PATHOGENICITY OF CANDIDA[1]

SAH'L MOURAD[2] AND LORRAINE FRIEDMAN

Departments of Tropical Medicine and Public Health, and Microbiology, Tulane University School of Medicine, New Orleans, Louisiana

Received for publication August 15, 1960

Laboratory studies of candidiasis have been hampered by inability to produce consistently a prolonged infection in laboratory animals. For the most part investigators have used exceedingly high dosages which have resulted in acute and rapidly fatal disease (e.g., Redaelli, 1924; Benham, 1931; Segretain, 1947). Others have triggered equally acute disease by lowering the resistance of the host with a variety of agents such as antibiotics and cortisone (Seligmann, 1953), low temperature (Scherr, 1953), radiation (Roth, Friedman, and Syverton, 1957), and mucin (Strauss and Kligman, 1951). Salvin, Cory, and Berg (1952) were able to produce prolonged infections in mice inoculated intraperitoneally by adding mucin to a relatively large number of cells. Mankowski (1957) was able to obtain relatively long lasting infections by inoculating intravenously an unstated number of cells, without mucin. Louria and Brown (1960), studying the effect of cortisone in Candida infected mice, employed a strain which possessed low virulence when inoculated intravenously. Subsequent to the initiation of the studies reported herein, Hasenclever (1959) investigated the virulence of *Candida albicans* in mice and rabbits when administered intravenously.

Although animals infected intravenously with *C. albicans* have been used in the evaluation of antimycotic drugs (e.g., Solotorovsky et al., 1954), we found it difficult to produce prolonged but fatal infections. Therefore, preliminary to other studies we found it desirable to investigate more fully the properties of virulence of *C. albicans*. By proper selection of strain, it was possible to produce prolonged infections with moderate dosages and without added substances such as mucin. Since any such studies of the genus *Candida* inevitably become entangled with the problem of toxicity, this also was investigated. Although an endotoxin in *C. albicans* has been reported, its action was demonstrated only when enhancing substances were added (Salvin, 1952) or when the host was pretreated with antibiotics (Roth and Murphy, 1957).

MATERIALS AND METHODS

Source of strains. Species used were of nonhuman or human origin. *Candida robusta* has been isolated from alcoholic beverages and is thought by Diddens and Lodder (1942) to be the imperfect form of *Saccharomyces cerevisiae*. *Candida reukaufii* is usually found in nectar (Lodder and Kreger-Van Rij, 1952). These nonpathogenic strains were kindly supplied by Dr. L. J. Wickerham of the U. S. Department of Agriculture.

All isolates of human origin formed chlamydospores and conformed in fermentation reactions to *C. albicans*. They were obtained from vaginal and skin lesions, from blood of patients with systemic candidiasis, and from sputa. The two strains which were used most extensively in this study (no. 266 and no. 4899) were isolated from the sputa of patients with respiratory disease of unknown etiology.

Preparation of inocula for titrations of virulence. Cultures of *C. albicans* were maintained on slants consisting of 1% casamino acids, 1% yeast extract, 2% glucose, and 2% agar. All of the strains grew as smooth, glistening colonies on this medium. Growth less than 1 week old was used to inoculate 150 ml of casamino-yeast extract-glucose broth in 500-ml Erlenmeyer flasks, which were incubated with constant agitation at 37 C for 24 to 36 hr. The inocula of species other than *C. albicans* were prepared as above except that the temperature of growth was 25 to 28 C and the period of incubation of the broth cultures was 6 to 7 days.

An attempt was made by microscopic examination to determine the percentages of particles of varying sizes, i.e., composed of 1, 2, 3, 4, 5, and more than 5 cells. When the percentage of par-

[1] This study was supported by grants from the National Institutes of Health, U. S. Public Health Service (E-2882, 2E3-C2, and 2G4-C2).

[2] Postdoctoral research trainee in epidemiology and mycology.

ticles consisting of aggregates of 4 cells or more exceeded 15%, the broth culture was discarded.

