Reports

Isolation of Blastomyces dermatitidis from Soil

Abstract. Through the intravenous inoculation of soil suspensions in the tail vein of mice, *Blastomyces dermatitidis* was recovered from a Lexington, Kentucky, soil sample. The positive specimen was collected in a tobacco-stripping barn. The shed had sheltered a dog that died of blastomycosis 2 years before the sample was collected.

Although soil has been found to be the natural habitat for many of the fungi pathogenic to man (*1*), all previous attempts to isolate *Blastomyces dermatitidis* from this source have failed (*2*). In the present study, soil specimens collected from the environs of human and canine cases of blastomycosis in or near Lexington, Kentucky, were examined by means of a new technique developed at the Medical College of Georgia. *Blastomyces dermatitidis* was isolated from one of the samples.

This sample was collected during the course of a continuing investigation of the natural habitat of the fungus (*3*). Each sample was taken by pressing the open mouth of a sterile 4-oz screw-cap bottle into the soil. The first 600 samples were examined by a technique which is routinely employed by the Mycology Unit of the Communicable Disease Center for the isolation of pathogenic fungi from soil (*4*) and which involved the intraperitoneal inoculation of mice. In addition, soil suspensions were serially streaked on plates of three different media (*3*). Approximately every tenth specimen (54 in all) was sent to the Medical College of Georgia for re-examination by a different technique that involved the intravenous inoculation of mice.

Prior to inoculation, a heaping teaspoonful of soil was suspended in 50 ml of physiological saline containing 1000 units of penicillin G and 1 mg of streptomycin per milliliter in a large (38 by 200 mm) test tube. After vigorous stirring, the soil was allowed to settle for 1 hour. A 5-ml sample was removed with a pipette from the interface between the supernatant and the sediment. Two-tenths of a milliliter of a slightly turbid saline suspension of *Mycobacterium fortuitum* was added as an adjuvant to each milliliter of sample. After thorough mixing, 0.5 ml of the specimen was inoculated intravenously into the tail vein of each of five adult white Swiss mice.

Three weeks after inoculation the mice were killed, and their lungs and small portions of their livers and spleens were cultured. Both lungs, each lung on a different plate, were cultured on Sabouraud dextrose agar containing 100 units of penicillin, 0.1 mg of streptomycin, and 1 mg of cycloheximide per milliliter. Pieces of liver and spleen from each mouse were cultured on tubes of brain heart infusion blood agar containing 100 units of penicillin and 0.1 mg of streptomycin and on neutral dextrose agar with the same antibiotics plus cycloheximide. Thirty cultures were made for each soil sample. To prevent drying, the plates were sealed with cellophane tape, the tubes with rubber stoppers. The Sabouraud agar plates and tubes of natural dextrose agar were incubated at 25°C, while the brain heart infusion blood agar, without cycloheximide, was incubated at 37°C. The cultures were examined after 30 days for the presence of pathogenic fungi.

Blastomyces dermatitidis was isolated from one of the 54 soil specimens examined at the Medical College of Georgia. Of the five mice inoculated with this soil, two yielded isolates of the fungus. Both Sabouraud agar plate cultures of the lungs from each mouse were positive for *B. dermatitidis*. The liver and spleen cultures were negative. At autopsy, gross lesions were not detected in the lungs.

The strain of *B. dermatitidis* isolated from soil was typical of the species (*5*). Growth on Sabouraud dextrose agar after 30 days at 25°C was similar to that of cultures isolated from human beings and dogs with respect to gross colony characteristics and microscopic morphology. When two subcultures were made on brain heart infusion blood agar and incubated at 37°C, they were converted to the typical yeastlike form in 10 and 15 days, respectively. Subcultures of the yeastlike form on brain heart infusion blood agar incubated at 37°C grew rather slowly and sparsely. When transferred to a 25°C incubator they grew much more rapidly and were converted to the mycelial phase.

