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Experimental Oral Candidiasis in Animal Models

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ABSTRACT

Oral candidiasis is as much the final outcome of the vulnerability of the host as of the virulence of the invading organism. We review here the extensive literature on animal experiments mainly appertaining to the host predisposing factors that initiate and perpetuate these infections. The monkey, rat, and mouse are the choice models for investigating oral candidiasis, but comparisons between the same or different models appear difficult, because of variables such as the study design, the number of animals used, their diet, the differences in *Candida* strains, and the duration of the studies. These variables notwithstanding, the following could be concluded. (i) The primate model is ideal for investigating *Candida*-associated denture stomatitis since both erythematous and pseudomembranous lesions have been produced in monkeys with prosthetic plates; they are, however, expensive and difficult to obtain and maintain. (ii) The rat model (both Sprague-Dawley and Wistar) is well proven for observing chronic oral candidal colonization and infection, due to the ease of breeding and handling and their ready availability. (iii) Mice are similar, but in addition there are well characterized variants simulating immunologic and genetic abnormalities (e.g., athymic, euthymic, murine-acquired immune deficiency syndrome, and severe combined immunodeficient models) and hence are used for short-term studies relating the host immune response and oral candidiasis. Nonetheless, an ideal, relatively inexpensive model representative of the human oral environment in ecological and microbiological terms is yet to be described. Until such a model is developed, researchers should pay attention to standardization of the experimental protocols described here to obtain broadly comparable and meaningful data.

Candida species are ubiquitous, human fungal pathogens capable of initiating a variety of recurring superficial diseases especially in the oral and vaginal mucosae ([129](#), [167](#)). In the late 1950s there was a steadily increasing number of reports on superficial *Candida* infections associated with the administration of broad-spectrum antibiotics such as tetracycline ([91](#), [178](#)). In subsequent years, the extensive use of steroids, immunosuppressive agents in organ transplant recipients ([158](#), [192](#)) myeloablative radiation therapy ([70](#), [74](#), [205](#)), and antineoplastics in patients with hematologic malignancies ([20](#), [62](#), [106](#)) contributed to the increasing morbidity associated with *Candida*. More recently, mucosal *Candida* infections have received profuse attention due to the advent of the human immunodeficiency virus (HIV) infection. For instance, it is now known that up to 90% of HIV-infected individuals suffer from oropharyngeal candidiasis ([161](#)). This condition is a key feature in staging HIV disease and was once included as a marker in disease classification ([64](#)). Curiously, HIV-infected patients appear to be more susceptible than immunocompetent individuals to oropharyngeal but not vaginal or disseminated candidiasis ([168](#), [177](#)). The other general risk factors for oral candidiasis are age (mainly the very young and the very old), denture prostheses, smoking, diabetes mellitus, iron and vitamin deficiencies ([159](#)), and salivary gland hypofunction ([166](#)).

Candida albicans is the species most often associated with oral lesions, but other, less pathogenic species such as *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei* are also occasionally but regularly isolated

(105, 170). Recently, a novel species, *C. dubliniensis*, closely related to *C. albicans*, has been isolated, particularly from mucosal lesions in HIV-infected patients (39).

An important cofactor associated with the pathogenesis of oral candidiasis appears to be the virulence of the infecting organism (113, 153). The specific features of the fungus that contribute to the development of oral candidiasis include its ability to adhere to and colonize the oral mucosa (87), its ability to form cylindrical appendages termed germ tubes (33), and its cell surface hydrophobicity (68). In addition, phenotypic and genotypic switching (176, 186), extracellular aspartyl proteinase secretion (44, 208), and phospholipase production (98) appear to play a subsidiary role in the pathogenicity. Nonetheless, the hierarchy of the importance of these predisposing attributes is little known, although some animal studies described here have shed some light on this issue.

CLINICAL EPIDEMIOLOGY OF HUMAN ORAL CANDIDIASIS

Isolation of *Candida* from the oral cavity does not imply disease, since its asymptomatic prevalence in healthy persons ranges from 3 to 48% (12) and is even higher in healthy children, 45 to 65% (129). In many epidemiological studies of oral candidiasis, the most commonly isolated yeast species is *C. albicans* (166). A median carriage rate of 38.1% has been observed for *C. albicans* alone in a number of surveys in community-dwelling outpatients (129), while a higher carriage rate (up to 78%) has been observed in hospitalized elderly patients (41, 204). Yeast carriage is even higher in those who are HIV seropositive and rises as the CD4⁺ T-cell count falls (67, 164, 196). Other pathogenic members of the genus *Candida* often isolated from the oral environment are (in descending order of virulence) *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. pseudotropicalis*, *C. krusei* and *C. guilliermondi* (129). *C. dubliniensis* is a recently discovered novel species, and its virulence potential is much like that of *C. albicans* due to their close genomic relatedness (190). Despite such diversity among the non-*albicans* species (143, 189), it is the general belief that they are of low virulence and that disease manifestation is determined mainly by the health of the host (128, 173, 183). Neither colonization with *Candida* species alone nor a significant increase in their salivary concentration (53) is necessarily a precursor of the development of oral candidiasis (121). Therefore, other local or systemic factors must be present for the organisms to initiate infection and cause disease.

Oral candidiasis may present in a variety of clinical forms, and the three main variants are the pseudomembranous type, commonly known as thrush, and the erythematous and hyperplastic variants (14) (Fig. 1). When two or more of these variants appear in unison, the term “multifocal candidiasis” is used (169). Other common lesions include *Candida*-associated denture stomatitis, angular cheilitis, and median rhomboid glossitis.

The recognition that *Candida* is an important pathogen, particularly in the immunocompromised host, has resulted in a vast body of in vitro investigations evaluating its virulent attributes in an attempt to elucidate the pathogenesis of the disease. The progress made in understanding some of these features, such as the mechanisms that result in adherence to host tissues (88), cell surface hydrophobicity (69), switching phenomena of the yeast (186, 187), secretion of aspartyl proteinases (208), and phospholipase production (98), is very impressive. Nonetheless, in vivo studies either in live humans or in animals are essential to elucidate and fully comprehend the mechanisms leading to candidal infection.

The host oral defenses against *Candida* essentially fall into two categories: nonspecific immune mechanisms (e.g., integrity of the mucosae, commensal bacteria, polymorphonuclear leukocytes, macrophages, and salivary factors) and specific immune mechanisms (e.g., serum antibodies, secretory antibodies, and cell-mediated immunity) (38).

The stratified squamous epithelium of the oral mucosa forms a continuous surface that protects the underlying tissues and functions as an impervious, mechanical barrier. The protection so provided is dependent on the degree of keratinization and the continuous desquamation or shedding of epithelial cells. Indeed, the latter mechanism is considered to play a pivotal role in maintaining a healthy oral mucosa and in limiting candidal colonization and infection. The interaction between *Candida* species and the commensal microbial flora is perhaps the next critical mechanism modulating oral candidal colonization (166). The commensal flora regulates yeast numbers by inhibiting the adherence of yeasts to

oral surfaces by competing for sites of adherence as well as for the available nutrients. A number of studies have also shown, both in vivo in gnotobiotic mice and in vitro, that candidal colonization of epithelia could be suppressed by streptococci, which are the predominant resident commensals of oral mucosal surfaces ([99](#), [123](#), [163](#)).

The human oral cavity is a unique ecological niche because it is constantly bathed in saliva, a biological fluid with potent antifungal and antibacterial activity. In addition, the constant salivary flushing action mechanically inhibits the accumulation of microorganisms in various oral niches. A quantitative reduction in saliva or salivary flow, for instance in Sjögren's syndrome, leads to a xerostomic state with a concomitant increase in oral candidal carriage and infection, indicating the importance of salivary defenses against invading fungi ([109](#), [162](#)). Elements in saliva that inhibit the growth of *Candida* include nonspecific factors such as the histidine-rich proteins, the proline-rich proteins, the salivary peroxidase system, lactoferrin, and lysozyme ([142](#), [171](#), [174](#), [193](#)). The anticandidal nature of histidine-rich polypeptides in particular is noteworthy. Pollock et al. ([142](#)) found that the antifungal activity of purified salivary histidine-rich polypeptides is akin to that of imidazoles ([104](#), [134](#), [142](#)). Lysozyme and lactoferrin are two further nonimmunoglobulin salivary proteins that contribute to the regulation of oral *Candida*. A number of studies have documented the fungicidal effect of apolactoferrin against *Candida* ([125](#), [171](#), [188](#), [197](#)), while the relative sensitivity of different *Candida* species to lysozyme has been demonstrated by Tobgi et al. ([193](#)) using six different species.

It is known that two independent systems, the systemic and the secretory immune systems, are both involved in defending the oral cavity against *Candida*. Lehner ([100](#)) was the first to suggest that salivary (secretory) immunoglobulin A (sIgA) may contribute to ameliorating the disease process. Individuals with lowered levels of sIgA are more often afflicted with mucosal candidiasis, and functional sIgA appears to prevent the attachment of *C. albicans* to the mucosal epithelium ([200](#)). Polymorphonuclear leukocytes and macrophages have the ability to phagocytose and kill *Candida* cells. However, the full expression of their activity is dependent on augmentation by cytokines synthesized or induced by T cells ([13](#)) and the length of time they survive in the hostile oral environment bathed in saliva.

Mucocutaneous and systemic candidiasis are both typically associated with defects in the cell-mediated immune response ([129](#)). A multiplicity of defects in cell-mediated immunity in subjects with chronic mucocutaneous candidiasis have been examined and defined ([150](#)). This is further exemplified in patients infected with the HIV, an agent which causes an impairment of the CD4⁺ T-helper lymphocytes, leading to frequent recurrences of oropharyngeal candidiasis ([73](#)). These and other host defenses against *Candida* have been reviewed recently by Greenfield ([63](#)).

A number of antifungal agents are available for the management of candidal infections ([115](#)). The major agents that are currently used for oropharyngeal candidiasis belong to either the polyenes (amphotericin B and nystatin), the imidazoles (clotrimazole, econazole, ketoconazole, and miconazole), or the triazoles (fluconazole and itraconazole) ([52](#)). Nystatin is ideal for topical treatment of oral infections since it is not absorbed from the gastrointestinal tract and hence the adverse effects are minimal. Amphotericin B is less widely used for this purpose due to its treatment-limiting adverse effects such as nephrotoxicity ([86](#)).