The concentration of particles in each culture was first estimated by means of a hemacytometer and then more precisely determined by the pour plate method. Based upon the resultant viable particle count, a sample of the culture was diluted with saline to achieve the desired volume and concentration for animal inoculation. When the estimated dosage was 100 viable particles, the number of colonies found by the pour plate method was of the order of 100 ± 5.

The volume of the inoculum was varied with the route, viz., 0.5 ml for intravenous, intraperitoneal, and peroral routes, 0.2 ml for the subcutaneous, 0.1 ml for the intramuscular, 0.05 ml for the intranasal, and 0.03 ml for the intracerebral routes.

Preparation of inocula for studies of toxicity. Broth cultures of each species, prepared as described for the virulence studies, were divided into two equal parts. One part was used to prepare saline suspensions containing 2×10^6 and 2×10^8 viable particles per ml. The remaining part of the broth culture was centrifuged. The sediment was separated from the supernatant, washed three times with saline, and then diluted to contain 2×10^9 particles per ml. These washed cells were then exposed to sonic vibrations for 6 to 8 hr in a 10 kc Raytheon oscillator, operated at maximum efficiency. Significant breakage did not occur earlier. Breakage of the cells was verified by microscopic examination of a wet preparation and the complete loss of viability was determined by culture. A portion of this suspension was then diluted, using the same dilution factor as was used to prepare the suspension of living, intact cells. Another portion was centrifuged at 3,000 rev/min for 30 min, separating a translucent supernatant from the broken packed cells. After appropriate dilution, 0.5 ml of each preparation was inoculated intravenously or intraperitoneally into mice.

Animal experiments. Six-week-old, male, white Webster mice, obtained from Taconic Farms, were used in all experiments. For the initial screening with a single dose to select strains of *C. albicans* of high and low virulence, groups of 5 mice per strain were employed. For the comparison of routes, 10 mice were used per dose and for the more extensive studies on virulence, 20 mice were inoculated with each preparation.

Intranasal inoculations were made by the instillation of the inoculum in the nostrils in tiny amounts with a blunt 27 gauge needle. (Since an anesthetic was not employed, it is certain portions of the inocula did not reach the lungs of the mice.) A polyethylene infant feeding tube, approximately 1.5 mm in diameter and 4.5 cm in length, adapted to a 1-ml syringe, was used for peroral administrations. The number of animals dead was counted daily through a period of 10 weeks. The experiment was then terminated and the surviving animals killed. When possible, autopsies were carried out shortly after death; otherwise the material was kept at -15 C and processed later.

RESULTS

Studies on virulence. To rule out a mechanical factor that might obscure recognition of the true characteristics of virulence, studies were made to establish the particle sizes suitable for virulence studies. Approximately 75% of the particles were composed of one or two cells; 15 to 20% had 3 cells; 8 to 14%, 4 or 5 cells; and less than 1% had more than 5 cells. In general, the cells of the nonpathogenic cultures (*C. robusta* and *C. reukaufii*) were larger than those of *C. albicans*. At the dose of 1×10^8, even these Candida of nonhuman origin caused 100% mortality in a few hours when more than 25 to 30% of the particles of a culture consisted of more than 4 to 5 cells.

To select strains of high and low virulence for more extensive studies, 32 isolates of *C. albicans* were screened by intravenous inoculation of 1×10^4 viable particles. This route was chosen empirically. The dosage was selected to lessen the possible occurrence of death due to toxic substances in larger inocula, and yet to permit detection of virulent strains. With 11 strains, no mice died, whereas with 7 others, 3 or 4 died in each group of 5 over a period of 10 weeks. The 14 remaining strains produced 1 or 2 deaths per group of mice.

To select a route of inoculation most suitable for more extensive studies on virulence, the intravenous, intracerebral, intraperitoneal, intranasal, intramuscular, subcutaneous, and peroral routes were compared, using 1×10^2, 1×10^4, 1×10^6, and 1×10^8 particles of a strain of relatively high virulence, no. 266, as shown in Table 1.

