

Oral and Esophageal *Candida albicans* Infection in Hyposalivatory Rats

SEAN W. MEITNER,¹ WILLIAM H. BOWEN,^{1,2} AND CONSTANTINE G. HAIDARIS^{1,2*}

Departments of Dental Research¹ and Microbiology and Immunology,² The University of Rochester, Rochester, New York 14642

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The opportunistic fungus *Candida albicans* is a major cause of oral and esophageal infections in immunocompromised patients, individuals on drug therapy, and the chronically ill. Because it has been observed that persons suffering from hyposalivation have an increased prevalence of oral candidiasis, we developed an animal model of infection based on hyposalivation. The objectives of our studies were to understand the mechanisms by which *C. albicans* causes oral disease and to begin to elucidate the role played by saliva in controlling *C. albicans* in the oral cavity. Our results showed that (i) oral *Candida* infection was established by a small challenge inoculum, (ii) mucosal lesions developed in the oral cavities and esophagi of infected rats, and (iii) transmission of oral *Candida* infection from an inoculated rat to uninoculated cagemates occurred rapidly. In addition, we compared the abilities of a clinical isolate and a spontaneously derived morphological mutant from that isolate to infect hyposalivatory rats and to induce disease. Infection was induced by the morphological mutant in hyposalivatory rats; however, the morphological mutant took significantly longer to transmit oral infection to uninoculated cagemates than did the parental strain.

The opportunistic fungus *Candida albicans* is a major cause of oral and esophageal infections in immunocompromised humans, including those with acquired immune deficiency syndrome (AIDS) (7, 11, 13, 17, 34, 36). Other conditions which predispose individuals to oral *C. albicans* infection include hyposalivation (12, 33), diabetes mellitus, prolonged use of antibiotics or immunosuppressive drugs, and poor oral hygiene (41). Furthermore, oral candidiasis occurs in the terminally ill (14) and chronic candidiasis has been associated with the onset of malignancy (18).

Results of epidemiological surveys indicate that *C. albicans* is carried as a commensal organism in the oral cavities of approximately one-third of the population (29). The shift by *C. albicans* from commensalism to expression of virulence in the oral cavity is strongly correlated with impairment of immune function (29).

Saliva is a component of the common mucosal secretory defense system and a primary effector arm of this system in the oral cavity (28). Several salivary constituents are believed to play a role in the control of *C. albicans*. *Candida*-specific secretory immunoglobulin A from saliva can inhibit the adherence of *C. albicans* to oral epithelial cells in vitro (48). These results suggest that secretory immunoglobulin A can inhibit colonization and promote clearance of *C. albicans* from the oral cavity. Furthermore, human saliva contains several nonimmune molecules which inhibit the growth of *C. albicans* in vitro (25, 31, 32, 35); these include the histidine-rich polypeptides termed histatins (not present in rats) and lysozyme. It is likely that *Candida*-specific salivary immunoglobulin A, nonimmune effector molecules in saliva, and the cleansing properties of saliva act concomitantly to eliminate *C. albicans* from the oral cavity (12, 25, 31, 33, 35, 48).

Because it has been observed that persons with hyposalivation have an increased prevalence of oral candidiasis (29), we considered it appropriate to determine whether we could develop an animal model of infection based on hyposaliva-

tion. Progress in understanding the etiology and pathogenesis of oral candidiasis has been largely hampered by the lack of a suitable animal model in which both the role played by saliva in controlling oral *C. albicans* infection and the development of *Candida*-induced mucosal lesions can be studied in a systematic manner. The purpose of our study was to develop an animal model with hyposalivatory rats (HSR). In addition, we examined the abilities of a clinical isolate of *C. albicans* and a morphological mutant derived from that isolate to infect HSR and to induce disease. Information derived from this study will facilitate investigations of the etiology and pathogenesis of the disease and aid in the exploration of methods of treatment for oral candidiasis.

MATERIALS AND METHODS

***C. albicans* strains and isolation of a colony morphology mutant.** *C. albicans* 613 was isolated from an oral lesion and identified by the Department of Clinical Microbiology of Strong Memorial Hospital, Rochester, N.Y. We isolated a spontaneously derived morphological mutant from strain 613 by plating it on amino acid-rich medium (23) and screening for colonies displaying atypical morphologies. Mutants arose at a frequency of 10^{-3} to 10^{-4} per generation. The virulence of one of the mutants was compared with that of the parent strain in our rodent model. The particular mutant was chosen because it differed from its parent strain with regard to the morphological characteristics of the colony and cells and functional attributes of the cell wall, particularly adhesion to plastic and a tendency toward self-agglutination (see below). The parent and its mutant were biotyped with the *Candida*-Check serotyping and sugar utilization kit (Iatron Labs, Inc., Tokyo, Japan) (40). Strain 613 and its corresponding mutant were serotype B (data not shown).

Phenotypic characteristics of the parental strain and mutant with regard to morphologic characteristics and functional attributes of the cell wall. (i) **Overall cell morphology and capacity for germ tube formation.** The *Candida* strains were grown on yeast extract-peptone-glucose agar plates (YEPD)

* Corresponding author.

