Extracellular digestion by the growing cells is not ruled out, but phagocytosis is rendered likely by the observations of Bensch *et al*(7) on phagocytosis of DNA-protein complex.

The failure to observe any abnormal chromosomal structures in the acceptor cells, plus the observed lack of incorporation of any H^3TdR in the presence of an excess of unlabeled thymidine suggests that the label was not incorporated in the form of poly- or oligonucleotides. This is in agreement with the *in vivo* experiments of Robinson and Brecher (12) who offered the first proof of thymidine re-utilization *in vivo* at the nucleotide level using inhibition with unlabeled thymidine. Added thymidine may have competed in the metabolic pool with the labeled thymidine from the chromosomes at some stage in the synthesis of DNA. This differs from earlier suggestions by Hamilton(10) and others(11) that DNA is incorporated as polymer. The observed failure of the 10 hour culture to incorporate label in its chromosomes is presumably due to a time lag which is greater than the ordinary G2 period. This represents the period between addition of the labeled chromosomes and incorporation of the extracted label into the newly synthesized chromosomes. It is possible that only cells in the G1 period are able to digest the chromosomes and extract the label for incorporation into the newly synthesized DNA.

Summary. (1) A method for producing a suspension of pure mitotic chromosomes of Chinese hamster cells with insignificant contamination by interphase nuclei or their fragments is described. The yield obtained is high and the chromosome morphology is well preserved. (2) H^3 thymidine label from such chromosomes became specifically incorporated into the chromosomes of fresh Chinese hamster cells and of human leucocyte cultures. No gross alteration in the chromosomes of the receptor cells occurred, and uptake of label was inhibited by the presence of added carrier thymidine in the medium, in an amount insufficient to inhibit DNA synthesis. Therefore, most of the incorporation appears to have involved previous degradation of the DNA at least to the level of free nucleotide.

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The Susceptibility of *Blastomyces dermatitidis* to Drying. (32065)

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One of the major mycologic mysteries has been the determination of the natural reservoir of *Blastomyces dermatitidis*. Although it is assumed that this reservoir is the soil, the only workers who have isolated this fun-

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gus from the soil have not been able to substantiate their work(1,2).

The isolation of *B. dermatitidis* from sources such as soil may depend on the susceptibility of different species of laboratory animals to infection with this fungus. In the recent report by Smith *et al*(3) it was shown that the hamster was the more sensitive laboratory animal to infection. About two-thirds of the hamsters could be infected with an intraperitoneal dose in the range of 3,000-4,000 organisms. The conclusion was that to equal the hamster rate of infection in mice would require a minimal dose exceeding 100,000 viable particles. Thus the mouse, frequently used in attempts to isolate *B. dermatitidis* from soil would appear to be a relatively inefficient animal to use for this purpose. It is well known also that considerable time often elapses between the collection of soil in the field and inoculation of mice with soil specimens.

Experiments aimed at determining the susceptibility of *B. dermatitidis* to drying in soil are reported here. Studies were performed also using different methods of storing soil samples for extended periods of time.

Materials and methods. Screened loam soil was placed in flat metal pans one-half inch deep. The soil dampened with distilled water and covered with aluminum foil was autoclaved for 30 minutes on 3 consecutive days.

Humidity chambers modified from Hinton *et al*(4) were essentially one-half gallon widemouth jars fitted with metal screw cap lids. One hundred ml of distilled water was placed in each jar and a 250 ml Erlenmeyer flask with approximately 100 cc of sterilized soil was floated in the water. A hole one-fourth inch in diameter was punched through each lid and plugged with cotton. The chambers containing the soil were autoclaved for 20 minutes at 15 lb pressure.

A human isolate (Davis) of *B. dermatitidis* grown on Sabouraud's medium was harvested by swirling with glass beads and distilled water. One ml of the harvested suspension was inoculated through the hole in the lid onto the surface of each soil sample with a sterile syringe.

The fungus was allowed to grow for approximately 1 year. The flasks were then removed from the humidity chambers and several soils were combined in a rubber stoppered flask. The soil was mixed thoroughly by hand shaking. Sterile 50 ml graduated centrifuge tubes with relatively firm cotton plugs were pre-weighted on an analytical balance. Approximately 10 cc of the infected soil was placed into several of these tubes. Again the tubes with soil were weighted to determine the weight of the soil in each tube.