No mice died in the perorally inoculated group

and at autopsy no gross evidence of fungous dissemination was noted. With higher concentrations (1×10^6 and 1×10^8) inoculated subcutaneously and intramuscularly, abscesses developed at the sites of injection but healed before the end of the experiment. No deaths occurred among those given the lower dose. One death occurred in each of the groups of mice inoculated by the subcutaneous and intramuscular routes and these were among those given higher dosages. These two fatalities had abscesses of the kidneys, the cultures of which were positive for Candida. No gross lesions were noted among the mice killed at the tenth week.

Four of 10 mice, which received 1×10^8 cells intranasally, died between the sixth and seventh week after inoculation. Three of these animals showed numerous small lung abscesses, possibly of a bronchopneumonic distribution, and one mouse had extensive abscesses involving entire lobes. *C. albicans* was demonstrated in these lesions, but there was no evidence of dissemination to the kidneys or to other organs. Of the 6 animals killed at 10 weeks, the lungs were apparently normal and cultures for *C. albicans* were negative.

The intraperitoneal inoculations produced generalized and fatal infections with only the higher doses. One mouse died 24 days following inoculation with 1×10^6 particles, but all those which received 1×10^8 particles succumbed between 3 and 16 days after inoculation. At autopsy it was noted that the kidneys were enlarged, with numerous abscesses. Mesenteries had many pinpoint nodules and adhesions among the viscera. *C. albicans* was recovered in cultures from kidneys of the animals that died. Only a few of the surviving animals had any gross lesions but these were extensive and limited to the kidneys. Cultures of these lesions were sterile as were the cultures of material taken from animals which appeared entirely normal at autopsy.

When the inoculum was administered intravenously, the time of death ranged from an average of 12 hr after an injection of 1×10^8 particles to 5 to 7 weeks following 1×10^4 (Fig. 1). Gross lesions were always noticed on the kidneys. There was a tendency for the abscesses to be more numerous and larger when the dose was larger. All were positive for *C. albicans* upon culture. Kidney damage was either unilateral or absent in the survivors, depending upon the dose.

TABLE 1

Comparison of mortality of 6-week-old, male, white mice when inoculated with various doses of Candida albicans strain 266, in saline suspension using different routes of inoculation

No. of Viable Particles	Mortality of Mice According to Route of Inoculation						
	Intravenous	Intracerebral	Intraperitoneal	Intranasal	Subcutaneous	Intramuscular	Peroral
1×10^2	0*	1	0	0	0	0	0
1×10^4	6	2	0	0	0	0	0
1×10^6	10	10	1	0	0	0	0
1×10^8	10	10	10	4	1	1	0

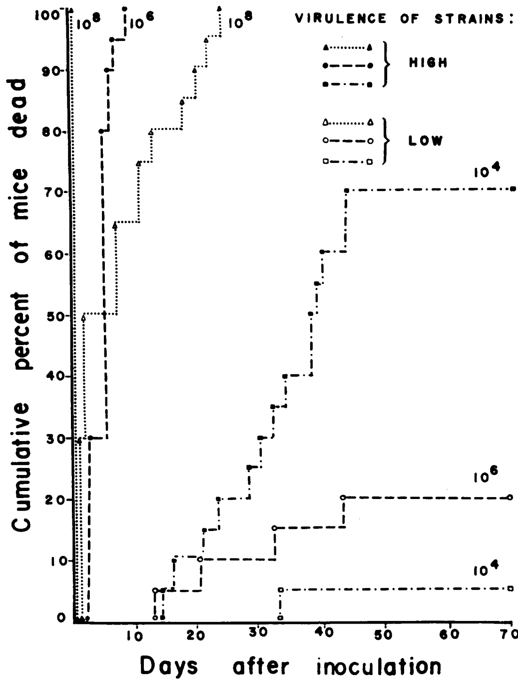
* This number and the following represent the fatalities among 10 mice.

Intracerebral inoculations produced fatalities at irregular intervals (Fig. 2). In several cases of early death it is probable that deaths were due to trauma because one death occurred among 10 control mice injected with saline. *C. albicans* was recovered from the brains, but not from other organs, of those which died during the first month.