(37) at room temperature. Cells from the plate were suspended in sterile distilled water, pelleted by centrifugation ($600 \times g$ for 10 min at room temperature) (Dynac), and washed twice. The suspension was adjusted to a concentration of 10^6 cells per ml and maintained at room temperature. A sample of this suspension was pelleted by centrifugation and suspended in an equal volume of unsupplemented M199 medium (GIBCO Laboratories), pH 6.7, prewarmed to 37°C . Germ tube formation was induced by incubation in M199 medium at 37°C for 2 h (9). The cell morphologies in the water suspension and M199 medium were determined by light microscopy.

(ii) **Self-agglutination in water.** A large loopful of cells from a YEPD plate was added to 1.0 ml of sterile distilled water in a microcentrifuge tube and vortexed vigorously. The resulting suspension was evaluated visually for the degree of agglutination and scored as plus or minus.

(iii) **Adhesion to a plastic surface.** The mutant strain used in this study was highly filamentous; hence, it was difficult to quantitate cell numbers by direct counting. Therefore, to compare the abilities of the mutant and parental strains to adhere, we used a quantitative technique described by Klotz et al. that assessed the amount of cell mass adherent to a culture well bottom (22). The abilities of our *C. albicans* strains to adhere to plastic microtiter wells were quantitated as follows. A 100- μl sample of a cell suspension (A_{540} , 0.2 with a 1-cm light path) in either YEPD broth or M199 medium was added to microtiter wells (Costar polyvinyl chloride 96-well tissue culture dishes; VWR Scientific) in triplicate. This cell concentration (approximately $10^6/\text{ml}$) and volume completely covered the microtiter well bottom in a uniform monolayer with a minimal amount of cell stacking as determined by light microscopy. The cultures were incubated for 2 h at either room temperature or 37°C . The wells were washed twice with double-distilled H_2O and allowed to dry. The A_{410} of the adherent cells was determined spectrophotometrically with an automated enzyme-linked immunosorbent assay plate reader (Dynatech Laboratories, Inc.) (22).

Animals. Female pathogen-free Sprague-Dawley rats were acquired from the Charles River Labs, Inc., Kingston, N.Y. Animals were received at age 14 days with their dams. Rats were weaned at 21 days and given Purina pellet rat chow and sterile distilled water ad libitum until age 26 days. At that time, the animals were divided into groups according to the experimental design. The animals were housed in filter-top cages and screened for the presence of *C. albicans* by plating oral swabs on YEPD agar. Our experience has been that carriage of *C. albicans* or acquisition of the fungus from the environment by rats does not occur.

Diet. Diet 2000 (56% sucrose) was fed to the rats ad libitum for the duration of the experiment. The rats also received sucrose (10% wt/vol) in their water (20).

Surgical hyposalivation. The rats were sedated with chloral hydrate (400 mg/kg of body weight; 50 mg/ml) administered intraperitoneally. The parotid salivary ducts of the animals were ligated, and the submandibular and sublingual salivary glands were surgically removed. The surgical sites were closed with wound clips which were removed after 4 days by procedures previously described (5, 26). Upon completion of the surgical procedure, the bulk of total salivary flow was inhibited because only the minor salivary glands were available for secretion of saliva into the oral cavity.

Microbiology. The oral cavities of the animals were swabbed on two successive days with a cotton-tipped applicator saturated with an actively growing culture of *C.*

albicans (approximately 10^7 cells per ml) in YEPD broth. Our experience has been that this mode of infection is more effective in establishing oral *Candida* infection than adding a volume of culture suspension directly to the mouth. We estimate that the maximum number of cells that can be delivered to the oral cavity by our technique is 5×10^6 , although the actual inoculum probably ranges from 10^5 to 10^6 cells per application.

The establishment of *C. albicans* infection was evaluated by swabbing the inoculated oral cavity with a sterile cotton applicator, followed by plating on YEPD agar. At the end of the experiment, the animals were killed by asphyxiation in a CO_2 atmosphere and then decapitated. Half of the lower jaw was removed by aseptic dissection, placed in 5 ml of sterile saline, and sonicated in a Braun-Sonic 1510 for 30 s to dislodge adherent microorganisms from the jaw. Sonication does not adversely affect the viability of *C. albicans*.

To determine the total number of cultivatable flora from the jaw and tissue sections, samples of the sonicated suspensions were plated on tryptic soy agar supplemented with 5% sheep blood (GIBCO); the plates were incubated for 48 h at 37°C .

Histology. The mandibular jaws and esophagi from the rats were rinsed in neutral buffered Formalin. The jaws were decalcified in 10% trifluoroacetic acid for 48 h, rinsed and processed for histology, embedded in paraffin, sectioned at a 5- μm thickness, and stained with hematoxylin and eosin Y (24) or phloxine and methylene blue (27).

Statistical analysis. An analysis of variance was used to make a statistical comparison of the number of CFU of *C. albicans* isolated from the jaws of the experimental groups. All statistical evaluations were performed by using the analysis of variance program of the STATVIEW II statistical software package (Abacus concepts, Berkeley, Calif.). *P* values of ≤ 0.05 were deemed statistically significant. All mean values in the text and tables include the standard deviations of the means.