The introduction of the imidazole and azole groups of antifungals during the last two decades has revolutionized the management of fungal infections ([86](#)). The approved azole antifungal agents for the treatment of oral candidiasis are miconazole, clotrimazole, ketoconazole, fluconazole, and itraconazole ([52](#)). Miconazole is effective for almost all oral manifestations of candidiasis including chronic mucocutaneous candidiasis. Until the introduction of the triazoles (itraconazole and fluconazole), ketoconazole (an imidazole) was widely used as an alternative to amphotericin B ([85](#)), but it suffered from the drawbacks of hepatotoxicity and endocrine toxicity. However the more recently introduced triazole agents, itraconazole and fluconazole, are far superior since they are orally active and water soluble and have a significantly lower toxicity than do the imidazoles ([85](#)). Indeed, fluconazole is the drug of choice in the treatment of candidiasis in HIV infection.

The euphoria surrounding the efficacy of the azoles has now been tempered by the realization of moderate or high-level resistance to fluconazole in some species, such as *C. glabrata*, *C. krusei*, and *C. albicans*

(148, 170). This phenomenon has been especially common in *C. albicans* isolated from patients in whom fluconazole has been extensively used, as in those infected with HIV (61, 138). In addition to these topical or systemic antifungals, antiseptic agents such as chlorhexidine gluconate have been used to supplement the drug regimens, especially in treating *Candida*-associated denture stomatitis. The animal models described herein have made major contributions to the evaluation of these drugs, especially during their developmental stages.

NEED FOR AND CLINICAL RELEVANCE OF ANIMAL MODELS

Apart from the ethical dilemmas associated with experimentation on live humans, humans are notoriously dissimilar in terms of their dietary and social habits, immune status, and oral physiology such as salivary function. These factors, plus the racial, ethnic, and cross-cultural variations in human demographics, add to the confounding matrix of factors influencing the etiology and pathogenesis of diseases such as candidiasis, where the invading organism is not a true parasite but an opportunistic pathogen. Hence, in theory at least, the development of an ideal animal model for oral candidiasis would provide a standardized tool which can be controlled and manipulated to derive universally comparable data on the etiopathology, diagnosis, and management of the disease process. Perhaps it is true that the available animal models have thus far successfully illuminated the pathogenesis of many variants of oral candidiasis from the points of view of both the host and the yeast. However, the diagnostic and management aspects of the disease processes have not been widely addressed, and the results have been mixed.

The most common form of oral candidiasis is *Candida*-associated denture stomatitis, seen in 50 to 69% of denture wearers at one time or another (29). Not surprisingly, therefore, the pathogenesis and management of this condition have been studied in a number of models by many investigators. Budtz-Jørgensen, who pioneered such studies, employed *Macaca irus* monkeys with custom-fitted acrylic plates (26) for this purpose, while others have used the Wistar rat model to study *Candida*-associated denture stomatitis and its histopathology (132, 179, 181). All workers who successfully initiated the disease in animal models have claimed a striking similarity between the human and animal lesions and have stressed the utility of the respective animal model. After reproducing *Candida*-associated denture stomatitis, the next step was to demonstrate the cofactors involved and the efficacy of topical antiseptics and antifungals in the management of the condition. These therapeutic approaches have included the incorporation of chlorhexidine acetate (97) and azole antifungals (126) to denture base materials, as well as the delivery of systemic imidazoles by this route, using the Wistar rat model (8, 108, 201). However, translation of these into human therapeutic trials has met with little success (50).

Thrush, or pseudomembranous candidiasis, is the best-known form of mucosal candidiasis and has currently come to the forefront due to HIV infection (161). Efforts to produce oral thrush in rats and mice have been successful to varying degrees, and the etiology and therapy of this ailment have been elucidated. For instance, one recent study has shown that superficial candidal invasion and initiation of thrush is favored by topical application of corticosteroids, which dramatically shifts the host-parasite relationship in favor of the yeast (47). Although it is possible to obtain mice that are deficient in the quality and quantity of CD4⁺ T cells, thus mimicking HIV infection, surprisingly little work has been performed to explore this intriguing area (31, 32, 46).

Other predisposing factors for oral candidiasis that have come under scrutiny in a number of animal models include broad-spectrum antibiotic therapy (5, 82, 155–157), carbohydrate-rich diets (66, 154, 155), topical use of corticosteroids (47), corticosteroid inhalation (28), trauma (131), iron deficiency (147, 185), diabetes (51), xerostomia (1, 83, 84, 119, 133), decrease in CD4⁺ T-cell counts and phagocytic function (31, 32), defective T-cell function (16), and immunosuppressive therapy (27, 172). Undoubtedly, these studies have helped us to understand the etiology of oral candidiasis and the development of the management protocols that are currently prevalent.

From a histopathological and diagnostic point of view, most of the lesions described in animal models have faithfully reproduced human candidal lesions. For instance, Budtz-Jørgensen and Bertram (30) and Budtz-Jørgensen (26) experimentally induced palatal candidiasis in the monkey model, which closely mimicked the nonspecific inflammatory changes of the oral mucosa seen in *Candida*-associated denture

stomatitis in humans. The palatal smears from the experimental infection yielded yeasts that were almost exclusively in the hyphal form, as in *Candida*-associated denture stomatitis (30, 34). Also, a number of studies by others with the Sprague-Dawley rat model of oral candidiasis have detected colonization patterns and lesions that were similar to human lesions both microscopically and histologically (4, 5). Earlier studies by Russell and Jones (156) and Jones et al. (82) using a rat model also demonstrated that *Candida* carriage and infectivity in this animal are similar to those in humans. The recently described murine acquired immune deficiency syndrome (MAIDS) mouse model (46) is an exciting new development resembling early stages of human HIV infection, which could be harnessed to elucidate the pathogenesis of oral candidiasis. Since 10 to 15% of candidal hyperplastic lesions progress to dysplasia and oral carcinoma (166) a few workers have attempted to investigate this relationship in animal models (117). Intriguingly, a putative correlation between specific biotypes of *Candida* and oral dysplasia has been demonstrated in one experiment (93) and yet no further studies have been conducted as a follow-up.

For all these reasons and more, animal models have served for more than five decades to illustrate the enigmatic and tempestuous relationship between this opportunist yeast pathogen and its human host. On perusal of the available literature, we were unable to find a comprehensive account of animal models in oral candidiasis. The following, therefore, is an attempt to review in detail the microbiological, histopathological, and therapeutic approaches and potential caveats pertaining to experimentally induced oral *Candida* infections in animal models described in the English language literature during the last half century.

EARLY TISSUE CULTURE SYSTEMS AND HISTOPATHOLOGIC STUDIES

In vitro tissue culture systems derived from nonhuman sources were used by a few investigators to study the pathological processes in candidal infection much earlier than the introduction of the in vivo experimental animal models. Partridge (136) was the earliest to confront this problem and used the chick chorioallantoic membrane to culture pathogenic fungi. Subsequently, Cawson (35) used the same assay to evaluate the hyperplastic response of the ectoderm to candidal invasion while Hurley and Stanley (77) experimented with cultured mucosal cells from the lingual dorsum of neonatal rats for the same purpose. They also assessed the yeast-induced cytopathic effect and the association between the growth phase of yeasts and the lethal effect on tissues.

As opposed to these animal systems, cultured human explants and tissues have been used by a few investigators. Pemberton and Turner (137) used cultured human gingival epithelium to investigate *C. albicans* invasion, while cultured explants from the lingual dorsum were used in ultrastructural studies by Miles (120) and Howlett (75) to compare the invasive potential of different *Candida* species. The findings by these workers were very similar to those for clinical (oral) candidiasis, supporting the notion that in vitro cell culture systems were an appropriate model for the study of the disease.

At about the same time, Montes and Wilborn (122) demonstrated in clinical histopathologic studies that *Candida* penetrates the human oral epithelium in both acute and chronic phases of the infection and essentially behaves as an intracellular parasite. These findings gave impetus for more detailed studies on oral mucosal invasion of *Candida*. Subsequent electron microscopic studies by Cawson and Rajasingham (36), with biopsy tissues from patients, also demonstrated clearly the invasion of the hyperplastic oral epithelium by candidal hyphae. The results of these investigations were barely adequate to unravel the complexities of the disease process, and animal models (e.g., monkeys, rabbits, rats, and mice) have been continually used since then to study the genesis of oral *Candida* infections. We review below the advantages and disadvantages of these animal models and then the experimental details and outcomes of investigations related to each model.

PROS AND CONS OF CURRENT ANIMAL MODELS

Monkey Model (*Macaca irus*)

Primates appear to be the ideal animal model for experimental *Candida* infections because of their relatively close kinship to humans. The composition of the oral microflora of monkeys, especially *M. irus*, is both qualitatively and quantitatively very similar to that of humans (23, 24), and *C. albicans* is a

frequent oral saprophyte in monkeys (23, 152). In addition, monkeys are able to retain acrylic plates resembling denture prostheses in place, a prerequisite for experimental studies on *Candida*-associated denture stomatitis. However, primates are relatively expensive and difficult to maintain, especially for large-scale experiments. Some workers have also reported that artificial oral infestation of monkeys with *Candida* is difficult and unreliable (133). Hence, the monkey model has been largely replaced by smaller mammals such as rats and mice, which have gained popularity.

Rat Model (Wistar and Sprague-Dawley)

Two species of rats—Sprague-Dawley (SD) and Wistar—have been widely used in experimental oral *Candida* infections. The two main advantages of the rat model are the low maintenance cost and the sufficient size of the oral cavity, which easily permits inoculation and sample collection. Furthermore, the tongue of this animal is fairly easily colonized by *Candida*, demonstrating conditions such as median rhomboid glossitis and atrophic candidiasis (2) (Fig. 1).

Candida infections in SD rats can be experimentally induced within a few weeks without traumatizing the mucosal epithelium, and a number of investigators claim this model to be satisfactory since it is known to yield consistently reproducible data (3–5, 54, 55, 82, 155, 156). However, the vast majority of these workers (with the exception of perhaps one group) had to provide antibiotic (e.g., tetracycline)-laced food to initiate the lesions. The clinical and histologic findings in experimental disease in SD rats are similar to those of humans. Clinically, small white patches of “thrush” can be visualized on the keratinized lingual mucosa and sometimes on the cheek mucosa.

According to some workers, rats are likely to harbor *C. albicans* in the oral cavity, albeit to a lesser extent than humans do (80). However, we were unable to find quantitative estimates of candidal colonization of the oral mucosa in wild-type rats. Therefore, one disadvantage of the rat model could be that the animal may harbor *C. albicans* as a low-level transient commensal (130, 206) and therefore the contribution of the innate immune response to the disease process would be difficult to fathom. However, it could be argued that such natural prevalence of oral *Candida* mimicks the human ecosystem since 30 to 50% of humans carry oral yeasts (166). Workers using this model for future studies should therefore bear in mind the critical importance of ruling out natural oral colonization by *Candida* prior to artificial inoculation.