The intravenous route of inoculation was selected for more extensive studies to characterize the virulence and infectivity of five strains, shown by the initial screening to possess differing degrees of virulence. The time of death following inoculation of these strains varied greatly according to the strain and to the number of viable particles in the inoculum, the higher doses causing the earlier deaths. Fig. 1 shows the cumulative death rates of two strains representative of high (no. 266) and of low (no. 4899) virulence. At the dose of 1×10^6 viable particles for example, only 20% of deaths occurred within 10 weeks with the strain of low virulence. By comparison, 100% deaths were observed in 9 days with the more virulent strain. No deaths occurred in less than 2 days with 1×10^6 particles of either of these strains. This observation of the difference in virulence was confirmed by the results obtained at the other dose levels.

Studies on toxicity. An attempt was made to determine whether the rapid death following the administration of large doses of *C. albicans* (e.g., 1×10^8) is due, at least in part, to the presence of toxic material in the original inoculum.

Table 2 shows the mortality of mice following



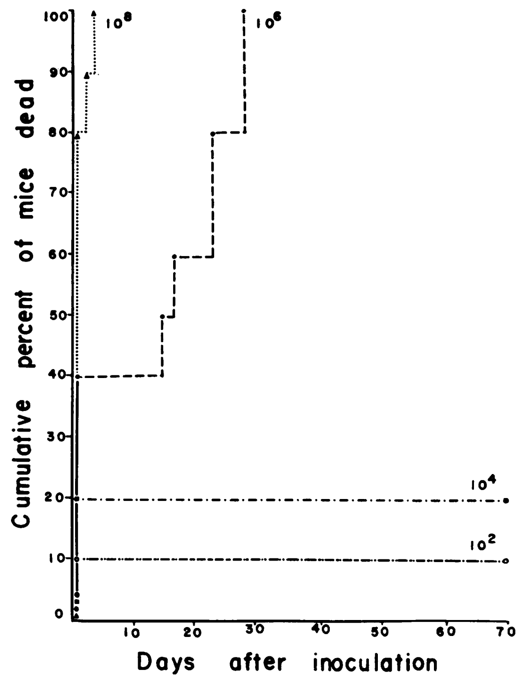


Fig. 1 (*left*). Comparative cumulative death rates for white mice after intravenous inoculation with varying concentrations of viable particles of *Candida albicans* strains 266 (high virulence) and 4899 (low virulence) in saline suspension.

Fig. 2 (*right*). Cumulative death rates for white mice after intracerebral inoculation with varying concentrations of viable particles of *Candida albicans* strain 266 in saline suspension.

TABLE 2

Mortality of 6-week-old, male, white mice following intravenous inoculation with various preparations from living cultures or sonically ruptured cells of Candida albicans strain 266

Dose (Expressed as Concn of Viable Particles in the Original Suspension)	Mortality in Mice (Groups of 10)*				
	Sonically ruptured cells			Living culture	
	A† Washed cells sonically ruptured	B Supernatant from A	C Washed sediment from A	Washed cells	Broth filtrate
1×10^6	0	Not done	Not done	10 (> 3 days)	
1×10^8	3	0	0	10 (< 12 hr)	
1×10^9	9	6	1 (immediate)	Not done	0

* Except where indicated, all deaths occurred within 6 hr after inoculation. Parentheses ( ) = time of death after inoculation.

† An average of two experiments.

the intravenous inoculation of various preparations from *C. albicans* strain 266 (high virulence). These were (A) suspension of washed living intact cells; (B) broth filtrate obtained from a culture of intact living cells; and (C) washed and mechanically ruptured cells at the same concentration as (A). Deaths occurred among mice inoculated with either the living intact cells or the nonviable sonically ruptured cells. Essentially the same results were obtained

TABLE 3

Mortality of 6-week-old, male, white mice following intravenous inoculation with intact living or sonically broken cells of two human and two nonhuman isolates of Candida