Development of the HSR model of oral *C. albicans* infection. Three studies were done with desalivated rats. In study 1, we explored the ability of the parental strain to induce frank candidiasis. In studies 2 and 3, we investigated the abilities of *C. albicans* parental strain 613 and the morphological mutant to transfer from one animal to another as an index of virulence.

Study 1: induction of oral candidiasis by the parental strain. Eighteen female rat pups (age, 27 days; Charles River) were screened for carriage of *C. albicans*. Following verification that the rats were free of *C. albicans*, they were divided into three groups of six animals each. In group 1, the salivary glands were left intact; in groups 2 and 3, the animals were desalivated and fed as already described.

Animals in group 1 (salivary glands intact) and one desalivated group (group 2) were infected with *C. albicans* parental strain 613 by swabbing of the oral cavity. The remaining desalivated animals (group 3) were not infected with *C. albicans*. The animals were killed 3 weeks postinfection, and evaluation of infection performed as already described.

Study 2: transmission of infection by the *C. albicans* parental strain. Forty weanling rats were screened and found to be free of *C. albicans*. At an age of 26 days, the rats were subdivided into four groups of 10 animals; they were either surgically desalivated or left intact and fed as already described. In each group, five donor animals were infected with *C. albicans* parental strain 613 by swabbing of the oral cavity. All of the animals that were swabbed with *C.*

albicans became infected. Infected donors were caged with uninfected recipients. Oral cultures from both donor and recipient animals were taken daily and plated onto Sabouraud agar plates to determine whether infection had transferred from donors to recipients. If infection was not transferred from infected to uninfected animals within 4 weeks, the animals were killed. The following parameters were evaluated: (i) the time required for transmission of infection from donors to recipients, (ii) the number of recipients infected in each group, (iii) the mean number of *C. albicans* CFU in the jaws of donor and recipient animals in each group, and (iv) the weight change of donor and recipient animals at the end of the experiment.

Study 3: comparison of virulence of the parental and mutant strains in the HSR model of oral candidiasis. We evaluated the ability of one of the morphological mutants spontaneously derived from the parental strain (mutant strain 613-m1) to (i) establish infection in the oral cavities of desalivated rats and (ii) be transmitted to uninfected recipient animals. Mutant strain 613-m1 and parental strain 613 were compared in the study. The protocol used was similar to that of the transmission experiment already described, and the experiment was terminated after 4 weeks. For a list of the different experimental groups, see Table 3. For each group, five pairs of 26-day-old rats were used, with one donor and one recipient per cage. The following parameters were evaluated: (i) the time required for transmission of infection from donor to recipient, (ii) the number of recipients infected in each group, (iii) the mean number of *C. albicans* CFU in the jaws of both donor and recipient animals in each group, and (iv) the weight change in grams in both donor and recipient animals.

RESULTS

Isolation and phenotypic characterization of *C. albicans* strains. The morphologies of the colonies and cells of strains 613 and 613-m1 are shown in Fig. 1, demonstrating the differences between the parental and mutant strains. Mutant strain 613-m1 differed from its parent in that its colony morphology was wrinkled and irregular, not smooth and circular. Furthermore, the cell morphology of the mutant was pleiomorphic, in contrast to the uniformly oval or round parental strain cells. In addition to differences in colony and cell morphology, 613-m1 differed from the parental strain in its tendency to self-agglutinate in water and by a diminished ability to adhere to a polyvinyl chloride plastic surface.

Parental strain 613 dispersed readily in water after vortexing, resulting in a uniform suspension. In contrast, mutant strain 613-m1 agglutinated into large clumps of cells that could not be completely dispersed by either vigorous vortexing or pipetting. However, a sufficient number of mutant cells could be dispersed for use in the plastic adhesion assay. The level of adhesion of mutant 613-m1 to plastic was consistently only 5 to 10% of the levels observed for the parental strain, regardless of the medium used or the temperature of incubation. We assayed the adhesion to plastic of the two strains suspended in either M199 medium or YEPD broth; adhesion was determined after incubation at room temperature and also at 37°C. A_{410} values for the parental strain ranged from 0.045 to 0.156. The highest values were obtained after incubation in M199 medium at 37°C. A_{410} values for mutant strain 613-m1 ranged from 0.003 to 0.007 under the assay conditions described. As a point of reference, an A_{410} value of 0.150 corresponded to a monolayer of closely spaced, adherent cells in the culture well. The