Mouse Model

As opposed to the rat, *Candida* is not a constituent resident oral microbe of the conventional laboratory mouse (96, 139). This appears to be a major advantage of the experimental mouse model of oral candidiasis. In addition, since the murine bacterial flora has been well characterized and recognized to consist of fewer than 20 species, (194), this model permits evaluation of the role of oral commensal bacteria in initiating or suppressing candidal infection. Moreover, the immunobiology of the healthy murine oral mucosa has also been fairly well characterized by a number of workers (48, 49, 94), making it ideal for unraveling adaptive immune responses of the mucosal tissues to candidal infection. Furthermore, mice are easily obtained in large numbers and their maintenance is cheap. Conventional infant mice can be readily colonized by topical inoculation of the oral mucosal surfaces with 10^8 pelleted *C. albicans* blastospores per ml (95). Their small size could be considered an added advantage as it facilitates routine daily monitoring, especially when large numbers are used. Nevertheless, the size of the murine oral cavity can also be considered a distinct drawback due to the difficulty in monitoring mucosal changes by naked-eye examination. Hence, some workers have cultured the tissues or organs of the whole animal to ascertain infestation or infection (15).

Mouse mutants are also extremely useful for experimental studies. The sex-linked anemia (*sla*) mutant, for instance, is ideally suited for experimental candidiasis since it shows a consistent and a prolonged degree of anemia without artificial dietary restriction or bleeding (18, 65). Another mutant, an inbred strain with a metabolic disease resembling diabetes mellitus in humans, has been reported (76). Since uncontrolled diabetes mellitus is well known to predispose individuals to oral candidiasis, this model could be of potential value in understanding diabetes-related oral candidiasis.

Other mutants of this model appear useful for studying the effect of inherited disorders on the

development of oral candidiasis. For instance, an autosomal recessive mutation responsible for severe combined immune deficiency (SCID syndrome) has been reported in mice (22). SCID is a rare congenital syndrome of humans that results in loss of both B- and T-cell immunity, and SCID mice are also severely deficient in these lymphocytes. Interestingly, the SCID-hu model has been used to study the infection of human lymphoid cells with HIV-1 a condition which is well known to initiate and aggravate mucosal candidiasis (114, 124). Perhaps the SCID mouse model may be used in future for studying oral candidiasis in HIV infection, together with the very recently described MAIDS model (see below).

Other immune disorders such as the athymic state, X-linked B-lymphocyte defects, and candidiasis related to these syndromes have been investigated using the mouse model (71, 175). A mutant mouse strain called the beige mouse, with a lysosomal defect resulting in deficient phagocytosis (60, 151) as well as deficient NK-cell activity (11, 57, 107, 149), has been used for additional studies. Indeed, beige mice handled thrushlike lesions less well than their littermates did. These mice, as expected from their lysosomal defect, which impairs phagocytosis, are also susceptible to systemic candidiasis. The foregoing mouse mutant variants have given us a fresh insight into the host defense mechanisms operational in superficial forms of oral candidiasis. Further details of oral *Candida* infections in these models are provided later in this review.

Hamster Model

Although not as popular as the preceding animal models, the cheek pouch of the hamster has been used by some workers to investigate experimental oral candidiasis (116). McMillan and Cowell, (116) found that a single inoculation of the organism (10^7 CFU per ml) was adequate to cause infection or infestation of the hamster cheek pouch mucosa. Artificial ligation of the cheek pouch with sutures after *Candida* inoculation was also noted to be a simple manouvere to retain the inoculum within the cheek pouch. These workers used the latter technique to study candidal infection in the hamster cheek pouch after induction of epithelial hyperplasia by turpentine (in liquid paraffin) application. The disadvantages of the hamster cheek pouch are its low oxygen tension and the lack of a natural salivary flow, which only poorly mimic the oral milieu. Hence, this model, for all intents and purposes is rarely used (118).

In the next section we sequentially review in detail the reports in the English literature on experimental oral *Candida* infections conducted in five different animal models, namely, monkey, rat (Wistar and SD), mouse, and hamster. Further experimental details of these studies are tabulated in chronological order in Table 1 for ease of reference.

ORAL CANDIDIASIS IN ANIMAL MODELS

Monkey Model

Denture stomatitis is the most common condition which affects the palatal mucosa of denture wearers, about 69% of whom are infected (29). The main etiologic agent responsible for the condition is *Candida*, which proliferates at the interface between the denture (almost always the upper prosthesis) and the mucosa (43). Budtz-Jørgensen (26), in an elegant series of studies using the monkey model, demonstrated that the palatal inflammatory changes observed in these animals resembled those of human denture wearers.

Early experiments were conducted by inoculating *C. albicans* under custom-made acrylic plates fitted to the palatal surface of monkeys (26). Candidal inoculation of control groups of monkeys without an acrylic plate, or those fitted with an uninoculated plate, did not reveal clinical or histologic changes in the palatal epithelium. However, when *C. albicans* was inoculated under the acrylic appliance, acanthosis and hyperplasia of the epithelium, together with a cellular infiltrate of the lamina propria, were noted. A diffuse erythema confined to the mucosa in contact with the acrylic plate was also observed, reminiscent of *Candida*-associated denture stomatitis (26, 30). Although all animals demonstrated *Candida* carriage, the yeast load on the palatal mucosa was not quantified by these workers. The authors also observed that (i) it was essential to cover the mucosa to produce the experimental infection and (ii) topical treatment with tetracycline enhanced candidal proliferation and the severity of the infection. The primary inflammatory lesion showed spontaneous healing 2 to 3 weeks after infection, clinically and histologically.

Interestingly, repeat inoculation leading to reinfection of the healed mucosa resulted in a more intense erythema in comparison with the primary lesion. These primary and secondary inflammatory responses appeared to indicate that delayed hypersensitivity may be involved in *Candida* infections of the oral mucosa, although too few animals were investigated to give statistically valid information.

In a subsequent study, the same author showed that the natural healing process after candidal infection can be temporarily suppressed by systemic immunosuppressive therapy with azathioprine (27). Experimental *Candida* infection of the palatal mucosa was induced in 14 adult *M. irus* monkeys by inoculating *C. albicans* (serotype A) under acrylic plates. Seven animals were given azathioprine, and the remainder acted as controls. The control animals demonstrated an atrophic and erythematous epithelium which resolved within 2 to 3 weeks. Cellular hypersensitivity to *C. albicans* was measured by an in vitro leukocyte migration test (27). In the normal animals, the migration inhibition was significant from 1 week to 5 months after infection. Cellular hypersensitivity developed concomitantly with clearing of the infection, while antibody was not yet detectable as assessed by an agglutination reaction. On the other hand, in azathioprine-treated animals, the infection persisted and cellular hypersensitivity did not develop until 1 to 3 weeks after the drug treatment was discontinued. The antibody titer also rose consistently after 4 weeks, reaching a maximum at 8 months. In immunosuppressed monkeys, a depressed migration inhibition reaction, together with a delayed cellular immune response (up to 2 to 3 weeks), was seen; the humoral immune response was early and of shorter duration. These monkeys also developed thrush-like lesions on the palatal mucosa and a mild inflammatory response in the lamina propria; *Candida* hyphae were visible in the stratum corneum, as in human lesions. This study demonstrated that cellular hypersensitivity to *Candida* plays a critical role in host resistance to experimentally induced candidiasis.

Oral thrush is relatively common in those using steroid inhalers for asthma and other allergic conditions. To investigate this condition, Budtz-Jørgensen (28) used monkeys injected with the corticosteroid triamcinolone acetonide. Of 13 monkeys in this study, 6 were injected with the steroid triamcinolone acetonide intramuscularly 2 weeks before and 2 weeks after inoculation. In the control group, acute atrophic candidiasis without hyphal invasion was noted and healed within a period of 2 to 3 weeks, while in the steroid-treated group, thrush was seen in all animals together with hyphal invasion of the ortho- and parakeratinized epithelium. A depressed inflammatory response also persisted in 50% of the animals in the steroid group for 5 to 6 weeks until the plates were removed. The inflammatory response was marked under the area covered by the acrylic plate. These studies confirmed that the local environmental conditions such as restriction of salivary flow due to the acrylic prosthesis, as well as the systemic immunity, are important in the initiation and aggravation of oral *Candida* infections.

Another group of workers used the same model but a different monkey species, *Cercopithecus aethiops*, to induce thrush using maxillary acrylic plates and inoculations of *C. albicans* (133). They investigated the effect of a reduced salivary flow induced by systemic oxyphenyclimine chloride on pseudomembranous lesions under a maxillary plate. They reported that monkeys with reduced salivary flow developed larger lesions while reaffirming that the acrylic plate is a prerequisite for initiation of oral candidiasis.

To conclude, due to reasons such as the purchase and maintenance cost cited above, the monkey model has fallen into disfavor. Nonetheless, the pioneering work of Budtz-Jørgensen (26–28) and Olsen and Haanaes (133) using this model was instrumental in defining the basic pathological processes involved, especially in *Candida*-associated denture stomatitis. It is noteworthy that the monkey model served as the “gold standard” for subsequent animal models—namely the rat, the mouse, and the hamster.

Wistar Rat Model

At almost the same time as the experimental studies with the monkey model were being conducted in Scandinavia, Jones and Adams (78) were investigating an alternative, the rat model, in the United Kingdom. These workers found that the Wistar rat was a simple and less expensive alternative to the monkey model for experimental oral fungal infections. In their first investigation, which was done with 26 Wistar rats and lasted 10 days, Jones and Adams (78) demonstrated asymptomatic colonization of the mouths of all the animals and histologic evidence of candidiasis in some 50% of the rats orally inoculated with *C. albicans*. To demonstrate asymptomatic colonization, they sampled the oral cavities of the rats

with sterile paper points (1 cm in length), which were then incubated in Sabouraud's broth for 24 h at 37°C to check for candidal growth. Histologically, infection occurred on the dorsal lingual surface, the buccal mucosae, and the free and attached gingivae (Fig. 1). Both the histology and the clinical appearance of the lesions closely resembled acute oral candidiasis in humans.

The authors subsequently extended these experiments to demonstrate the effect of xerostomia on oral candidiasis by desalivating the rats with hyoscine hydrobromide (1). A total of 42 Wistar rats were subjected to similar experimental conditions as before but for an extended period of 6 weeks. To determine oral candidal infection, one rat from each of the six groups was sacrificed weekly up to 6 weeks. The decapitated heads were fixed in 10% formol saline and decalcified, and sections were prepared for hematoxylin and eosin and periodic acid-Schiff staining, which showed evidence of candidiasis in 5 of 30 rats inoculated with *C. albicans*. The investigators observed epithelial abnormalities such as parakeratosis and thickening of the stratum corneum in lesional tissues, indicating that the model faithfully mimics the chronic hyperplastic variant of candidal infection.