Dose (Expressed as Concn of viable Particles in the Original Suspension)	Mortality in Mice (Groups of 20)*							
	Human isolates				Nonhuman isolates			
	C. albicans strain 266 (high virulence)		*C. albicans* strain 4899 (low virulence)		*C. robusta*		*C. reukaufii*	
	Sonically ruptured	Living	Sonically ruptured	Living	Sonically ruptured	Living	Sonically ruptured	Living
1×10^6	0	20 (> 3 days)	0	4 (> 3 wk)	0	0	0	0
1×10^8	7	20 (< 12 hr)	6	20 (< 12 hr)	11	8 (immediate)	10	7 (immediate)
1×10^9	18	Not done	17	Not done	20	Not done	19	Not done

* Except where indicated, all deaths occurred within 6 hr after inoculation. Parentheses ( ) = time of death after inoculation.

with the intraperitoneal route but the fatalities produced were less than were observed when the inoculations were intravenous.

We then tried to determine whether the rapid deaths were due to soluble substances liberated from the sonically broken cells or to the cell wall and other insoluble materials exposed by the rupture. Mice were injected intravenously with (A) washed and sonically ruptured cells at the 1×10^9 level, (B) with the supernatant of the sonically ruptured material, or (C) with the sediment after being washed three times and resuspended in saline so as to contain the same proportion of particulate matter as (A). Both the sonically broken cells and the supernatant therefrom were lethal; the washed sediment caused no apparent harm (Table 2).

Finally, we compared by the intravenous route the toxicity of two cultures of nonhuman origin, *C. robusta* and *C. reukaufii*, with two cultures of *C. albicans*, one of high and one of low virulence (Table 3). At dosages of 1×10^8 or greater, the sonically treated material, regardless of source, produced death and all of these deaths occurred within 6 hr after inoculation. The percentage of deaths obtained with the preparations of nonhuman source, however, was slightly higher than was observed after inoculation of similarly prepared *C. albicans*. There were no deaths from infection (i.e., occurring several days or weeks after inoculation) with the living cultures of nonhuman origin. Some deaths occurred following inoculation with 1×10^8 intact viable particles of these nonpathogenic strains, but only immediately after inoculation. By comparison, intact viable *C. albicans* at the same dose regularly caused death of all the mice with the deaths occurring not only immediately but up to 12 hr after inoculation.

DISCUSSION

As might have been anticipated, the severity of the disease produced varied with the strain, dosage, and route of inoculation. The acute and rapidly fatal disease observed by most investigators was produced by very high dosages (e.g., 1×10^8) of most of the strains. It was possible in this study, however, to demonstrate a more chronic infection with lower dosages of certain of the strains, i.e., those possessing invasive powers as well as toxic substances. As an example, when 1×10^6 particles of strain no. 266 were inoculated intravenously, all of 20 mice died, with 50% dead by the third day and the remainder dead by the ninth day. When 1×10^4 particles of this strain were similarly inoculated, however, a much less acute disease was observed with 70% of the animals dying within 7 weeks. Therefore, to see the virulence of *C. albicans* in its fullest array often required long periods of observation. It is worthy of note that although no deliberate attempt was made to detect changes in virulence with age of strain, the results obtained were reproduced regularly throughout the course of this study.

Death following large inocula has been ob-

served by most workers. Many hypotheses have been proposed to explain the mechanisms of this death and most include such factors as embolization (Redaelli, 1924), uremia (Winner, 1956), toxemia (Salvin, 1952), myocardial (Adriano and Schwarz, 1955), and pancreatic (Young, 1958) damage, or a combination of these factors. Stovall and Pessin (1934) ruled out vascular occlusion as a cause of death by demonstrating that intravenous injection of very large doses of related species such as *Candida parapsilosis* or *Candida tropicalis* was not pathogenic to laboratory animals. They observed that intravenous injection into a rabbit of 3 billion organisms of *C. parapsilosis* suspended in 10 ml saline caused only slight fever for 24 hr and no lesions were seen at autopsy.