Growth from a 6-day-old blood agar culture of the yeastlike form of the isolate was suspended in 3 ml of saline, and 0.5 ml of this suspension was injected intravenously into the tail veins of three adult mice. One of the mice died on the 7th day, the other two on the 8th. At autopsy the lungs of all three mice showed a massive embolic pneumonia with consolidation into a firm, gray, granulomatous mass. Direct examination of all portions of the lungs revealed numerous tissue-phase cells of *B. dermatitidis*. Cultures made of the livers and spleens, as well as of the lungs, were positive for *B. dematitidis*. The fungus grew in the yeast phase at 37°C and the mycelial phase at 25°C.

The virulence of 0.5 ml of a suspension of the yeastlike form, which showed 90 percent light transmission at a wavelength of 550 $m\mu$ on a Spectronic 20 colorimeter when it was injected intravenously, was compared with six other isolates of *B. dermatitidis*—five from human beings and one from the northern sea lion (*6*). The soil isolate was found to be the most virulent.

The positive sample was taken from the earth floor of a stripping shed attached to a tobacco barn located near Lexington, Kentucky. A wooden bench about 3 feet wide was attached to the wall along one side of the room. A stove completed the furniture. Fragments of tobacco left from the stripping process had accumulated on the ground, especially under the bench. The soil that yielded *B. dermatitidis* was taken from the floor just inside the door, at the narrow end of the room. This sample, a brown clay loam, contained little gross organic matter and had a *p*H of 5.8 as determined by a Beckman *p*H meter.

This shed at one time had been used to shelter a dog affected with blastomycosis. In December 1955, a diagnosis of blastomycosis was made in a female Weimaraner approximately 1 year old, living at a house in a nearby town. For much of the time, from the diagnosis of its infection until its death in July 1956, this dog was housed in the shed from which the positive soil was collected. She is reported to have bedded down often near the door and under the bench. Since the dog exuded purulent

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

material from many abscesses scattered over the feet and body, she had ample opportunity to inoculate the soil. However, from the time of her death in July 1956 until the sample was collected on 8 October 1958, there was no known subsequent contamination of the shed with *B. dermatitidis*.

Although *B. dermatitidis* was isolated from only a single soil specimen, it has been demonstrated for the first time that this fungus can and does exist in soil under natural conditions for a long period of time. Success in isolating it when numerous attempts by others, as well as ourselves, had failed is thought to be due to the improved technique employed. It is the general opinion, with some supporting evidence, that the laboratory mouse is not especially susceptible to infection with *B. dermatitidis* when the organism is inoculated intraperitoneally. However, Heilman (*7*) found that intravenous inoculation of small numbers of both the yeastlike and mycelial forms caused death, while intraperitoneal injection of comparable doses of the same strains did not cause any symptoms in the mice. Inoculation of *B. dermatitidis* by the intravenous route allows the organisms to be carried directly to the lungs, which appear to be the most favorable site for infection to develop (*8*). Whether or not the addition of *Mycobacterium fortuitum* enhanced the isolation of *Blastomyces dermatitidis* is not clear at this time (*9*).

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5. The identity of this isolate of *B. dermatitidis* was confirmed by the Mycology Unit, Communicable Disease Center, Atlanta, Ga.
6. This strain was kindly furnished by W. M. Williamson, Chicago Zoological Park, Brookfield, Ill.
7. F. R. Heilman, *J. Invest. Dermatol.* **9**, 87 (1947).
8. J. F. Denton, unpublished data.
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Ability of Some Black Sea Organisms To Accumulate Fission Products

Abstract. The coefficients of accumulation of strontium-90, cesium-137, and cerium-144 in seaweeds, eelgrass, actinia, mollusks, and crustaceans are presented. The discharge of strontium-90 into sea water from decomposing seaweed and the retention and additional absorption of cesium-137 and cerium-144 onto organic debris is discussed. Some observations are made about the ability of these elements to diffuse into sea water and about the relative hazard to man from strontium-90 and cerium-144 in marine life.

A great deal of importance is attached to the elucidation of the role of living organisms in the general problem of the diffusion of fission products into ocean and sea waters (*1, 2*). This suggests the need for an investigation of the ability of different sea animals and plant life to accumulate the most important radioactive substances (*3, 4, 5*).