In the first experiment, 2 of these soils were immediately suspended in sterile distilled water. Serial dilutions were plated on Sabouraud's dextrose agar medium. The number of viable particles per gram of soil was determined using standard colony plate count techniques after 20 days incubation at 27°C. Two tubes of the soil were placed again into a 100% humidity chamber, 4 were left on a table top and 2 were placed in a desiccator containing $CaCl_2$ crystals. All soils were left at room temperature. After 5 days, one of the tubes of soil from the table top and the 2 from the desiccator were weighed and cultured as before. The weight of each soil at 5 days was subtracted from its weight on the first day to determine the weight loss due to water evaporation. Again at 11, 14, and 18 days, a tube of soil left on the table top was weighted and cultured. The remaining 2 tubes of soil which had been replaced at 100% humidity were cultured as above after 18 days.

The second experiment was performed almost a year later. It differed from the first as follows: 1) Sampling was done at 3, 4, 5, 7, 10, 17, and 25 days later using 1 tube of soil left on a table top and 1 in a desiccator at room temperature for each of these days. 2) The 2 tubes of soil placed into a humidity chamber were kept at 4°C and cultured after 25 days.

In a third experiment, a flask containing 100 g of soil was removed from a humidity chamber and the soil mixed thoroughly. A portion of this soil was diluted and cultured as previously described to determine the number of viable particles of *B. dermatitidis* per gram. Two 10 g samples were each placed in a graduated cylinder and cysteine (0.1%)

TABLE I. Viability Studies of *B. dermatitidis* in Soil After Storage up to 18 Days in Different Environments (Exp. 1).

Time (days)	Environment	% Water loss	No. viable (per g)	% Viable
0	Initial control		11,486	100
5	Room	8.61	1,077	9.3
5	Desiccator	9.52	1,500	13.0
5	"	8.48	1,060	9.2
11	Room	9.47	563	4.9
14	"	9.07	495	4.3
18	"	9.77	567	4.9
18	" (100% humidity)		24,196	210.4
18	" (100% humidity)		24,933	216.8

physiologic saline added to a volume of 100 ml. Cultures were made from each after 7 and 30 days storage at 4°C to determine viability changes.

The remaining soil (70 g) was left at room temperature in the flask stoppered with a gauze plug. Cultures were made after 14 and 90 days to compare viability changes.

Results. The effect of drying on the viability of *B. dermatitidis* grown in sterilized soil is shown in Table I. As seen in the first column, the control (average of 2 tubes of soil) containing 11,486 viable particles of *B. dermatitidis* per gram of soil was considered to be 100% viable. After 5 days at room temperature on a table top the total number of viable particles per gram decreased from 11,846 to 1,077, a loss of 90.7%. A similar period of storage in the desiccator showed losses of 87% in one soil tube and 90.8% in the other. Another 50% loss of viability with less than 5% of the fungus particles remaining viable occurred after 11 days and 14 days at room temperature.

Two test controls, no. 7 and 8, replaced in the humidity jars and held for 18 days, showed an increase in organisms approximately doubling the count obtained at the beginning of the experiment.

The percentage of water loss as shown in the third column indicates a weight loss due to water evaporation ranging from 8% to 9% of the initial soil weight. There was a tendency for the water loss to be greater the longer the cultures were held at room temperature.

Experiment 2. The results of the second experiment are shown in Table II. The controls, removed from the humidity chamber, contained only 7,400 viable particles per gram which is somewhat less than in the previous experiment. The results of this experiment, however, are in general agreement with the previous one in that the cultures held for 3, 4, 5, 7, 10, 17, and 25 days on a table top or in a desiccator showed a marked loss of viability. This loss of viability was progressive from about 75% at 3 days to 90% at 5 to 10 days. The viability appeared to fall from about 90% loss at 10 days to 93% at 17 days and 95% at 25 days. The rate of viability loss in a desiccator was similar to that in the room environment. The controls held at 4°C in a humidity jar rather than at room temperature maintained a viability of about 86% (average) which was comparable to the initial controls.