Toxic substances have been demonstrated in other fungi, e.g., in the broth filtrate and the mycelium (Bodin and Lenormand, 1912), in the spores (Martins, 1928), and in the cellular sap (Henrici, 1939) of *Aspergillus fumigatus*, as well as in higher fungi (Abel and Ford, 1908). Henrici, who first investigated the presence of an endotoxin in *C. albicans*, failed to demonstrate it and wrote, "There are mechanical difficulties which have prevented me from expressing the small yeast-like cells of *Monilia albicans*, and extracts have proved non-toxic." Salvin (1952) was able to induce a high percentage of death with intraperitoneal inoculations of heat or chemically killed *C. albicans* cells (1×10^9 and higher) but only when an adjuvant such as killed *Mycobacterium tuberculosis* was added to the inoculum. When he attempted extraction of endotoxin by mechanical means, he apparently encountered the same difficulties as Henrici because he wrote, ". . . cells of *C. albicans* suspended in physiologic saline treated for 20 min in a Mickle tissue disintegrator failed to yield a soluble substance which would elicit discernible toxic reactions." Roth and Murphy (1957) also subjected cells of *C. albicans* to mechanical abuse but the supernatant was toxic only in mice pretreated with chlortetracycline. However, very few cells appeared ruptured after the 45 min of exposure in the sonic oscillator. In our experiments prolonged exposure in the sonic oscillator resolved this mechanical problem. By this means breakage of the resistant *C. albicans* cells occurred, but only after 6 to 8 hr.

Undoubtedly, there was sufficient toxic material in the very large inocula (1×10^8) of *C. albicans* which, in itself, caused at least some of the deaths. This was shown by the fatalities among the animals that received the soluble substances from sonically ruptured cells. Our experiments showed more deaths, however, when the cells of these large inocula were alive and intact. This would seemingly indicate another lethal factor, such as vascular occlusion, operative. Although the inocula were prepared from a broth culture incubated at 37 C with constant agitation, the percentage of particles composed of 4 or 5 cells or more was never less than 8%. With large inocula (1×10^8 particles), vascular occlusions may have occurred. Had histopathologic sections been examined they might have revealed emboli or cellular material in significant concentrations in critical areas. Nevertheless, it may still be that the deaths from the large doses of living intact cells were due to toxic substances. If prolonged exposure to sound waves destroyed a portion of these toxic substances, one might expect less mortality with the sonically ruptured cells, such as we observed.

The comparison of a virulent strain of *C. albicans* with another strain of lower virulence and with two species of nonhuman sources, *C. robusta* and *C. reukaufii*, demonstrated that the presence of toxic substances is not solely responsible for the virulence of *C. albicans*. The nonviable, sonically ruptured cells of the nonpathogenic strains killed more mice than even the most virulent of *C. albicans*. On the other hand, the intact cells of these cultures of nonhuman origin were relatively harmless. Deaths occurred only at the 1×10^8 dose level and only immediately after inoculation. *C. albicans*, however, caused death regularly at this and lower dosages. It is probable the deaths from *C. albicans* were not attributable to embolism inasmuch as for the most part the deaths did not follow immediately after inoculation. Furthermore, the particles of *C. albicans* were actually smaller than the nonpathogenic species. Since the sonically ruptured cells of the nonpathogenic species were lethal, the failure of intact living cells of these same species to cause death requires some explanation. It may be that the toxic substances were released from the living cells gradually in the body thus causing little or no damage.

SUMMARY

Mice were injected by various routes, using 1×10^2, 1×10^4, 1×10^6, and 1×10^8 viable

particles of a single strain of *Candida albicans*. The intravenous route was the route of choice because of resultant prolonged infection with smaller doses. In a screening of strains with 1×10^4 particles inoculated intravenously, 11 strains were avirulent, 7 caused fatal infection in 3 to 4 of 5 mice, and 14 were of intermediate virulence. Five strains were selected for more extensive studies. Very large dosages of most strains caused death almost immediately, but by proper selection of the strain and the dosage, it was possible to induce death from infection within 2 to 7 weeks.

Soluble substances, obtained by sonic oscillation of *C. albicans,* were lethal for mice when inoculated intravenously. Similarly, toxic substances were demonstrated also in species of nonhuman origin.