The following different marine plants and animals were selected for investigation in the present study: green algae (*Ulva rigida, Enteromorpha minor*), brown algae (*Dictyota fasciola, Padina pavonia, Cystoseira barbata*), red algae (*Corallina rubens, Ceramium rubrum, Polysiphonia elongata, Phyllophora rubens, Laurencia obtusa*), eelgrass (*Zostera marina*), coelenterata (*Actinia equina*), mollusks (*Mytilus galloprovincialis*), and crustaceans (*Pachygrapsus marmoratus, Carcinus moenas, Leander squilla*). The concentrations of Sr^{90} (in 15 species), Cs^{137} (in 7 species), and Ce^{144} (in 12 species) in these marine organisms were studied.

Specimens of the various organisms were placed in separate glass vessels filled with 2 to 5 liters of filtered sea water which contained concentrations (10μc/lit.) of Sr^{90}, Cs^{137}, and Ce^{144} (solutions of chlorides). Samples of water and organisms were collected simultaneously after 3, 6, and 12 hours and 1, 2, 4, 8, 16, 32, and 64 days had elapsed after the start of the study. One-milliliter samples of the sea water, placed in standard aluminum planchets, were evaporated under an infrared lamp. The samples of plant and animal organisms were rinsed in clear sea water, dried on filter paper, weighed immediately, and then placed in drying ovens at 80° to 90°C, after which their dried constant weight was determined. The desiccated organisms were powdered, and 10-mg paired samples of the powder were placed in standard planchets for counting. There was no self-absorption of radiation in these samples. Correction for radioactive decay was introduced for samples with Ce^{144}. The counting of Sr^{90} in the samples was done only after Sr^{90} and Y^{90} had reached equilibrium. Radiation was measured with a Geiger-Müller type counter, with a possible error of 3 percent.

The mean values of the coefficients of accumulation (the ratio of radioactivity in the organism to that of an equal weight of water) were calculated by averaging a number of coefficients, beginning with the samples of Sr^{90} collected on the second day, and with samples of Cs^{137} and Ce^{144} on the eighth day, after the experiment began. This was because from this time on (occasionally even earlier) these values were practically constant in all samples. One exception was the uninterrupted increase of Sr^{90} accumulation in the crustaceans and in the mollusk (*Mytilus*) shells. In these two, only the final values—that is, the highest coefficients of accumulation—were considered. The studies continued for as long as the organisms remained alive. The coefficients of accumulation of Sr^{90} for the crustaceans were studied through the 8th-day samples, and for the mollusk shells through the 64th day. The coefficients for Cs^{137} and Ce^{144} in the crustaceans and the mollusk shells were calculated through the 16th-day samples.

The coefficients of accumulated values for Sr^{90}, Cs^{137}, and Ce^{144} for certain species of marine organisms are shown in Table 1. The data for the coefficients of accumulation of Sr^{90} in

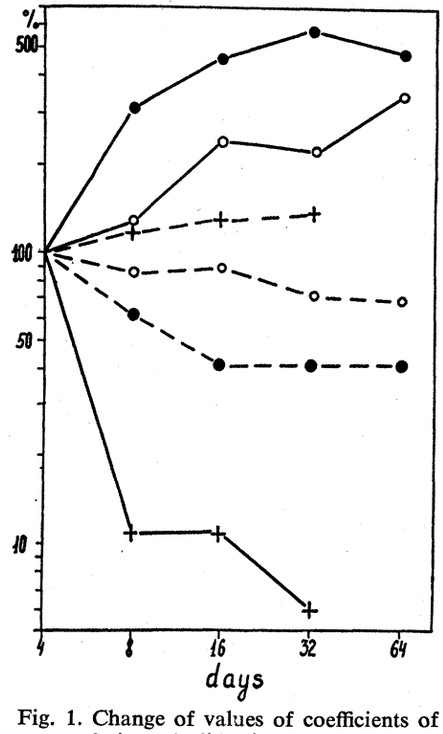

Fig. 1. Change of values of coefficients of accumulation (solid lines) of Sr^{90}(+), Cs^{137}(○), and Ce^{144}(●) in the process of dying off of *Cystoseira*. Concentration of activity in sea water is shown by dashed lines. The times are from the beginning of the experiment. Dying began after 4 days in the absence of fresh sea water.