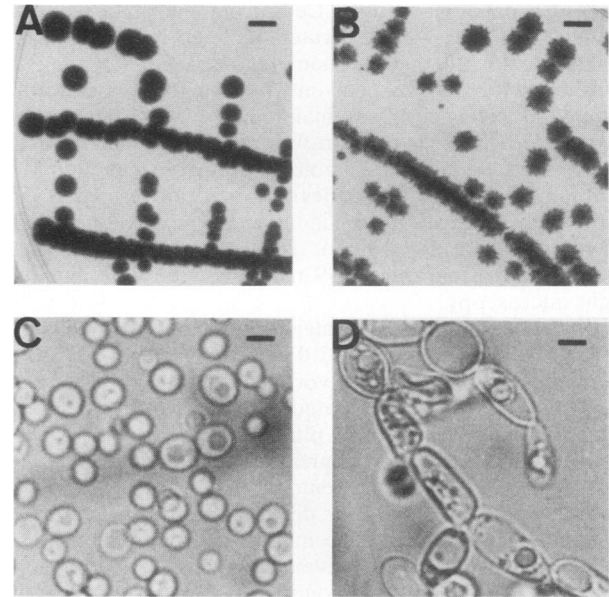


FIG. 1. Photographs of colonies and cells of *C. albicans* parental strain 613 and spontaneously derived morphological mutant 613-m1. (A) Colonies of parental strain 613. (B) Colonies of morphological mutant 613-m1. Photographs were taken with backlighting to emphasize the morphological differences between the colonies of the two strains. Bars, 4 mm. (C) Cells of parental strain 613. (D) Portion of a cluster of cells of mutant 613-m1. Clusters of cells in which complete separation of daughter cells did not occur were commonly observed. Bars, 4 μ m.

parental and mutant strains were similar, however, in that both strains formed germ tubes after incubation in M199 medium at 37°C (9).

Induction of oral candidiasis by the parental strain in HSR. With regard to clinical appearance, the animals in groups 1 (intact, infected) and 3 (desalivated, uninfected) gained weight throughout the experiment. However, the desalivated, infected animals in group 2 gained only minimal amounts of weight (Table 1). By macroscopic observation, the mucosa of the jaws of infected animals with salivary glands intact (group 1) showed increased roughness of the surface epithelium, appearing as small raised, opaque areas. The desalivated, infected animals in group 2 showed substantial formation of plaque on the teeth, caries, and the presence of raised white areas on both the marginal gingiva and alveolar mucosa (Fig. 2). The animals in group 3, desalivated but uninfected with *C. albicans*, appeared normal, with smooth and pink gingival tissues and alveolar mucosa.

TABLE 1. Oral infection by *C. albicans* 613 in intact rats and HSR

Group	Mean (SD) no. of <i>C. albicans</i> CFU/jaw (10^4)	Mean (SD) total cultivatable CFU (10^6)	Mean (SD) wt change ^a
1. Intact, infected	1.1 (1.7)	8 (4)	+76.5 (6.7)
2. Desalivated, infected	37.9 (35.5)	9 (3)	+4.4 (5.2)
3. Desalivated, uninfected	0	18 (9)	+47.8 (21.8)

^a Mean total body weight change in grams per rat at the end of the experiment.

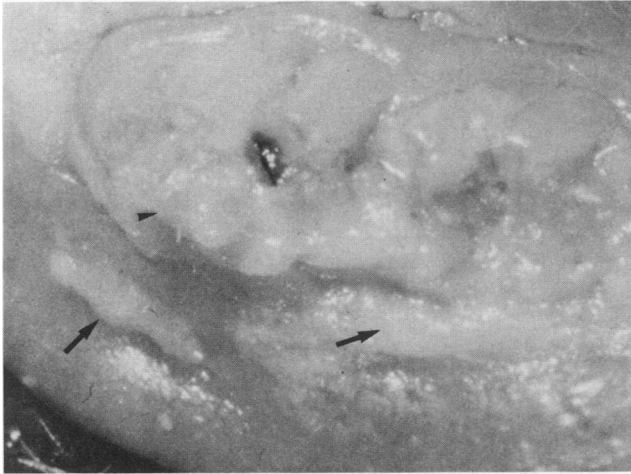


FIG. 2. Clinical appearance of mandibular teeth of a desalivated rat infected with *C. albicans* parental strain 613. The arrows indicate raised, white *Candida*-induced plaques on the marginal gingiva and buccal alveolar mucosa; the arrowhead points to a tooth.

Evaluation of the histologic sections of the marginal gingiva and the alveolar mucosa from the desalivated, uninfected animals revealed a normal keratinized layer of stratified squamous epithelium. In the desalivated, infected group, there was evidence of necrosis and desquamating parakeratotic epithelium penetrated by both candidal filaments and yeast forms (Fig. 3 and 4). *C. albicans* invaded the stratum corneum but not the stratum spinosum.

Below the layers of epithelial desquamation seen on the surface of the gingival and alveolar mucosa, ulceration of the epithelial barrier and interepithelial infiltration by polymor-

phonuclear leukocytes were observed in the granular layers of the stratified squamous epithelium. The pathologic changes observed were similar to those of human oral candidal plaques (6, 21) and are an indication of the validity of the HSR model.

Microscopic examination of histological sections from intact, infected animals revealed a few layers of stratified squamous epithelium lining a patent esophageal lumen (Fig. 5). Sections from desalivated, uninfected animals displayed hyperplasia of the epithelial layers which partially occluded the esophageal lumen (data not shown). *C. albicans* was not observed in esophageal sections from desalivated, uninfected (data not shown) or intact, infected (Fig. 5) animals. In contrast, esophageal sections from desalivated, infected animals were characterized by an increase in the number of layers of the stratum corneum. Furthermore, *Candida* yeast and filamentous forms were observed in the esophageal lumen and stratum corneum (Fig. 6).