Experiment 3. The viability changes occurring with *B. dermatitidis* in soil suspended in cysteine saline at 4°C are shown in Table III. The number of viable particles in one suspension had increased 10% and the other 34% after 7 days. However, after 30 days both suspensions showed a loss of 95% of the viable particles.

Viability changes after 90 days storage of

TABLE II. Viability Studies of *B. dermatitidis* in Soil After Storage up to 25 Days in Different Environments (Exp. 2).

Time (days)	Environment	% Water loss	No. viable (per g)	% Viable
0	Initial control	0	7,451 7,459	100
3	Room	3.99	2,091	28.0
	Desiccator	3.33	2,222	29.8
4	Room	4.40	768	10.3
	Desiccator	3.76	1,946	26.3
5	Room	4.51	800	10.7
	Desiccator	4.15	830	11.1
7	Room	4.64	708	9.5
	Desiccator	4.29	745	9.4
10	Room	4.24	780	10.4
	Desiccator	4.25	745	9.99
17	Room	5.07	541	7.25
	Desiccator	4.41	645	8.38
25	Room	4.74	454	6.09
	Desiccator	4.47	360	4.82
	100% humidity (4°C)		6,889 5,928	92 79.5

TABLE III. Survival of *B. dermatitidis* in a Saline Soil Suspension Stored at 4°C (Exp. 3).

Time (days)	Viable particles (per ml)	% Viable
0	3,800	100
7	4,200	110.5
	5,100	134.5
30	160	4.5
	170	4.7

B. dermatitidis in soil in an Erlenmeyer flask at room temperature are shown in Table IV. A loss of 90% of the viable particles occurred after 14 days going to 95% loss after 90 days storage.

Discussion. The results of these experiments illustrate clearly the detrimental effects of drying on *Blastomyces dermatitidis* in sterilized soil. The first experiment was performed during May in a quonset building where the room humidity was uncontrolled and usually low, whereas the second experiment was performed during February in a different building with constant heat and humidity control.

One important conclusion is that time between collection of soil samples and inoculation of animals may be vital in survival of the fungus. Indeed, it appears that as little as 3 days may result in a 72% loss of viability with a 90% loss of viability of organisms in the soil for 5 days. It is interesting to note that the maximum viability loss in all experiments did not exceed 95% even after 3 months storage.

There was no loss in viability of the fungus when stored up to 7 days at 4°C in a saline suspension. There was only a small loss when the soil was returned to a humidity jar at 4°C for 30 days. Both of these methods may be satisfactory for storing soil for periods of time prior to isolation. Whether the fungus would multiply as rapidly when stored at 100% humidity at room temperature with competitive microflora present is not known. This method, possibly with use of a plastic bag to retain humidity, should be investigated further.

TABLE IV. Survival of *B. dermatitidis* in Soil After 14 and 90 Days at Room Environment (Exp. 3).

Time (days)	Viable particles (per g)	% Viable
0	38,000	100
14	4,000	10.5
90	2,000	5.5

Although it is clear that results with sterilized soil must be interpreted with caution, it is obvious that other organisms in soil mixtures probably would act to increase loss of viability. The marked losses reported here, therefore, would appear to be minimal rather than maximal.

Summary. The effect of drying on the loss of viability of *Blastomyces dermatitidis* in sterile soil is reported. 1. An average of 90% loss of viability occurred after 5 days storage at room temperature in cotton plugged tubes or in a desiccator. The loss increased to 95% after 11 days or longer. Aliquots of this soil mixed and stored for 10 days in a humidity chamber at room temperature showed a 2-fold increase in viable particles. 2. The loss of viability from storage of soil in tubes after 3 days was 75% with an increase to a 90% loss by 5 days. A 95% loss after 25 days when stored at room temperature or in a desiccator was observed. When soil was placed in a humidity chamber and stored at 4°C for 25 days, only 14% loss in viability occurred. 3. Although *B. dermatitidis* in soil may lose 90-95% viability after 5-10 days at room temperature, longer storage of up to 90 days does not affect the remaining 5% viable particles. Little change was noted in soil suspended in saline at 4°C after 7 days, but after 30 days, a 95% loss of viable particles was detected. 4. The degree of loss of viability appeared to be correlated with water loss by evaporation.

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