plates and 25 silicone slides (*6*) in the slit sampler. Exposure length was 1–3 hr for the filters; for all others, 15–30 min over land and 30–60 min over water. Complete data for each sample were obtained from the navigator's log and from the main meteorological office of the Department of Transport at the Dorval Airport at Montreal, Quebec. Averages were made of the numbers of fungi and bacteria in the various air masses by each sampler and are summarized in Tables 1–4. Numbers of bacteria are based on colonies, those of fungi on colonies and numbers of fungus spores.

Wide variation in numbers of fungi and bacteria/cu ft is apparent from these data; it is due in part to the season of the year, the kind of samplers used, and the type of air mass encountered. More organisms were obtained in the August trip (Tables 3, 4) than in the June trip (Tables 1, 2) but the differences were not significant. The samplers varied considerably in their efficiency: for sampling fungi the filters were very inefficient, but for the bacteria, whose numbers with one exception were less than 1/cu ft, the filters were fairly satisfactory. Samples taken over land masses, particularly from Montreal to Goose Bay, had higher numbers than those taken over the ocean.

There was no diminution of organisms in the samples taken over the ocean. Colonies of fungi were present in all plates obtained, confirming the observations of Newman (*7*) over the Pacific Ocean. Bacteria were present in all samples in the August trip; in June, however, some plates had no colonies (Table 1). Fungi were more numerous than bacteria in all samples taken. The numbers obtained over the ocean are probably correlated with air masses. Polar air had very low numbers of both bacteria and fungi, whereas tropical air had higher numbers, with fungi greatly outnumbering bacteria. In one tropical air mass 8.8 fungi/cu ft were recorded from plates exposed in the slit sampler (Table 4).

Silicone slide studies revealed high numbers of fungus spores in the air. Comparison with plate counts clearly indicates the presence of large numbers of spores which are either non-viable or are unable to grow on our media. Concentrations up to 15.1/cu ft were obtained over the ocean (Table 2), and up to 361.4/cu ft over land (Table 4), while in the corresponding plates viable colonies of fungi were less than 1/cu ft. The latter air mass was identified by meteorologists as polar, but the high numbers of fungus spores indicated a tropical continental origin. It is believed that this technique might prove useful in the identification of air masses. Additional information concerning these flights will be published separately (*8*).

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## Effect of Fungicidin (Nystatin) in Mice Injected with Lethal Mixtures of Aureomycin and *Candida albicans*


**Rachel Brown, Elizabeth L. Hazen, and Alice Mason**

***Division of Laboratories and Research, New York State Department of Health, Albany* and *New York City***


Seligmann (*1*) recently demonstrated that sublethal doses of *Candida albicans* became highly lethal when mixed with aureomycin. Fungicidin[1] has both strong fungistatic and fungicidal activity *in vitro* against *C. albicans* (*2, 3*); therefore it seemed important to investigate its effect in association with aureomycin since moniliasis is believed by some to be a significant complication of aureomycin therapy.

The fungicidin used for this work was prepared as follows: Methanol extracts of *Streptomyces noursei*[2] mycelia were concentrated *in vacuo,* and the dry residue suspended in a mixture of equal parts of 0.85% sodium chloride solution and butanol. The fungicidin suspended at the interface was collected, washed with saline, and dissolved in methanol by warming at 50–52° C. After chilling, the clarified extract was precipitated with an equal volume of saline. The precipitate was again dissolved in methanol, and the chilled and clarified solution was precipitated by the addition of 4 vol of ethyl acetate. This final precipitate was dried quickly with ether. In cup tests 3.1 μg/ml showed inhibition against *C. albicans.* For *in vivo* tests a fungicidin suspension containing 5 mg/ml was prepared as previously described (*2*). The subcutaneous dose was 0.6 ml.

The strain of *C. albicans* (No. 4657) used in the animal tests was obtained from Rhoda Benham. It was isolated from a case of generalized cutaneous moniliasis. (This culture, since its isolation in 1946, has maintained its high virulence for rabbits: 0.2 ml of a 1:100 suspension in sterile 0.85% sodium chloride solution injected intravenously regularly kills rabbits of 2 to 2.5 kg between the second and third day, with the production of multiple abscesses in the cortex of the kidney.) The growth from a 48-hr culture on Sabouraud's agar slants was washed off with sterile saline and centrifuged at 1500 rpm for 30 min. The packed cells were resuspended in sterile saline (0.1 ml cells plus 2.4 ml saline) (*1*). A dose of 0.2 ml of this suspension corresponded to approximately 100 million viable cells.

[1] The senior authors have given the name Nystatin to their product fungicidin. It is being manufactured by E. R. Squibb and Sons under this name.