Quantitation of microorganisms from the tissues gave the following results. All desalivated animals that were inoculated became infected. The mean number of *C. albicans* CFU per jaw in desalivated, infected animals were over 30-fold greater than that in intact, infected animals, 3.8×10^5 versus 1.1×10^4 CFU (Table 1). These values were statistically significantly different ($P = 0.05$). *C. albicans* was not detected in tissues from desalivated, uninfected animals. However, the total numbers of cultivatable organisms from the jaws were not statistically significantly different among the groups (Table 1). Therefore, the increased numbers of *C. albicans* organisms recovered from the jaws of desalivated, infected animals reflects specific *Candida* overgrowth compared with the resident bacterial flora in the oral cavity and does not reflect a simple increase in the total number of oral flora as a consequence of desalivation.

The data show that desalivated rats in our model were

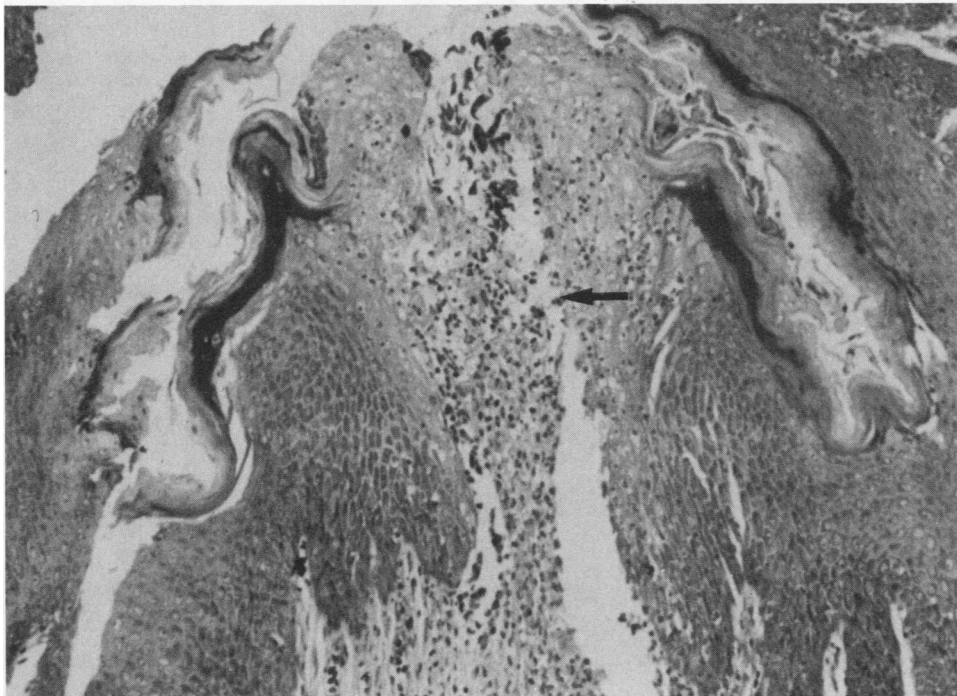


FIG. 3. Histological section (magnification, $\times 40$) of marginal gingiva and alveolar mucosa from a desalivated rat infected with *C. albicans* parental strain 613, revealing ulceration of the epithelial barrier at the junction of the sulcular fold and the alveolar mucosa (arrow).

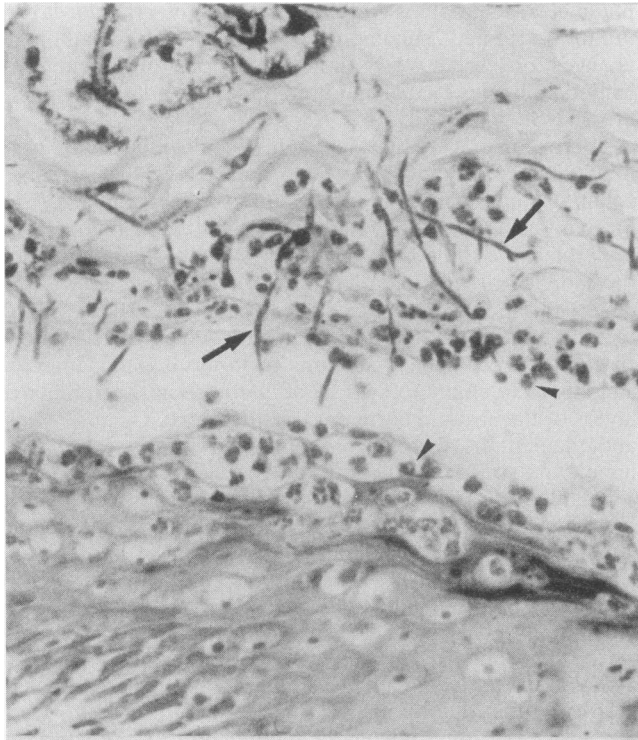


FIG. 4. Histological appearance (magnification, $\times 102$) of the epithelial layer of the marginal gingiva of a desalivated rat infected with *C. albicans* parental strain 613, revealing desquamating parakeratotic epithelia; candidal filaments (arrows) penetrating the stratum corneum and infiltration by polymorphonuclear leukocytes (arrowheads) were observed.

susceptible to oral infection by *C. albicans*. Candidal lesions appeared in the oral mucosa, and histological examination confirmed the clinical impression (Fig. 3 and 4). The esophagi of inoculated, desalivated animals were also infected with *C. albicans* (Fig. 6).