Subsequently, Olsen and Bondevik (132) used the Wistar rat as an alternative model to study *Candida*-associated denture stomatitis. They used 38 Wistar rats in two experiments, each with an observation period of 2 weeks. The rats in the control and test groups were fitted with uninoculated or *Candida*-inoculated acrylic plates, respectively. After 1 week, a generalized simple palatal inflammation similar to that of humans was seen in the test group, and its histopathology resembled that of palatal inflammatory lesions in humans.

A similar but more extensive study, conducted by Shakir et al. (179) using 77 male albino Wistar rats, lasted for 6 weeks, in comparison to the 2-week observation period of Olsen and Bondevik (132). The results were similar since they observed that both an acrylic appliance and *C. albicans* inoculation were prerequisites for inducing palatal inflammation. The epithelial changes intensified with the duration of the experimental period, and after 6 weeks focal areas of the palatal mucosa were atrophic and markedly hyperplastic with hyphal penetration, resembling the later stages of *Candida*-associated denture stomatitis (Newton's type III) seen in humans. This study also helped dispel the theory that trauma alone from ill-fitting dentures can induce palatal inflammation since the presence of *C. albicans* was essential to the induction of inflammatory changes. Using a similar experimental design, Shakir et al. (179) further observed that *C. albicans* serotype A is more pathogenic than serotype B in inducing palatal candidiasis. Also, a single strain each of *C. tropicalis* and *C. glabrata* failed to induce pathologic changes (180), implying a hierarchy of virulence in *Candida* species. Although this simple experiment is indicative of the relative pathogenicity of *Candida* species, more comprehensive animal studies to illustrate this phenomenon are needed.

In another investigation with the Wistar rat, the same group observed that after inducing palatal candidiasis (with *C. albicans* CA 3091 serotype A) by using an acrylic appliance, removal of the appliance resulted in complete resolution of the lesion, although *Candida* still persisted as a commensal for up to 2 weeks (181). Nonetheless, the organisms transformed into the pathogenic form when the appliance was refitted without further inoculation. Microbiological sampling was conducted by swabbing the palatal mucosa immediately after killing and observing the resultant growth on Sabouraud's agar. To confirm whether the recovered yeasts were *C. albicans* 3091 serotype A, the isolated colonies were serotyped. This experiment, which parallels the clinical experience in denture wearers, confirms the critical role of the denture in initiating *Candida*-associated denture stomatitis and the importance of good denture hygiene in the management of the disease.

The association between filament formation in yeasts and oral candidiasis is still unclear. The superior virulence of both forms of *Candida*, i.e., *Candida* blastospores and hyphal forms (129), in human tissue has been reported. Germ tube formation, which precedes hyphal growth in *C. albicans*, is generally associated with increased adherence to epithelial cells (89) and resistance to phagocytosis by virtue of their large physical dimensions. Some studies also suggest that the hyphal structures are better than the individual yeast cells of *Candida* at gaining a foothold during the primary invasion process of the host (111, 184). These views have generally led to the belief that germ tube and hyphal formation in *C. albicans* accentuates disease induction in humans (127). To investigate this phenomenon, Martin et al. (111)

compared the pathogenic potential of two germ tube-negative strains and a single germ tube-positive strain of *C. albicans*. When inoculated into three groups of rats fitted with an appliance covering the palatal mucosa, the germ tube-negative strains (MS997 and XTM2) did not produce palatal histologic changes; no changes were observed in rats not fitted with an appliance. In contrast, the germ tube-positive strain (*C. albicans* 3091 serotype A) elicited a chronic inflammatory response together with hyphal invasion and epithelial hyperplasia. Nonetheless, in the absence of an appliance, no pathologic changes were noted. These results reinforced the contention that hyphal formation or filamentation is an important pathogenic attribute of *Candida* species ([42](#), [184](#), [209](#)).

Candida species have a predilection for specific anatomical sites of the oral cavity. They commonly reside on both the nonkeratinized and keratinized oral mucosae of humans, particularly the lingual dorsum and the buccolingual surfaces, while gingivae are not normally favored. The oral colonization profile of *Candida* was determined by Fisker et al., following short-term oral inoculation of *C. albicans* in Wistar rats on a tetracycline-laced diet ([54](#)). This experiment revealed preferential *C. albicans* colonization of four main areas of the oral mucosa. Almost 98.8% of infective foci evidenced by pseudohyphal penetration of mucosal epithelium were found on the buccal mucosa, the buccal and lingual sulci, and the crest of the molar gingivae, and in the interpapillary areas of the dorsum of the tongue (Fig. [1](#)). The remaining foci (1.2%) were in the mucosa of the hard palate and the attached gingivae. Associated ultrastructural studies clearly revealed that the lingual surfaces which show preferential yeast colonization, particularly the interpapillary areas, were characterized by an uneven irregular epithelium with a loosely structured stratum corneum ([54](#)). The investigators surmised that the loss of cell cohesion and the abundant intercellular clefts between keratinized cells having a microplicated surface facilitated the colonization and initiation of hyphal penetration ([140](#)). The contention that the profile of oral candidal colonization and hyphal penetration is related to the degree of keratinization and/ or surface morphology of the mucosa was well supported by these findings.

The Wistar rat palatal candidiasis model has also been used to evaluate the therapeutic efficacy of topical oral antiseptics and antifungals used to treat this condition. Lamb and Martin ([97](#)) incorporated chlorhexidine acetate into an autopolymerizing resin appliance at a sufficient concentration to prevent palatal candidiasis in the Wistar rat and proposed that the effect was due to the slow release of the antiseptic. Similarly, Norris et al. ([126](#)) examined the therapeutic efficacy of the azole antifungal miconazole incorporated into autopolymerizing acrylic resin and observed that palatal candidiasis could be prevented by fitting Wistar rats with appliances supplemented with 10% (wt/wt) miconazole in acrylic polymer powder. The appliances were well tolerated, since the rats remained healthy during the experimental period, and indeed the rats wearing drug-laced appliances gained weight more rapidly than did their drug-free counterparts. In contrast, in the investigation with chlorhexidine acetate, the test animals lost weight, probably due to the adverse effect of chlorhexidine ([97](#)).

The efficacy of imidazole and triazole antifungals (ketoconazole and fluconazole) in denture stomatitis has also been studied in Wistar rats using palatal acrylic appliances inoculated with *C. albicans* ([108](#)). The authors observed that a ketoconazole dose of 7.0 mg/kg of body weight⁻¹ and a fluconazole dose of 0.75 to 1.0 mg/kg of body weight⁻¹ for 14 days was necessary to prevent the recrudescence of palatal candidiasis. Although human trials of drug-laced palatal appliances have not been conducted to our knowledge, some workers have used this principle and incorporated antifungal agents into denture-lining materials with some degree of clinical success in *Candida*-associated denture stomatitis patients ([50](#)).

The oral mucosa serves as a rugged, impenetrable barrier against a multitude of physiological and pathological insults. This primary host defense mechanism is highly effective due to the prolific and incessant epithelial cell turnover, and it has been postulated that this activity may accelerate under slowly progressing chronic disease conditions. However, histologic investigations of *Candida*-associated denture stomatitis patients have revealed that the mitotic activity of the palatal epithelium is similar to that of the healthy palatal mucosa ([198](#), [203](#)). Nonetheless, experiments by Shakir et al. ([182](#)) with Wistar rats indicate that *Candida* infection results in a significant increase in the mean numbers of mitotic figures per unit length of basement membrane in the palatal epithelium of the inoculated animals fitted with appliances. Since this increased epithelial proliferation and desquamation could be considered a

protective measure that wards off systemic fungal invasion, Van Mens et al. (198) have suggested that hyperplastic lesions in *Candida*-associated denture stomatitis are defense mechanisms of the host. Indeed, the exuberant granulomas of chronic mucocutaneous candidiasis and similar syndromes could be considered an extreme evasive reaction of the body to fungal invasion (129).

Since the presence of an oral prosthesis traumatizes the palatal epithelium (19, 29), some workers have conducted Wistar rat studies to investigate the effect of candidal infection on the barrier properties and permeability of the palatal epithelium (110). They observed that in the healthy rat, the palatal epithelial barrier was impermeable to the passage of lanthanum, whereas in the presence of candidal infection, the permeability barrier was selectively operational, with a predominant leakage of low-molecular-weight proteins and selective permeability of macromolecules. Furthermore, an electron-dense material was noted throughout the subepithelial tissue. Removal of the prosthesis resulted in healing of the epithelium and a reversal of the barrier properties to its original state, implying that permeability changes are intimately associated with palatal inflammation in *Candida*-associated denture stomatitis. The pathological effects, if any, of the loss of permeability of the diseased human palatal epithelium are unknown.

As stated above, diabetes mellitus is a common disease that predisposes to oral candidiasis (160). Dourov and Coremans-Pelseneer (51) conducted experimental studies with streptozotocin-treated diabetic rats to investigate the oral candidal carriage and histopathology induced over a 40-week experimental period. The oral flora was quantified before and after inoculation. Tongue swabs were taken and cultured on Sabouraud's agar for candidal growth. Results were scored according to the yield of CFU. Diabetic rats given a single lingual inoculation of *C. albicans* remained positive for the yeast throughout the 40 weeks, in contrast to three other control groups, namely, nondiabetic rats inoculated with *C. albicans*, normal rats, and diabetic rats without *C. albicans* inoculation. Moreover, these controls were devoid of the histologic changes seen in the test group that were consistent with long-term mycotic lesions of the lingual mucosa, such as loss of filiform papillae, parakeratosis, irregular thickening, and a diffuse lymphocytic infiltration of the deeper layers of the epithelium. Although this model appears useful for investigating diabetes-induced oral candidiasis, no other researcher to our knowledge has exploited in full the etiopathology of this condition using the Wistar rat.

Pathologic changes in salivary glands due to diseases such as Sjögren's syndrome, cytotoxic therapy, and irradiation may lead to reduced salivary flow and xerostomia (102, 103, 109, 162, 191). Oral candidiasis is a common manifestation of xerostomia, which also promotes chronic candidal colonization (101). The relationship between xerostomia and oral candidiasis was investigated in the monkey model (133), as described earlier in this review. Jorge et al. (83) used Wistar rats to further study this phenomenon. They rendered 20 Wistar rats xerostomic by surgical removal of the major salivary glands (parotid, sublingual, and submandibular) and orally inoculated them with *C. albicans* three times a week for 32 weeks. When the rats were sacrificed and examined, candidiasis and hyphal infiltration of the lingual mucosa were found in 70% of the sialoadenectomized animals compared with 20% of the controls, confirming the critical importance of saliva and salivary flow in preventing oral candidiasis.

