortisone-Treated Mice				Controls			
		Initial wt.(g)	Final wt.(g)	% wt. gain	Mycological Findings	Initial wt.(g)	Final wt.(g)	% wt. gain	Mycological Findings
59	Ant Hill	16.8	24.0	42.8	<u>T. glabrata</u>	15.8	28.2	78.5	
60	Ant Hill	15.5	23.2	49.7		15.0	28.5	90.0	
62	Under dead Cholla	13.0	22.8	75.4		16.5	29.5	78.8	<u>T. glabrata</u>
64	Under dead Cholla	13.4	28.5	112.6	<u>T. glabrata</u>	13.0	26.0	100.0	<u>T. glabrata</u>
66	Under plank	12.4	22.8	83.8		13.0	23.2	78.5	<u>T. glabrata</u>
70	Under animal dung	17.5	23.8	36.0	<u>T. glabrata</u>	13.7	26.8	95.6	<u>T. glabrata</u>
71	Under dead Century Plant	15.5	18.3	18.1	<u>M. apiospermum</u> <u>Scopulariopsis sp.</u> <u>Asp. flavus</u> <u>Asp. quercinus</u> <u>Asp. wentii</u> <u>Asp. flavipes</u> <u>Asp. ochraceus</u> <u>Asp. sydowii</u>	16.1	23.6	46.5	<u>M. apiospermum</u> <u>Scopulariopsis sp.</u> <u>Asp. flavus</u> <u>Asp. quercinus</u> <u>Asp. wentii</u>

<u>Cortisone-treated mice</u>	<u>Controls</u>
<u>Monosporium apiospermum</u>	<u>Monosporium apiospermum</u>
<u>Aspergillus flavus</u>	<u>Aspergillus flavus</u>
<u>Aspergillus quercinus</u>	<u>Aspergillus quercinus</u>
<u>Aspergillus wentii</u>	<u>Aspergillus wentii</u>
<u>Aspergillus flavipes</u>	
<u>Aspergillus ochraceus</u>	
<u>Aspergillus sydowii</u>	
<u>Scopulariopsis sp.</u>	<u>Scopulariopsis sp.</u>

Nocardia asteroides was isolated from both the cortisone-treated mice and from the controls inoculated with soil sample #79 which was collected from the trunk zone of a palo verde tree.

A completely unexpected finding in this investigation was the repeated occurrence in culture of the yeast, Torulopsis glabrata. This yeast was found in cultures from 36 of the 57 cortisone-treated groups and in 24 of the controls. Of the total 213 cortisone-treated mice subjected to autopsy, 115 yielded cultures positive for this yeast. Of 225 control mice 69 yielded positive cultures.

Table III lists the pathogenic organisms isolated from the cortisone-treated mice and from the controls, the source of the sample and the number of samples from a similar source that were included in the test.

Both Sabouraud-glucose agar and Bacto-Mycological agar proved to be highly satisfactory for the growth of Torulopsis glabrata but Mycosel agar was by comparison extremely poor.

TABLE III

The Microorganisms Obtained by the Passage of Soil
Through Cortisone-Treated and Untreated Animals

Organism Isolated	Number of Times Isolated from		Source of Soil Sample
	Cortisone-Treated	Controls	
<u>Coccidioides immitis</u>	2	0	Rodent Burrows (9))
<u>Coccidioides immitis</u>	1	0	Near trunk zone of Palo Verde (14)
<u>Nocardia asteroides</u>	1	1	Near trunk zone of Palo Verde (14)
<u>Aspergillus fumigatus</u>	1	0	Termite Tunnel (17)
<u>Monosporium apiospermum</u>	1	1	Root zone of a dead Century plant (1)
<u>Aspergillus flavus</u>	1	1	
<u>Aspergillus quercinus</u>	1	1	
<u>Aspergillus wentii</u>	1	1	
<u>Aspergillus flavipes</u>	1	0	
<u>Aspergillus ochraceus</u>	1	0	
<u>Aspergillus sydowii</u>	1	0	
<u>Scopulariopsis sp.</u>	1	1	The soil samples came from a vari- ety of sources. The yeast is not believed to have been derived from the soil
<u>Torulopsis glabrata</u>	36	24	

*Total number of samples from similar source

DISCUSSION

From the preliminary studies reported herein, it appears that the use of cortisone-treated mice in the screening of soil for pathogenic fungi might have certain advantages over the conventional method. Six soil samples yielded cultures of potentially pathogenic fungi or Nocardia when passed through the cortisone-treated mice while only two soil samples were similarly positive when inoculated into the control non-cortisone-treated animals.