Transmission of infection with the *C. albicans* parental strain. Results in Table 2 show the importance of saliva in resistance to oral *C. albicans* infection. All of the animals that were swabbed with *C. albicans* became infected. Transfer of infection from one desalivated animal to another desalivated animal occurred very rapidly, and all five recipient animals became infected. In contrast, transfer from an intact donor to an intact recipient took significantly longer ($P = 0.05$) and only three of five intact recipients became infected.

Statistically similar numbers of *C. albicans* CFU were isolated from the jaws of infected animals in both desalivated and intact recipient groups. The total number of *C. albicans* CFU isolated from the jaws of infected rats was substantially higher than the numbers of *C. albicans* CFU isolated from jaws in the other two studies described herein (Table 2). We also observed a smaller difference between the number of *C. albicans* CFU isolated from the jaws of intact, infected animals and the number of CFU isolated from desalivated animals (Table 2). The basis for the increased recovery of *C. albicans* is unclear, but the increase may reflect a general increase in susceptibility to infection in the particular litter of rats used for the experiment. Nonetheless, 4 of the 10 intact donor animals inoculated with *C. albicans* cleared the organism and, in addition, did not transmit the infection to

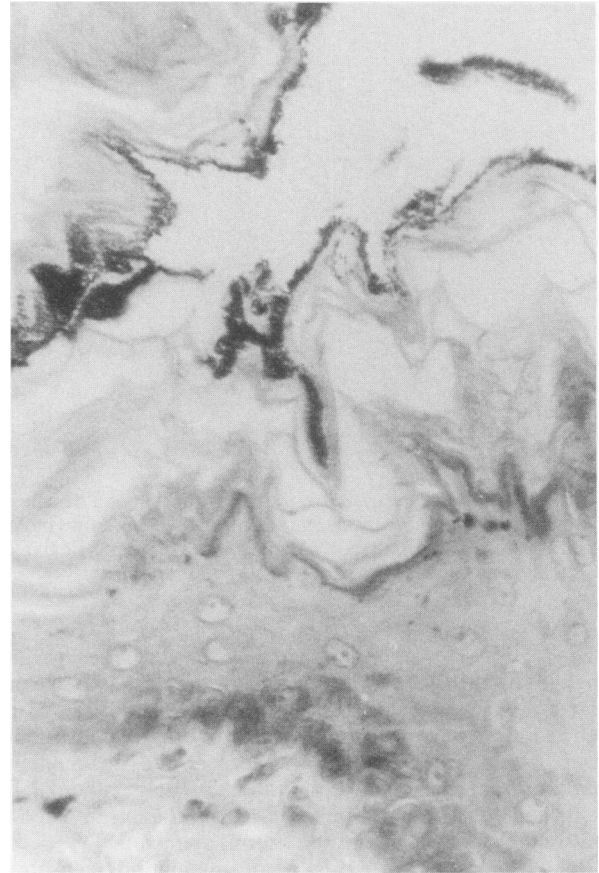


FIG. 5. Histological appearance of esophageal sections (magnification, $\times 134$) from an intact, infected animal. No *C. albicans* organisms were observed in these sections.

recipients. This observation is consistent with the results obtained in the final transmission study (Table 3).

Comparison of the infectivity, pathogenicity, and virulence of parental strain 613 with those of spontaneously derived morphological mutant 613-m1 in the HSR model of oral candidiasis. The experiment with mutant strain 613-m1 demonstrated that a spontaneously derived morphological mutant from a clinical isolate was also capable of establishing infection in our HSR model and was transmitted to uninfected recipient animals (Table 3). The numbers of *C. albicans* CFU recovered from the jaws of animals infected with either the parental strain or the mutant were not significantly different ($P = 0.05$). However, the time required for transmission of the mutant to a recipient animal was significantly ($P = 0.05$) longer than the time required for transmission of the parental strain. Hence, considering the time required for transmission of infection as an indicator of infectivity, pathogenicity, and virulence, strain 613-m1 appeared to be deficient in these attributes compared with its parental strain in the HSR model. Nevertheless, the morphological mutant persisted long enough in donor animals to permit transmission to recipients.

The results of our investigation demonstrate the utility of the HSR model in the study of oral *C. albicans* infection. The data show that oral *Candida* infection was established with a small challenge inoculum and that mucosal lesions developed in the oral cavities and esophagi of the infected rats. We also showed that transmission of oral *Candida*