The vast majority of workers to date have resorted to the unnatural use of antimicrobials to eradicate the antagonistic population pressure of the commensal oral flora and thus initiate oral candidal colonization (Table 1). Since the broad-spectrum antibiotics used, such as tetracycline, adversely affect the immune response, a model that obviates the use of antimicrobials is a desirable alternative to mimic the clinical status. Jorge et al. (84) claim that the sialoadenectomized Wistar rat fits this requirement, since they noted 100% oral *Candida* carriage in xerostomic rats (after consecutive once-weekly oral inoculation for 5 weeks) compared with 50% carriage in controls. *Candida* was totally eradicated from the latter group within 18 weeks, whereas 66.6% of sialoadenectomized rats continued to harbor the yeasts. This model therefore appears suitable for the investigation of oral candidiasis since it maintains the normal oral flora with its competitive, colonization pressure akin to the clinical conditions in humans. However, since no researcher thus far has substantiated the claims of these authors, further studies with the sialoadenectomized Wistar rat models are urgently warranted.

Sprague-Dawley Rat Model

As can be seen above, the Wistar rat model has been used by a number of workers to induce experimental oral candidiasis. However another species, the SD rat, has been more extensively used by others and appears to be the most popular model by far for the study of mucosal candidiasis (2–5). Jones and Russell, who pioneered *Candida* studies with the SD model, demonstrated that animals that succumb to infection show histologic changes similar to chronic candidiasis of the posterior dorsum of the human tongue (80). Furthermore, they have also shown ultrastructurally that *C. albicans* changes into the mycelial phase and penetrates the cornified layer of the rat lingual epithelium as in humans (81). The following studies with the SD model have contributed significantly to our understanding of the host-fungus interactions in oral candidiasis.

In early investigations, Bowen and Cornick (25) demonstrated that a carbohydrate-rich diet (CRD) positively encourages the oral carriage of *C. albicans* in SD rats. A number of workers have confirmed this finding using both in vitro and in vivo studies and have elucidated the role of dietary carbohydrates in the pathogenesis of oral candidiasis (66, 91, 154, 165). Therefore, Russell and Jones (154) tested the effect of a CRD containing 42% powdered icing sugar and 30% starch against the “standard 3/8” mouse and rat diet on the oral carriage of *C. albicans*. In experiments with both the mycelial- and yeast-phase *C. albicans*, the organism was recovered more frequently from the CRD-fed animals than from the controls on a normal diet. It was also observed that oral infection, noted as mycelial penetration of the superficial epithelial layers, was more frequent in CRD-fed animals.

Armed with this information and the effect of broad-spectrum antibiotics on the genesis of candidal infection, Russell and Jones (155) further studied the effect of both tetracycline-laced drinking water and a CRD on disease progression. The persistence of *Candida* in the mouth of each rat was examined by swabbing the tongue and mucosal surfaces throughout the experimental period. The oral yeast carriage was monitored semiquantitatively by counting the number of oral swabs positive for *C. albicans* (and not by quantifying the yeast growth). Tetracycline administration resulted in oral persistence of *C. albicans* in all rats over a period of 24 days. The prolonged carriage induced by a tetracycline-laced diet was far superior to that achieved by feeding a CRD alone. Furthermore, increased frequency and severity of lingual infection were seen in tetracycline-fed rats compared with the controls. For instance, in contrast to controls, the tetracycline-fed rats sacrificed on day 13 demonstrated lingual, gingival, and buccal mucosal infections. The posterior of the oral cavity was affected more than the anterior (Fig. 2), and candidal infection was seen with pseudomembranes, mimicking human oral candidiasis. Histologically, mycelia penetrated the orthokeratotic epithelium, which also demonstrated inter- and intracellular edema, and there was a marked inflammatory cell infiltration of the corium. A concomitant increase in the severity of infection was also seen with prolongation of the experiment.

The onset of thrush in neonates is usually seen 4 days after birth (129), and the predisposing conditions are thought to be immature immune defenses, antibiotic therapy, maternal cross-infection, and cross-infection from nursery staff (166). Jones and Russell (79) explored the importance of these host factors, including infancy, leading to the transition of *C. albicans* from saprophytism to parasitism. When infant (12-day-old) SD rats were inoculated with the yeast form of *C. albicans* (without tetracycline and a CRD), they were unable to demonstrate either candidal carriage or infection. However, inoculations of the mycelial form produced histologically demonstrable infection after 15 days. This study suggests that infancy per se may not be a predisposing factor in the initiation of candidal infection and reaffirmed the generally held belief that the mycelial form of *C. albicans* is more pathogenic than the yeast form. Since the authors did not include a control adult group of rats in this study, the question of high oral carriage of *Candida* due to infancy per se remains unresolved.

After these preliminary experiments, Russell and Jones (156) studied the effect of prolonged candidal inoculation and tetracycline treatment on murine oral infection. This experiment is perhaps the most extensive animal study to date, conducted over a period of 12 months with 60 rats to identify oral histologic changes that were wholly due to candidal infection. The oral carriage of *C. albicans* after fortnightly inoculation and recorded at 1-, 3-, 6-, 9-, and 12-month intervals was 58.6, 48.3, 38.3, 40.0, and 45.0% respectively. These results were rather disappointing since the animals were inoculated regularly. Nonetheless, the authors recorded in detail the lingual histologic changes and, after 21 weeks,

observed a loss of papillae together with flat-surfaced hyper- or parakeratotic stratified squamous epithelium. Changes in the deeper layers included a mononuclear cell infiltrate of the corium, degenerative changes of superficial muscle cells with a giant cell reaction, and sarcolemmal proliferation and perivascular inflammatory infiltrate in deep muscle layers. These observations tended to suggest that the candidal infestation, though restricted to the superficial cornified epithelium, may also produce pronounced histologic changes in the deeper corium and the underlying muscle. This was the first observation in an animal model that *Candida* may elicit pathologic effects in subjacent tissues in addition to the immediate vicinity of hyphal infiltration. Other studies have now confirmed that candidal extracellular enzymes, such as secreted aspartyl proteinases and phospholipases, may account for such effects (21, 144).

Since the administration of tetracycline encourages the oral carriage of *C. albicans* in SD rats, it was postulated that a germ-free gnotobiotic would be ideal for the study of oral candidiasis. To test this hypothesis, Jones et al. (82) compared the oral carriage of *C. albicans* in germ-free and conventional (specific-pathogen-free) SD rats with and without tetracycline treatment. The mouth and the rectum of the rats were swabbed and the swabs were cultured for *Candida* in Sabouraud's agar at the beginning of the experiment, before inoculation, and at regular intervals afterwards, and the number of positive swabs was recorded. The authors observed that the oral cavity of all germ-free animals, whether treated with tetracycline or not, remained colonized with *C. albicans* until the end of the experimental period. In contrast, only 50% of the tetracycline-free, as opposed to 85% of the tetracycline-treated, conventional rats harbored *C. albicans*, reconfirming that the antibiotic does favor oral yeast carriage ($P < 0.05$). Infection was clearly evident in both the germ-free and conventional rats as mycelial penetration of the cornified epithelium, particularly the dorsal lingual surface.

Further experiments by Jones et al. (82) reconfirmed that (i) germ-free animals can remain colonized for up to 19 weeks with or without receiving tetracycline and (ii) colonization in conventional rats receiving tetracycline is longer lasting than in those without the antibiotic ($P < 0.01$). Interestingly, they found no evidence of oral infection, as opposed to superficial infestation, in any of the conventional rats whereas they saw histologic evidence of infection in gnotobiotics. Contradictory findings on infectivity have been reported by others using conventional rats (157). The latter group tested the effect of different schedules of tetracycline administration in two groups of SD rats (60 in each group) that were either maintained on tetracycline throughout the experimental period of 22 weeks or given the drug only during the first fortnight. All animals were inoculated with *C. albicans* orally on three alternate days in the second week. The results showed that initial administration of tetracycline fosters long-term oral candidal colonization with no significant difference in the incidence of infection.

Further studies by Hassan et al. (66) have shown that oral carriage of *C. albicans* of SD rats rapidly diminished when animals were fed a normal diet free of tetracycline and given only an initial inoculum of the yeast at the beginning of the experiment, compared with the following combinations (i) CRD, (ii) normal diet and tetracycline, and (iii) CRD and tetracycline. Significant differences in the recovery of *C. albicans* between the last three groups of rats were maintained irrespective of whether the inoculum was continuous or given only once at the beginning of the experiment.

To conclude, the foregoing studies indicate that a combination of tetracycline treatment and a CRD favor the oral carriage of *C. albicans* in SD rats regardless of whether an adequate inoculum of the challenge strain is administered once or on several occasions. It should, however, be noted that at least one group has found that tetracycline exposure is not prerequisite for oral *Candida* colonization provided that the SD rats are infected with a mucosally virulent strain of *C. albicans*. Allen et al. (5) studied the development of oral candidiasis in test and control groups of 20 SD rats each, receiving tetracycline-laced and drug-free water, respectively. The animals were all inoculated with a mucosally pathogenic strain of *C. albicans* that was noted to produce infection in 80% of the animals in an earlier study (141). There were no significant differences in *C. albicans* carriage rate in the two groups, and after 20 weeks grossly visible lesions were seen in 50 to 55% of both the test and control groups. Nonetheless, the lesions in the tetracycline-treated group were significantly larger than those in the controls ($P < 0.05$). These results suggested that the establishment of yeast infection is not necessarily dictated by antibiotic

exposure. However, the degree and severity of infection are likely to be related to the synergistic effect of tetracycline and the virulence of the infecting strain.

Van Wyk et al. (199) also investigated the possibility of using germ-free SD rats as a model for oral candidiasis. They observed that the daily inoculation (10^6 CFU) of *C. albicans* in drinking water for 14 days was adequate to infect the oral epithelium of the gnotobiotics sufficiently to produce epithelial changes such as acanthosis, loss of papillae, and a chronic inflammatory infiltrate of the lamina propria. In a second experiment, they investigated the chronological events leading to oral candidiasis in germ-free animals supplied with *C. albicans*-laced drinking water, over a period of 36 days. Invading yeasts were seen in the superficial lingual, palatal, and cheek epithelium within 72 h but were scanty after day 8. The resulting lesions were pronounced from 72 h to 6 days and resolved after day 15. This implied that host immunity to the invading pathogen was the major force in eliminating the infection in the SD model. They also proposed that the genetic differences among the rats may result in variant oral lesions and that genetically homogeneous inbred animals should be used to reproduce similar lesions.