[2] Actinomyces No. 48240.

TABLE 1

EFFECT OF FUNGICIDIN (NYSTATIN) IN MICE INJECTED WITH A LETHAL MIXTURE OF *Candida albicans* AND AUREOMYCIN

| Material injected | Treatment with fungicidin: No. of doses | Treatment with fungicidin: Time of initial treatment | Results: Deaths* | Results: Time of death (days) | Results: Autopsy findings: Gross lesions +† | Results: Autopsy findings: Gross lesions None | Results: Autopsy findings: Cultural +‡ | Results: Autopsy findings: Cultural Unsatisfactory§ |
|---|---|---|---|---|---|---|---|---|
| *C. albicans* + aureomycin | | | 10/10 | 1 | 5 | 5 | 10 | |
| *C. albicans* + aureomycin | 1 | At infection | 4/10 | 3–8 | 4 | | 2 | 2 |
| " " | 10 | " " | 2/10 | 2,13 | | 1‖ | 1‖ | |
| " " | 1 | 4 hr before infection | 5/ 5 | 1–6 | 4 | 1 | 5 | |
| " " | 1 | 2 " " " | 1/ 5 | 12 | 1 | | 1 | |
| " " | 10 | 2 " " " | 1/ 5 | 3 | | 1 | | 1 |
| " " | 1 | 2 hr after infection | 4/ 5 | 1–3 | 1 | 3 | 3 | 1 |
| " " | 10 | 2 " " " | 2/ 5 | 2,14 | | 1‖ | 1‖ | |
| " " | 1 or more | 4 " " " | 10/10 | 1– 7 | 3 | 7 | 8 | 2 |
| *C. albicans* | | | 7/10 | 2–12 | 4‖ | | 3 | 1 |
| Aureomycin | | | 0/10 | | | | | |
| Fungicidin | | | 0/10 | | | | | |

The *C. albicans* suspension was administered intraperitoneally in a dose of 0.2 ml containing approximately 100,000,000 viable cells.

The *C. albicans* and aureomycin mixture was administered intraperitoneally in a dose of 0.2 ml containing approximately 100,000,000 viable cells and 1.9 mg of aureomycin.

The aureomycin was administered intraperitoneally in a dose of 0.2 ml containing 2.0 mg.

Fungicidin was administered subcutaneously in doses of 3 mg/0.6 ml, a single dose on the 1st day, two on the 2nd, 3rd, and 4th days, and one on the 5th, 8th, and 9th days.

* Numerator = number of dead mice; denominator = total number of mice.
† Appearance of very small white masses on surfaces of liver, spleen, or kidneys, or on all three organs.
‡ Isolation of *C. albicans* from peritoneal cavity.
§ Examinations unsatisfactory owing to overgrowth with a spreading microorganism.
‖ Remaining mice too decomposed for autopsy.

Crystalline aureomycin was dissolved in sterile saline solution, 10 mg/ml. For the combination of aureomycin and *C. albicans* 2.4 ml of this solution was added to 0.1 ml of packed cells. A dose of 0.2 ml of this mixture contained 100 million cells and 1.9 mg of aureomycin.

Ninety-five white mice (Albany strain) were employed in two separate but similar experiments, 45 mice in one and 50 in the second. Sixty-five were injected intraperitoneally with the mixture of aureomycin and *C. albicans*; 10 were given intraperitoneal injections of the saline suspension of *C. albicans* alone, and 10 aureomycin solution alone; 10 were injected subcutaneously with fungicidin alone.

Of the sixty-five mice injected with the mixture of *C. albicans* and aureomycin, 10 received no fungicidin, and the remaining 55 were divided into groups and treated at different time intervals with single or multiple doses of fungicidin. The mice were observed for 16 days. The results of the two experiments are shown in Table 1.

The 10 mice that received the mixture of aureomycin and *C. albicans* were dead within 24 hr, whereas 7 of the 10 that received *C. albicans* alone survived from 2 to 12 days, and 3 were still alive and appeared well at the end of 16 days.[3] When a single injection of fungicidin (3 mg) was administered 2 hr before infection or at the time of infection, 10 of 15 mice were still alive at the end of 16 days. Subsequent repeated injections of fungicidin did not significantly change the nature of the results. When the administration of fungicidin was delayed until 2 hr after infection, a single dose saved only 1 out of 5 mice, whereas continued treatment saved 3 out of 5. Fungicidin administered 4 hr before or 4 hr after infection showed no protective effect beyond delay in the time of death.

A single dose of fungicidin administered subcutaneously 2 hr before the inoculation of an otherwise lethal mixture of *C. albicans* and aureomycin was highly protective.

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[3] This strain of *C. albicans* is more virulent than that used by Seligmann.