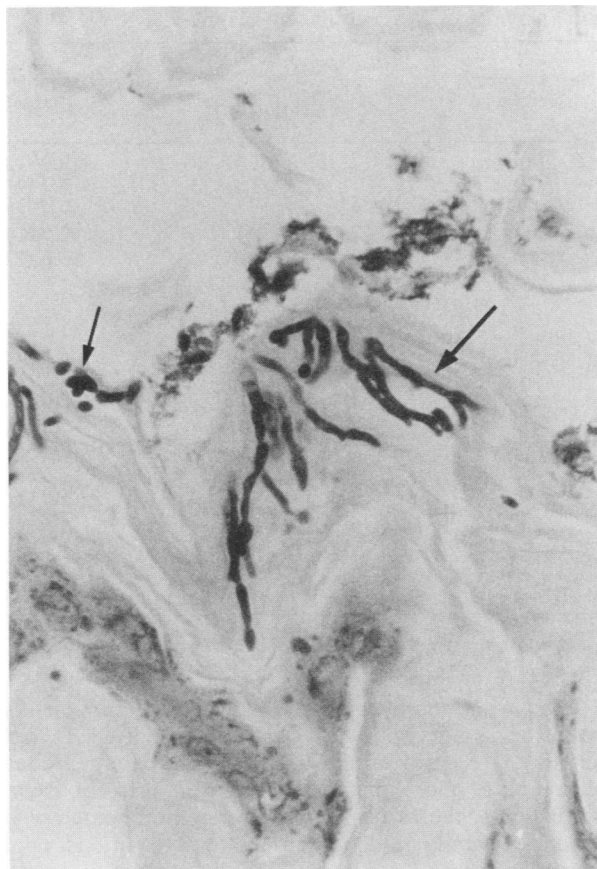


FIG. 6. Histological appearance of an esophageal section (magnification, $\times 134$) from a desalivated, infected animal. Note the *C. albicans* yeast (short arrow) and filamentous forms (long arrow).

infection from an inoculated rat to uninoculated cagemates occurred rapidly. Finally, we demonstrated that the infectivity, pathogenesis, and virulence of different strains can be assessed both qualitatively and quantitatively with the HSR model.

DISCUSSION

We developed a model of oral *C. albicans* infection in HSR which can be used to assess and compare the virulence of different *C. albicans* strains. By using this approach, our understanding of the mechanisms by which *C. albicans* induces oral disease will be enhanced. In addition, we will elucidate the role played by saliva in controlling *C. albicans* infection in the oral cavity.

In the HSR model of oral candidiasis, pretreatment of the animals with tetracycline or other antibiotics (1, 2, 15) is unnecessary to initiate infection. Infection is established by using a small inoculum of *C. albicans*, as opposed to models in which continual exposure to the fungus is required to establish oral infection (1, 2, 15). Furthermore, infection can be established by transmission from one animal to another. There are at least two possible explanations for the more rapid transfer of *C. albicans* from desalivated donors to desalivated recipients than from intact donors to intact recipients (Tables 2 and 3). Conceivably, the environment in the oral cavity may select for variants of *C. albicans* with enhanced infectivity, pathogenicity, and virulence; this pos-

sibility is suggested by the similar transmission times observed between desalivated donors to desalivated recipients and desalivated donors to intact recipients. Although we did not take isolates of *C. albicans* 613 obtained after oral infection and reinoculate them into desalivated rats, we did observe enhanced virulence in *Streptococcus sobrinus* reisolated from desalivated animals (W. H. Bowen, et al., submitted for publication). Alternatively, desalivated donors may carry and have available for transmission increased numbers of *C. albicans* organisms than do intact donor animals, although this was not a factor in our studies with *S. sobrinus* (26). In our model, the rats displayed a progressive oral infection over a period of several weeks and mucosal lesions developed. Histological examination showed that lesions that develop in HSR are similar to those that occur in humans (6, 21).

The ability of *C. albicans* to establish infection in the oral cavity has been associated with diminished salivary flow (29, 41); the HSR model parallels clinical conditions in which salivary flow has been reduced. A recent clinical study has shown that oral defense mechanisms are impaired early in human immunodeficiency virus type 1-infected patients (50). In particular, several indicators of submandibular salivary gland dysfunction were detected. Furthermore, the impairment of oral defense mechanisms was correlated with a high incidence of oral candidiasis in human immunodeficiency virus type 1-infected patients. Not surprisingly, patients with AIDS who have oral candidiasis frequently have esophageal *Candida* infections as well (7, 11, 13, 17, 34, 36). Esophageal infection in the desalivated, infected rats in our model was also quite severe. It is a distinct possibility that the esophageal infection makes ingestion of food difficult, resulting in the weight loss observed in HSR. It is also possible that a similar condition in patients with AIDS who have oral and esophageal candidiasis contributes to weight loss in those individuals. The HSR model will also be useful in furthering our understanding of the etiology, and pathogenesis of oral candidiasis in patients without AIDS in whom disease, drug treatment, or radiation therapy has induced xerostomia or dry mouth (41), as well as in patients in whom occlusion of the mucosal epithelium by prolonged denture wear has resulted in candidal stomatitis (12).

It is possible that both hyposalivation and composition of the diet contribute to the establishment of frank candidiasis in the HSR model. A high-sugar diet has been shown to promote oral candidiasis (8, 16, 30, 39). The acidic environment created by the sucrose-rich diet used in our model may facilitate the growth of *C. albicans* in the oral cavity. Earlier

TABLE 2. Transfer of *C. albicans* infection in 26-day-old animals

Group (donor/recipient)	Mean (SD) transfer time (days)	No. of recipients infected/total	Mean (SD) no. of <i>C. albicans</i> CFU/jaw (10^7)	Mean (SD) wt change ^a
Desalivated/desalivated	1.2 (0.4)	5/5	1.9 (0.4)	+1.8 (3.4)
			2.8 (1.9)	+2.4 (3.5)
Desalivated/intact	3.2 (2.2)	5/5	1.7 (0.6)	+1.8 (3.0)
			1.4 (0.7)	+19.3 (10.4)
Intact/desalivated	4.3 (2.08)	3/5	1.5 (0.9)	+55.7 (54.3)
			1.3 (0.7)	+30.7 (25.0)
Intact/intact	6.7 (0.6)	3/5	1.1 (0.5)	+68.3 (56.3)
			0.9 (0.2)	+65.3 (39.3)

^a Mean total body weight change in grams per rat at the end of the experiment.