An animal model that resembles human oral candidiasis is of value not only for studying the pathogenesis of the disease and the virulence of the organism but also for evaluating new antifungals which are introduced from time to time. The SD rat model has therefore been evaluated for antifungal drug testing by a few workers. Walrath and Blozis (J. Dent. Res. Spec. Issue, abstr. 975, 1986) produced clearly visible hyperkeratotic tongue lesions in female SD rats using tetracycline-laced drinking water and oral inoculation of *C. albicans*. (The authors observed continuous oral carriage for up to 8 months and tongue lesions for 7 months.) The rats were then treated with food pellets laced with the antifungal clotrimazole, and the lingual lesions resolved within 1 week. Hence, the authors suggested that the SD rat model was a satisfactory *in vivo* tool to study the effect of antifungals in the management of chronic oral candidiasis.

A study was also designed by Allen et al. (8) to investigate the effect of ketoconazole on lingual candidal infection in SD rats. Two control and two test groups of animals were orally inoculated with a *C. albicans* isolate known to produce mucosal lesions. Several animals in the test and control groups developed lingual candidal lesions (9 of 20 and 8 of 20) respectively. All lesions in the test group of animals treated with ketoconazole resolved, while 2 of 9 animals in the untreated control group also showed spontaneous resolution of lesions. These findings confirmed that the observed leukoplakic lesions were indeed caused by the *Candida* inoculum and that the SD model was suitable for testing antifungal therapy. However, it should be borne in mind that natural resolution of the lesions is common with this model and that appropriate controls need to be used to obviate spurious results. Despite the availability of this satisfactory model, very few workers appeared to have ventured into studies of the efficacy of newer antifungals using the SD rat model.

There is a comprehensive body of data on the oral histopathology of *Candida* infection in SD rats, and these are discussed below. A number of workers have reported that most candidal lesions are concentrated on the posterior midline dorsum of the tongue (6). In studies by Allen et al. (6), hyphae were present in the parakeratotic layer, together with chronic inflammation of the underlying connective tissues. The authors speculated that the topography of the conical papilla region might favor the retention of yeasts in the interpapillary crevices and thus provide them with an increased opportunity to invade the epithelium. Hence, it would seem that the surface architecture of the mucosa plays a role in selective candidal colonization, a view that has been echoed by Fisker et al. (54) and Philipsen et al. (140). Further investigations were performed by Fisker et al. (55), who subjected SD rats to prolonged oral candidiasis to localize the infection foci and evaluate the mucosal response. Candidal infection confirmed by histologic examination was observed in 15 of 60 animals; the majority of the infective foci were localized in the buccal sulcular folds, the gingival margin, the cheek, and the interpapillary area of the tongue. These areas accounted for 92.2% of the infective foci, and the remainder were in densely keratinized attached gingivae and palatal epithelium. A noteworthy observation was the apparent similarity of the tongue lesions and the histologic features of human median rhomboid glossitis (40, 207). The mucosal response in median rhomboid glossitis comprises an inflammatory reaction without degenerative changes in the subepithelial tissues.

Other histopathologic features of experimental oral candidiasis in animal models that have been

documented thus far include increased epithelial mitotic activity, epithelial proliferation leading to hyperplasia (Fig. 3), and rapid desquamation of the oral mucosa. The factors causing enhanced epithelial proliferation are not clear, although they could involve either host immune mediators or enzymes or metabolites released by the organism. To determine the impact of the latter attributes of *Candida* on epithelial cell turnover, Reed et al. (145) injected yeast-free culture supernatants into the buccal epithelium of young adult SD rats and assessed the mitotic activity by using a metaphase arrest technique at 11 and 31 h. They observed a significant rise in mitotic activity 31 h after injection of a 5-h culture supernatant of *C. albicans*, indicating that extracellular products of the yeast may induce proliferation of the buccal epithelium. Although they postulated that a range of candidal products such as hydrolytic enzymes and cell wall polysaccharides may adversely affect the epithelial and connective tissue cell turnover, they have performed no further experiments to substantiate these assertions.

It has been stated that the different incidences of infection seen in different animal models, and even within the same model, could be due to strain variations and the related virulent attributes of *C. albicans*. Hence, studies have been conducted to elicit differences in pathogenic traits among *C. albicans* isolates (3). In one study, four groups of SD rats (with 10 animals per group) were orally inoculated weekly for 25 weeks with four disparate strains of *C. albicans*. Of these, oral candidal carriage of various degrees was seen in three groups while the fourth group was completely devoid of infection. Further, it was observed that some strains exhibited consistently high colony counts while others invariably produced low colony counts. In addition, histologic evidence of infection was observed only in two of the three groups exhibiting candidal carriage and only 4 of 10 (40%) and 2 of 10 (20%) rats in each such group exhibited characteristic candidal lesions of the lingual mucosa. Allen and Beck (4) extended their experiments with 16 strains of *C. albicans* and an experimental period of 16 weeks without tetracycline supplements. They demonstrated significant variations in the oral recovery of *Candida*, ranging from 0 to 65%, and intraspecies differences in pathogenicity in terms of lingual infection, yielding results consistent with their earlier study (3).

Allen et al. (7) also observed that a single oral inoculation with a mucosally virulent strain of *C. albicans* without the help of any antibiotics or immunosuppressive agents was adequate to induce dorsal tongue lesions in SD rats. In a study of 210 animals sequentially killed over a 20-week period to follow up the clinical evolution of the lesions, they observed the most extensive epithelial changes, such as papillary atrophy and the destruction of dorsal lingual papillae, during the weeks 2 and 3 of infection (Fig. 4). Between 4 and 20 weeks, the percentage of animals with clinically evident lesions ranged from 10 to 30% although after week 18 all tongue lesions had been resolved. These observations and the histopathology concurred well with those seen in human mucosal lesions in candidal infections while reaffirming that *Candida* is an opportunistic pathogen easily overcome by innate body defenses. It should be noted that this is one of the few experiments described in the literature where the authors were able to induce infection without antibiotic or carbohydrate supplements in the food.

The same workers noted the distinct strain-related patterns of *C. albicans* infections on the dorsal lingual mucosa of immunocompetent rats (3, 4). They showed that while some isolates produced lesions particularly on the posterior-dorsal lingual mucosa accompanied by flattening of the normal papillary architecture of the epithelium, another group failed to produce any mucosal lesions. Allen et al. (9) further evaluated a lesion-inducing isolate and a nonpathogenic isolate by using both normal and cyclosporin-immunosuppressed rats. The lesion-inducing isolate showed a significantly increased rate of infection in normal as well as cyclosporin-treated rats compared with the nonpathogenic strains.

An association between oral candidiasis and iron deficiency has been documented by a number of investigators (34, 56, 159). The SD rat model has an added advantage for use in studies of this relationship, since there are several dietary methods for producing iron deficiency in these rats (10, 112). In one such study, Rennie et al. (147) demonstrated that iron deficiency may not necessarily predispose SD rats to oral candidiasis since some malnourished animals did not acquire the infection. Further, they observed a reduced capacity of anemic rats to recover from candidal infection. Similar results have been reported by Sofaer et al. (185) using anemic mice (see below).

The protective role of saliva in preventing oral candidiasis has been studied previously in the Wistar rat

model (83, 84). To further investigate this in SD models, hyposalivatory rats have been used (119). The latter authors conducted a series of studies and observed that all desalivated rats were susceptible to *C. albicans* infection and that the oral carriage in the infected animals was 30-fold greater than that in the normal control animals (i.e., 3.8×10^5 and 1.1×10^4 CFU, respectively) ($P < 0.05$). The importance of an intact salivary response in preventing *C. albicans* infection was clearly shown when transmission of infection from one desalivated animal to its counterpart occurred in 1.2 days while transfer from a normal donor to a recipient took 4.3 days. Interestingly, in the hyposalivatory model of oral candidiasis, pretreatment with tetracycline was unnecessary to initiate infection. The authors therefore concluded that the hyposalivatory-rat model is useful to assess the infectivity, pathogenesis, and virulence of different *Candida* strains in both qualitative and quantitative terms.

A relationship between *Candida* infection and mucosal trauma has been addressed by other workers (30, 131, 195). When the role of thermal trauma to the oral mucosa was investigated, it was noted that thermal ulceration facilitated candidal invasion of the dorsal lingual mucosa (131). These results also suggested that mild, long-term trauma due to chronic irritation from unstable dentures may contribute to the initiation or aggravation of *Candida*-associated denture stomatitis. The authors further postulated that the inflammatory exudate consequential to trauma might enhance the adhesion of the yeasts and thus facilitate infection.

One major host factor preventing fungal infections in general and oral candidiasis in particular is the cell-mediated limb of the immune system. The number of patients with immunological problems and hence susceptible to candidiasis is increasing in the community and includes those undergoing organ transplantation, those undergoing cancer therapy, and HIV-infected individuals (38). Recent experimental studies to investigate the impact of immunosuppression on mucosal candidiasis have been carried out by Samaranayake et al. (172), using both *C. albicans* and *C. krusei*. They noted that oral colonization by *C. albicans* was 12-fold greater than that of *C. krusei* prior to immunosuppression during an initial experimental period of 21 weeks with weekly inoculation of organisms. However, none of the animals succumbed to candidal infection. This was confirmed by histopathologic studies of a few selected animals within each group. However, when the animals were immunosuppressed with cyclophosphamide, the leukocyte counts of all the animals were significantly depressed and both *Candida* species produced histopathologic changes on the lingual mucosa characteristic of mucosal candidiasis (Fig. 5). *C. albicans* produced 100% infection in animals (three of three), while only 25 to 30% infection was observed with two different *C. krusei* isolates. Both species produced fungal hyphae that penetrated the lingual epithelium and stopped short of the prickle cell layer. However, the *C. albicans* hyphae penetrating the lingual mucosa were longer than *C. krusei* hyphae (17 and 8 μm , respectively) and tended to be relatively more profuse (Fig. 6). The results of this study substantiated that (i) immunosuppression facilitates candidal infection and (ii) *C. krusei* is capable of transformation into an invasive pathogen in the setting of immunosuppression. The latter finding is consistent with recent clinical epidemiologic data which indicate a reemergence of *C. krusei* infections among debilitated persons (168).