REFERENCES

ABEL, J. J., AND W. W. FORD 1908 Further observations on the poisons of *Amanita phalloides*. Arch. exptl. Pathol. Pharmakol., Supplement-Band, 8–15.

ADRIANO, S. M., AND J. SCHWARZ 1955 Experimental moniliasis in mice. Am. J. Pathol., **31,** 859–873.

BENHAM, R. W. 1931 Certain monilias parasitic to man. J. Infectious Diseases, **49,** 183–215.

BODIN, E., AND C. LENORMAND 1912 Recherches sur les poisons produits par l'*Aspergillus fumigatus*. Ann. inst. Pasteur, **26,** 371–380.

DIDDENS, H. A., AND J. LODDER 1942 *Die anaskosporogenen Hefen*, II. Teil. N. V. Noord-Hollandsche Uitgevers Maatschappij, Amsterdam.

HASENCLEVER, H. F. 1959 Comparative pathogenicity of *Candida albicans* for mice and rabbits. J. Bacteriol., **78,** 105–109.

HENRICI, A. T. 1939 An endotoxin from *Aspergillus fumigatus*. J. Immunol., **36.** 319–338.

LODDER, J., AND N. J. W. KREGER-VAN RIJ 1952 *The yeasts. A taxonomic study*. North-Holland Publishing Co., Amsterdam.

LOURIA, D. B., AND H. BROWNE 1960 The effects of cortisone on experimental fungus infections. Ann. N. Y. Acad. Sci., **89,** 39–46.

MANKOWSKI, Z. T. 1957 The experimental pathogenicity of various species of *Candida* in Swiss mice. Trans. N. Y. Acad. Sci., **19,** 548–570.

MARTINS, C. 1928 Études experimentales sur l' *Aspergillus fumigatus*. Compt. rend. soc. biol., **100,** 525–528.

REDAELLI, P. 1924 Experimental moniliasis. J. Trop. Med. Hyg., **27,** 211–213.

ROTH, F. J., JR., J. FRIEDMAN, AND J. T. SYVERTON 1957 Effects of roentgen radiation and cortisone on susceptibility of mice to *Candida albicans*. J. Immunol., **78,** 122–127.

ROTH, F. J., JR., AND W. H. MURPHY 1957 Lethality of cell-free extracts of *Candida albicans* for chlortetracycline-treated mice. Proc. Soc. Exptl. Biol. Med. **94,** 530–532.

SALVIN, S. B. 1952 Endotoxin in pathogenic fungi. J. Immunol., **69,** 89–99.

SALVIN, S. B., J. C. CORY, AND M. K. BERG 1952 The enhancement of the virulence of *Candida albicans* in mice. J. Infectious Diseases, **90,** 177–182.

SCHERR, G. H. 1953 The effects of environmental temperature on the course of systemic moniliasis in mice. Mycologia, **45,** 359–363.

SEGRETAIN, G. 1947 Étude de la maladie experimentale d'un lapin provoquee par un *Candida albicans* agent probable d'une mycose pulmonaire. Ann. inst. Pasteur, **73,** 674–676.

SELIGMANN, E. 1953 Virulence enhancement of *Candida albicans* by antibiotics and cortisone. Proc. Soc. Exptl. Biol. Med., **83,** 778–781.

SOLOTOROVSKY, M., E. J. IRONSON, F. J. GREGORY, AND S. WINSTEN 1954 Activity of certain diamidines against blastomycosis, and Candida infection in mice. Antibiotics & Chemotherapy, **4,** 165–168.

STOVALL, W. D., AND S. B. PESSIN 1934 Pathogenicity of certain species of *Monilia*. Am. J. Public Health, **24,** 594–602.

STRAUSS, R. E., AND A. M. KLIGMAN 1951 The use of gastric mucin to lower resistance of laboratory animals to systemic fungus infections. J. Infectious Diseases, **88,** 151–155.

WINNER, H. I. 1956 Immunity in experimental moniliasis. J. Pathol. Bacteriol., **71,** 234–237.

YOUNG, G. 1958 The process of invasion and persistance of *Candida albicans* injected intraperitoneally into mice. J. Infectious Diseases, **102,** 114–120.