TABLE 3. Comparison of transfer of infection with 613 parental strain versus mutant 613-m1

Group	Infecting strain	Mean (SD) transfer time (days)	No. of recipients infected/total	Mean no. of <i>C. albicans</i> CFU/jaw (10^3)	Mean (SD) wt change ^a
Desalivated donor Desalivated receiver	Parental	3.8 (0.8)	5/5	133.0 (61.3) 42.5 (47.1)	-12.9 (7.0) -7.4 (7.1)
Intact donor Intact receiver	Parental	27.0	1/5	3.1 (2.5) 0.02 (0.04)	92.7 (10.6) 93.6 (22.4)
Desalivated donor Desalivated receiver	613-m1	15.4 (7.0)	5/5	59.7 (62.2) 35.6 (39.6)	11.1 (27.2) 2.6 (29.1)
Desalivated donor Intact receiver	613-m1	21.0 (8.5)	2/5	127.6 (110.4) 1.3 (2.2)	12.6 (36.3) 83.8 (28.8)
Intact donor Desalivated receiver	613-m1	No transfer	0/5	0.6 (0.9) 0	87.3 (32.8) -1.0 (22.0)
Intact donor Intact receiver	613-m1	18.0	1/5	0.01 (0.02) 0.05 (0.09)	90.6 (15.6) 94.9 (16.0)

^a Mean total body weight change in grams per rat at the end of the experiment.

studies in our laboratory (4) have demonstrated that in primates receiving their essential nutrition by gastric gavage, the *Candida* population in the oral cavity can be increased by addition of sugars to the diet. Studies are under way to determine the contribution of diet to the development of oral *Candida* infection in rats. We are presently comparing the degree of oral infection in rats fed Diet 2000 containing sucrose (20) with that of rats fed the same basic diet containing an isocaloric amount of wheat starch.

C. albicans mutants, in combination with our animal model, should serve as a powerful tool to facilitate our understanding of the mechanisms by which the fungus causes disease. We have compared the virulence of a spontaneously derived morphological mutant of *C. albicans* with its respective parental strain in the HSR model. The mutant we chose for analysis differed from its corresponding parental strain with regard to morphological characteristics and functional attributes of the cell wall. In light of the importance of the cell wall in attachment to mucosal and prosthetic surfaces in the oral cavity, it is reasonable to expect that mutants in cell wall morphogenesis may differ from the parental strain in the ability to establish infection. This has been reported for mutants of *C. albicans* in animal models of systemic (19) and vaginal (43) infections in which the mutants were of diminished virulence. We obtained a similar result with a morphological mutant in the HSR model of oral *Candida* infection. The morphological and functional characteristics of isolates of mutant 613-m1 recovered from the oral cavities of rats did not differ from those passaged in vitro (data not shown). We did not determine whether the overall virulence of the mutants isolated from rat oral cavities was increased.

There are several reasons for using a spontaneously derived morphological mutant in our study. DiMenna reported that a high percentage (13%) of *C. albicans* isolates from the oral cavity contained rough colony forms (10). This observation associated variant colony forms with clinical material for the first time. *C. albicans* morphological variants from patients with systemic (46) and vaginal (44, 45) infections have also been characterized. Recently, isolation

of *C. albicans* colony morphology variants from the oral cavities of patients with AIDS has been reported (D. Coleman et al., J. Dent. Res. 68:893, 1989). Despite the frequent occurrence of morphological variants in primary isolates from patients, the relationship between morphologic variation and *Candida* infection in the oral cavity is poorly understood.

C. albicans apparently lacks a sexual cycle; therefore, it has no obvious mechanism for genetic recombination (49). However, colony morphological variation, which occurs at a frequency of approximately 10^{-4} per generation (42), has been correlated with chromosomal rearrangements in *C. albicans* (38, 47). It has been proposed that chromosomal rearrangements in *C. albicans* provide a mechanism by which the fungus generates diversity within its population (38). Chromosomal rearrangements have been shown to regulate gene expression in both lower and higher eucaryotes (3). However, understanding the relationship between chromosomal rearrangements and the variety of alterations observed in *C. albicans* mutants awaits the identification of appropriate genetic markers. No correlation has been made between a specific colony morphology and a given karyotype. Nonetheless, by examining the virulence of different morphological mutants in the HSR model, we can begin to correlate morphologic and functional attributes of the mutants with pathogenicity. Spontaneously derived morphological mutants isolated from virulent parental strains of *C. albicans* are likely to be highly complex in their alterations. However, isogenic mutants of *C. albicans* in genes that control colony morphology have not been engineered. Until such mutants can be constructed, the molecular basis for the altered morphologic and functional characteristics observed in the mutants will remain unclear.

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