Mouse Model

One of the earliest investigations with the mouse model was a study by Sofaer et al. (185) to define the role of iron deficiency in oral candidiasis. They used the mouse mutant sex-linked anemia (sla) model and conducted two experiments with normal and anemic mice. In the first experiment, three groups of mice were tested: (i) untreated mice, (ii) mice receiving *Candida* by oral inoculation, and (iii) mice receiving *Candida* by oral inoculation together with tetracycline in the drinking water. In the second experiment, the effect of combined tetracycline and cortisone administration was tested, where one group of animals received hydrocortisone in the drinking water in addition to receiving *Candida* and tetracycline. At different stages of the experiments, *Candida* was recovered by oral swabs and immediately plated on malt agar for yeast growth. A standard technique was used throughout so that an approximate comparison of colony counts between different groups of animals could be made. In the first experiment, there was no significant difference in *Candida* isolation between normal and anemic mice and no histologic evidence of candidal infection. In the second experiment, all mice in the cortisone group yielded significantly higher *Candida* counts, indicating enhanced yeast colonization potentiated by hydrocortisone. Furthermore,

hydrocortisone- and tetracycline-treated mice showed increased histologic evidence of lingual infection.

Corticosteroids are commonly used for their anti-inflammatory and immunosuppressive properties. A major side effect associated with their use is oral and pharyngeal candidiasis (160). Apart from the foregoing study, others have used the mouse model to evaluate the effect of corticosteroids on oral candidiasis. Holbrook et al. (72) determined the colonization potential and infectivity of a pathogenic and a nonpathogenic strain of *C. albicans* in the mouse model. Two groups of 40 male mice (inbred strain CBA/CA) were given chlortetracycline and hydrocortisone; one of the groups was orally inoculated with a known virulent strain of *C. albicans*, and the other was inoculated with a known attenuated strain. The authors found significantly pronounced lingual colonization, disruption of keratin, and inflammatory response after administration of a virulent strain compared to an attenuated strain.

More recently, the effect of topical local corticosteroids was experimentally demonstrated in the mouse model by Deslauriers et al. (47). In this study, two groups of mice (34 and 41 mice per group) were inoculated with a *C. albicans* strain that established a low-level, long-term carrier state. Quantification of the longitudinal yeast carriage in the oral cavity was carried out from days 1 through 49 postinoculation, using Calgiswabs. The alginate tips were immersed in 2 ml of Ringer's citrate buffer, and the CFU were enumerated on selective agar medium. The carrier state was established in less than 10 days and persisted for at least 3 months at oscillating recovery levels. Subsequent administration of topical corticosteroids resulted in increased carriage of up to 40-fold higher than the controls; by day 21, this rose to 400-fold. The carrier state was restored to normal 10 to 15 days after cessation of drug administration.

Immunological investigations revealed three- to fourfold greater persistence of intraepithelial CD4⁺ T cells in infected animals than in control animals. However, in animals treated with the topical corticosteroid, these cells virtually disappeared from the epithelium. Within 24 h after cessation of treatment, CD4⁺ T cells were massively recruited (7 to 10 times the number seen in control carrier mice), first in the subepithelial capillary beds and then in the epithelium. The authors also noted that a significant reduction of the local CD4⁺ T cells level paralleled an increase in the oral carriage of *C. albicans*. This elegant study with an animal model clearly demonstrates the critical relationship between cellular immunity and oral candidiasis—a relationship all too frequent in HIV infection.

The mouse model has also been used to observe candidal colonization patterns and the inflammatory response in chronic recurrent candidal infection. When a group of mice was orally challenged with topical application of a *C. albicans* strain isolated from a patient with systemic candidiasis, the yeast population plateaued to a constant titer (approximately 300 ± 100 CFU per g of excised mucosal tissue) after 7 days postinoculation (95). However, this primary infection stimulated the cellular immunity, and a secondary topical challenge 30 to 43 days later failed to produce a mucosal reaction comparable to the first. The authors also observed a 10-fold difference between colony counts 24 h after the first and second inoculations. These results confirmed the findings of a number of previous studies (55, 82, 96) on the ability of mice and other animals to acquire resistance to the pathogen, which led to only a transient carrier state. This response in healthy animals has frustrated the attempts of many workers to produce a faithful model reproducing chronic oral candidiasis. Indeed, debilitation of the animal via artificial means (e.g., drugs and radiation) is essential to elicit such a response in normal inbred mice.

In another study, normal adult CD-1 mice were orally inoculated with *C. albicans* (10⁸ cells/ml in sterile phosphate-buffered saline) or by topical application. The yeast could be recovered from both the digested oral mucosa and saliva samples for up to a week. Although maximal colonization was noted at 48 h, the candidal infection greatly decreased after 2 to 3 days. Histologically, yeasts were seen attached to the oral epithelium 3 h after inoculation and hyphal penetration reached a maximum around 48 h. After 2 days of microbial challenge, a strong inflammatory reaction characterized by polymorphonuclear infiltrates was seen in the epithelium, together with parakeratosis. The infection involved a large area of the cheek mucosa and sometimes reached the muscle layer. However, the oral mucosa returned to normal after 7 to 13 days, indicating a self-limiting infection. The most important observation was that oral *Candida* colonization could be induced in normal adult mice without the aid of compromising agents, as previously shown by a few studies with Wistar and SD rat models (5, 82). Further, the microbiological and histopathologic data suggested that the mouse model is suitable to unravel the adaptive immune

responses of the oral immune system. However, it is disappointing that none of these models mimics the human oral carriage of *Candida*, where chronic carrier states persist in up to 40 to 50% of healthy adults. Thus, an animal model which resembles human oral candidal carriage is needed to further clarify the host-parasite interactions in oral candidiasis.

Since a defective cellular immune response is a precursor of mucosal candidiasis, Balish et al. (15, 16) conducted extensive investigations of this aspect of the disease using the mouse model. For this purpose, they used adult athymic and euthymic nude mice bearing *nu/nu* and *nu/+* genotypes, respectively. When *C. albicans* was given in the drinking water (10^5 cells/per ml), the yeasts colonized both groups of mice in large numbers with minimal hyphal invasion of the oral mucosa. Scanning electron microscopy revealed superficial yeast infestation of the ventral surface of the tongue and the cheek mucosa with no visible candidal infection. Therefore, these investigators concluded that both genotypes of the mice manifested resistance to extensive mucocutaneous candidiasis and that thymus-matured T cells may not be obligatory for resisting mucosal candidiasis in this particular murine model (15).

They extended this experiment with mice (*nu/nu* or *nu/+*) that were intraperitoneally injected with cyclophosphamide and noted extensive infection and hyphal penetration of the tongue and cheek mucosa within 5 to 7 days. Hence, it appears that *nu/nu* or *nu/+* mice that are artificially debilitated may serve as a model that can be manipulated to study the role of nutrition, endocrine function, and immunosuppression in mucosal candidiasis.

Balish et al. (16) also used athymic and euthymic gnotobiotic mice to investigate the immune response to *Candida* in the absence of colonization pressure by oral bacteria. In one study where adult mice were orally inoculated with *C. albicans*, such hyphal penetration of the dorsal lingual surface was seen after 14 days in both *nu/nu* and *nu/+* gnotobiotics and remained for period of 24 weeks in the *nu/nu* mice, while *nu/+* mice cleared candidal hyphae within 10 weeks. Furthermore, spleen cells from *nu/+* mice showed a positive in vitro lymphocyte proliferative response from 3 to 22 weeks after colonization and infection with *C. albicans* while *nu/nu* mice could not mount such a blastogenic response. This study with the gnotobiotic mouse model indicated that although *nu/nu* and *nu/+* mice are both apparently equally susceptible to oral candidiasis, the former cannot mount a lymphoproliferative response to *Candida*. This result is not surprising since *nu/nu* mice lack functional T cells. However, the authors clearly demonstrated that the *nu/nu* and *nu/+* murine models could serve to mimic mucosal candidiasis observed in individuals with defective T-cell function.

Mucosal candidiasis is also the commonest opportunistic infection in HIV-infected persons, and a decrease in the $CD4^+$ T-cell count is a hallmark of this disease (45, 59, 135). It is also known that patients with defective phagocytic function often become infected with *C. albicans* (90, 202). A mouse model that is claimed to exemplify both these conditions was introduced by Cantorna and Balish (31). This, the congenitally immunodeficient germfree mouse model (*bg/bg nu/nu* mouse), appears to be naturally susceptible to oral (hard palate and tongue) and esophageal candidiasis even without supplements of antibiotics, immunosuppressives, or cytotoxic drugs. Cantorna and Balish (31) investigated the ability of athymic (*nu/nu*), euthymic (*nu/+*), beige (*bg/bg*), black (*bg/+*), beige athymic (*bg/bg nu/nu*), and beige euthymic (*bg/bg nu/+*) germ-free mice to contract systemic as well as superficial candidiasis. Adult germ-free mice were orally inoculated by administering *C. albicans*-laced drinking water (10^5 yeasts per ml). On histopathologic testing, the hard palate and esophagus of the immunocompetent mice and the singly immunodeficient mice did not show signs of infection, although small numbers of *C. albicans* organisms colonizing the outer keratinized layers of the tongue were observed. However, yeasts and hyphae were seen in large numbers attached to and penetrating the keratinized portions of the tongue and hard palate of *bg/bg nu/nu* mice. Interestingly, colonization with *C. albicans* was accompanied by death in these doubly immunodeficient (*bg/bg nu/nu*) mice (22 of 73) within 4 weeks. The dead mice had macroscopic, plaquelike hyperkeratotic lesions on the dorsal lingual surface and the hard palate. As expected, little inflammation was observed in the infected tissues of *bg/bg nu/nu* mice which survived *C. albicans* colonization, and they had fewer yeast and hyphae on the palate, tongue, and esophageal surfaces as well as less hyperkeratosis. This shows that although *bg/bg nu/nu* mice were susceptible to mucosal candidiasis, a proportion were able to mount an inflammatory response, primarily of

polymorphonuclear leukocytic origin.

Cantorna and Balish (32) extended their immunological studies by using *bg/bg nu/nu* and *bg/bg nu/+* mice to evaluate the role of CD4⁺ T cells in mucosal candidiasis. When the mice were orally inoculated with *C. albicans* (B311 type A)-laced drinking water, *bg/bg nu/nu* mice were highly susceptible to oral (mainly lingual) and esophageal candidiasis during the first 4 weeks of the experiment, and this susceptibility diminished over a period of 20 weeks. In contrast, although the *bg/bg nu/+* mice showed oral infection during the first week, the yeast was cleared promptly, since no infected tissue was observed in mice sacrificed after 2 and 4 weeks into the experiment. This clearly indicated that the CD4⁺ T cells play a critical role in protecting against mucosal candidiasis.