The isolation of Coccidioides immitis from rodent burrows might be expected since it has been reported also by Emmons (1942), Ajello, Reed, Maddy, Budurin and Moore (1956), Egeberg and Ely (1956) and Elconin, Egeberg and Lubarsky (1957). These investigators believe that the rodent burrows favor the growth of the fungus for they not only protect it from the extreme heat of the sun but also from the washing of heavy rains. The 9 samples from animal burrows included in this investigation gave positive results in 2 cases. The lack of any positives in the control groups could reflect a low infectivity since the samples were collected in late September and late October after the rainy season. However, some rain had fallen a week or two before the dates of collection. The two positive samples from rodent burrows came from different sites for one was collected in the Tucson Mountain Park west of Tucson while the other was collected on Pantana Ranch about 8 miles east of Tucson.

Egeberg and Ely (1956) and Elconin, Egeberg and Lubarsky (1957),

have pointed out the importance of rain to the growth of Coccidioides immitis in soil. This may also be true for other pathogenic fungi. The 3rd isolate of Coccidioides immitis came from a sample adjacent to the trunk zone of a palo verde tree, a plant very prevalent in the desert area. However, 13 samples from similar sources were negative.

Aspergillus fumigatus is a pathogen and "an extremely cosmopolitan mold that occurs with particular frequency in soil containing appreciable organic material," (Thom and Raper, 1945). From one soil sample (#54), 1 of 17 obtained from termite tunnels, Aspergillus fumigatus was isolated but only by passage through the cortisone-treated mice. It has not previously been reported from Arizona soil.

Nocardia asteroides, considered by some to be a fungus-like organism, has been isolated from soil in various places. Although disease due to this organism does occur in Arizona, its isolation from the soil of this state has not previously been reported. In this investigation, it was isolated from both cortisone-treated mice and from the controls inoculated with a soil sample that came from the trunk zone of a palo verde tree (#79).

Monosporium apiospermum, the imperfect form of Allescheria boydii, is a cause of mycetoma and has recently been reported by Scharyj, Levene and Gordon (1960) to be the cause of a pulmonary infection. This organism was found in one soil sample (#71) that came from the root zone of a recently dead century plant. It was isolated from both the cortisone-treated and the control animals. There appear to be no reports of the occurrence of disease due to Monosporium apiospermum, or to its perfect form Allescheria boydii, in Arizona nor of previous isolations of this

organism from local soils. The isolation of this mold, together with a variety of others, from this particular soil sample as well as the serious effects observed in both cortisone-treated and control mice immediately following inoculation would appear to be due to toxic substances in the soil capable of overwhelming the resistance of the mouse to the invasion of organisms that are considered to be non-invasive or only mildly invasive.

Cryptococcus neoformans, which has been observed as a cause of disease in this area, was not found in any of the soil samples.

The yeast, Torulopsis glabrata, was originally isolated by Anderson (1917) from the intestinal tract of man and has not been reported from any source other than humans. It has never been found to be a soil inhabitant. Benham (1935) found it to be non-pathogenic for rabbits, but Black and Fisher (1937) noted its pathogenicity for rats. Wickerham (1957) called attention to recent observations of Torulopsis glabrata as a serious pathogenic agent for humans. Apparently, it, like Candida albicans, may become a serious invader in patients who have undergone extensive antibiotic treatment. Since, in this investigation, the antibiotic-treated soils were inoculated into all animals (cortisone-treated and controls) and this yeast was isolated from both groups, it is difficult to determine from the data whether antibiotics, or cortisone, or the two in combination altered the resistance of the mouse so that an organism presumably saprophytic in the intestinal tract of the animal was able to invade the tissues. Cultures of the spleens, lungs and livers from one group of normal mice did not yield this yeast.

Most of the mice in both the cortisone-treated and control groups

survived the experimental period. However, it appears that cortisone did exert an unfavorable effect on the mouse for in 41 of the 57 groups, the percent weight gain in the cortisone-treated mice was depressed. This effect on weight was also noted by Mankowski and Littleton (1954). In every instance in which pathogenic molds were isolated, the percent weight gain was markedly less than that of the controls.

The true usefulness of this method can only be determined by further investigation which should include studies of the effect of different dosages of cortisone, the effect of different routes of administering cortisone, and the effect of different routes of soil inoculation as well as the effect of different adjuvants.

SUMMARY

(1) The use of mice in which resistance to disease is reduced by cortisone appears to have certain advantages over the conventional method in which non-treated animals are used.

(2) Six out of the total of 57 soil samples tested yielded pathogenic fungi or Nocardia asteroides. Four of these, from which Coccidioides immitis was isolated in 3 instances and Aspergillus fumigatus in one, gave positive results in the cortisone-treated mice only. Two soils containing Nocardia asteroides and Monosporium apiospermum gave positive results in both the cortisone-treated and the control mice. From one sample, a variety of fungi was obtained in both the cortisone-treated and control animals but the variety was greater in the cortisone-treated groups than in the controls.

(3) The yeast, Torulopsis glabrata, was recovered from 69 of the 225 control mice and from 115 of the 213 cortisone-treated mice. It is not considered to have been derived from the soil.

(4) In 41 of 57 groups, the percent weight gain was less in the cortisone-treated mice than in the controls.

(5) The results appear to justify the conclusion that this method of soil screening deserves further investigation.

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