SCID mice have provided valuable information on the roles of both the B and T cells in mucosal candidiasis (15, 16, 32). For this purpose, Balish et al. (17) used adult immunodeficient SCID mice that were orally inoculated with *C. albicans* (10⁵ cells/ml)-laced drinking water. Although the mice were chronically colonized with large populations of yeast cells (10⁶ to 10⁸/g), no oral mucosal lesions were apparent. Thus, in the absence of T- and B-cell function, SCID mice seem to compensate by using innate mechanisms such as phagocytic cell function to resist extensive mucosal candidiasis. Nevertheless, when SCID mice were given cyclophosphamide (100 mg per kg of body weight at 11 weeks after oral colonization with *C. albicans*), they showed more severe lingual candidiasis than did saline-injected controls. Thus, cyclophosphamide, which causes severe neutropenia and further impairment of innate immune mechanisms, enhanced the susceptibility of SCID mice to mucosal candidiasis. Future workers studying innate and acquired immunity in *C. albicans* infection could usefully employ the gnotobiotic SCID mouse model as a tool to explore immune events involved in oral candidiasis, especially those associated with immunosuppressive therapy.

It is known that the carrier state of *Candida* is associated with an intrinsic cellular immune response, as described above. To further demonstrate the association of different T-cell subsets and the histocompatibility complex (*H-2*) in *Candida* infection, Chakir et al. (37) conducted studies with DBA/2 and BALB/c mice. These two strains of mice with the same major histocompatibility complex haplotype (*H-2^d*) were inoculated by topical application of pelleted blastospores with *C. albicans* (LAM-1). Oral *Candida* populations were established in all mice postinoculation. Both DBA/2 and BALB/c mice demonstrated a peak of infection and a residual *Candida* population during the early period of the experiment. The infection was of shorter duration in BALB/c mice than in DBA/2 mice. Furthermore, in the latter, the infection was sustained, with a reproducible second peak of proliferation on day 5 postinoculation, after which the *Candida* counts dropped sharply (a 60-fold-lower residual level) but were present for at least 25 days. The comparative kinetics of the oral *Candida* infection in DBA/2 mice was significantly different from that in BALB/c mice on days 3, 4, 5, and 6 after yeast inoculation.

These workers also quantified *Candida* organisms in digested cheek, palatal and lingual mucosal tissues. Analysis of these data revealed that the DBA/2 and BALB/c patterns were significantly different from days 3 through 6 postinoculation. Furthermore, the cheek and palatal mucosal tissues of infected mice showed that a first peak of proliferation occurred on day 2 at this site whereas it occurred on day 3 on the tongue. A similar recruitment of CD4⁺ and CD8⁺ T cells and of MAC-1 cells in mucosal tissue of both strains of mice was noted during candidal infection. The carrier state of *Candida* was associated with the persistence of intraepithelial CD4⁺ T cells. However, there was a time-specific recruitment of $\gamma\delta$ T cells that coincided with a dramatic decrease in viable *Candida* organisms in the mucosal tissue, which occurs on day 3 in BALB/c mice and on day 6 in DBA/2 mice. Taken together, these results indicate that the two strains of mice sharing the same major histocompatibility complex (*H-2^d*) display different kinetics of *Candida* clearance and primary oral infection after topical application of a standardized inoculum. Further, it appears that the priming modalities of T cell subsets in the oral mucosa are not associated with the *H-2* complex in the mouse model.

Oral candidiasis is a criterion in most, if not all, staging systems for HIV infection (12, 34, 51). HIV infection leads slowly but inexorably to a loss of immune competence, the most striking feature of which is a depletion of CD4⁺ T cells (23, 45). Mice infected with the Du5H(G6T2) mixture of mouse leukemia viruses develop a syndrome, MAIDS, that has many of the immune abnormalities found in HIV infection.

Using this model, Deslauriers et al. (46) studied retrovirus-infected C57BL/6 mice and examined them for their ability to resist the development of oral candidiasis, as indicated by development of the carrier state after a self-limiting acute infection, and to clear a subsequent, secondary oral inoculum of *C. albicans*. After oral inoculation with *Candida*, the carrier state was established in both control and test mice in less than 10 days and remained stable at <100 CFU for more than 6 months in both the control and 70% of virus-infected animals. The carrier state fluctuated in the remainder (30%) of the retrovirus-infected mice especially from day 100 after virus inoculation, sometimes reaching acute-infection values and remaining at these high levels for 2- to 3-week periods interspersed by episodic transient returns to the carrier state, reminiscent of recurrent oral candidiasis in human HIV infection. The isolation frequencies of CD4⁺ and CD8⁺ lymphocytes were unchanged and significantly decreased ($P < 0.05$), respectively, in both the cervical lymph nodes and spleens of coinfecting mice compared with the *C. albicans*-carrying, virus-free, age-matched control animals. Furthermore, the secretion of gamma interferon by concanavalin A-stimulated spleen cells from retrovirus-infected mice was significantly decreased ($P < 0.05$) compared to that from virus-free mice, in parallel with known abnormalities associated with MAIDS. These data are consistent with a role for Th1 cells in host resistance to mucosal candidiasis but suggest that CD8⁺ and/or $\gamma\delta$ T cells, or NK cells, may also contribute either through the production of gamma interferon (15, 24, 55) or through the recently described direct antifungal activity of CD8⁺ cells against *C. albicans* (39).

Although not directly relevant to oral candidiasis, a model which demonstrates thrushlike candidal lesions has been described in artificial pneumatized cysts in mice (92). To produce these candidal lesions, subcutaneous cysts were formed by injecting 3 to 3.5 ml of air through a hypodermic needle into the subcutis of the back of a mouse. The cysts, which were lined by an epithelial cell layer of mesenchymal origin within a 5- to 7-day period, were challenged with 10^6 cells/ml of yeast suspension in saline. The mice were immunosuppressed with cyclosporin A, which selectively impairs T-cell immunity and NK-cell activity without affecting nonspecific phagocytic activity. Mice thus immunosuppressed developed distinct, macroscopic thrushlike lesions in the cysts within 4 to 6 days, while no lesions were visible in control animals. These experiments have not been substantiated, and it is doubtful whether they provide a useful alternative to the less cumbersome models described above.

The foregoing illustrates the multifaceted exploitation of the mouse model by various researchers to elucidate clinical, therapeutic, and immunological features of oral candidiasis. Taken together, the many variants of the mouse model appear especially suited to study the short-term yet complex humoral and cellular immune responses associated with the disease. The recently described MAIDS mouse model, in particular, is worthy of special mention since it offers promising scope for workers interested in the immunobiology of oral candidiasis in HIV infection.

Hamster Model

The cheek pouch of hamsters has been a favorite site for studies of oral carcinomas. Hence, a few investigators have experimented with this model to evaluate oral *Candida* infection but without much success. McMillan and Cowell (116) carried out an investigation by inoculating *Candida* into the cheek pouch of 64 adult hamsters. A single strain each of *C. albicans* and *C. tropicalis* were used. While only one-third of the animals treated with either organism showed pathologic lesions in the pouch mucosa, microabscesses reminiscent of candidal infection were found only in animals treated with *C. albicans*. Other histopathologic changes included inflamed epithelium with neutrophils, and a lymphocyte and macrophage infiltrate in the connective tissue. Epithelial thickening with increased thickness of the stratum corneum and random distributions of yeast and hyphae were also noted, with no hyphal invasion.

Chronic hyperplastic candidiasis is characterized by invasion of a thickened epithelium by candidal hyphae. However, it is not clear whether the hyperplasia is caused by *C. albicans* or whether the organism invades an already hyperplastic epithelium (146). To investigate this rather enigmatic phenomenon Franklin and Martin (58) induced epithelial hyperplasia in the hamster cheek pouch mucosa by application of 50% (vol/vol) turpentine in liquid paraffin. Afterward, the pouches were inoculated with *C. albicans* and the inoculum was retained by sutures. In six animals which satisfactorily retained *Candida* for up to 1 month, the investigators observed increased mitotic activity and both a hyperplastic and an atrophic epithelium. The pathologic features of the hyperplastic epithelium resembled human candidal

leukoplakia. These preliminary results indicate that *C. albicans* is a prime agonist in initiating hyperplastic epithelial changes associated with candidal leukoplakia.

This model was once again used by McMillan and Cowell (117) in the hope of clarifying the role of *C. albicans* in leukoplakia, dysplasia, and neoplasia. They inoculated the cheek pouch of 80 hamsters with *C. albicans* (either CA UOI or CA ATCC 10261) once a week for 9 months. During this prolonged period of inoculation, hamsters were sacrificed at monthly intervals and examined for abnormalities. Although the histopathologic changes were similar at all ages, the number of abnormal foci was greater in hamsters inoculated for a minimum of 6 months. These changes were said to be similar to those observed by the same investigators (over a 6-week period after a single inoculation of *C. albicans*) in the cheek pouch of adult hamsters (116). However, a heavy chronic inflammatory response was more extensive than in the latter short-term study. Since the majority of candidal organisms were in the yeast form, it was apparent that hyphal penetration into the epithelium was not essential for the induction of pathologic changes observed in chronic hyperplastic candidiasis. Since invasion of the epithelium is probably an important contributory factor for dysplasia, these studies should be followed up over a much longer period to determine if epithelial hyperplasia does indeed lead to oral malignancy.

CONCLUSIONS AND FUTURE DIRECTIONS

The diverse attributes of *Candida* species as opportunistic pathogens and the multiple oral diseases they cause are well recognized. Therefore, carefully designed animal studies are critical to provide new insights into the complex biological and pathologic behavior of *C. albicans*, as well as non-*albicans* species of *Candida*. Further, a number of *Candida* species are either emerging or reemerging as agents of increasing morbidity and mortality in compromised patient cohorts. This represents another important arena in which animal models can contribute to unraveling the pathogenic mechanisms of this ubiquitous opportunist.

A number of animal models have been described to study oral candidiasis over the past half a century or so. These include the monkey, rat, mouse, and hamster models and their variants. Most of these studies have been performed under different experimental conditions, as a result of which the outcomes are difficult to compare. Other variables that confound data interpretation include differences in the numbers of animals, the *Candida* strains used, and the duration of the studies. In the foregoing review, we have attempted to evaluate the animal models (that are described in the English language literature) in order to objectively redefine the criteria for their use.

Most animal studies of experimental oral candidiasis have been conducted following alteration or manipulation of the oral environment by administration of antibiotics, special diets, or mechanical trauma, exclusion of the protective effect of a normal salivary flow, or accumulation of unwanted metabolites under an artificially inserted appliance. Furthermore, germ-free gnotobiotics have been used by some workers, since it has been difficult to study the interactions between the artificially introduced exogenous yeasts and the host in the presence of the population pressure exerted by commensal bacteria. A case has also been made for the use of inbred animals since outbred animals are genetically heterogeneous.

On the pathogen level, it has been evident that different strains show different potentials to cause infection. Although this strain disparity in the pathogenic attributes of *Candida*, even within the same species, is well established, only a few investigators have attempted to use a reference or a uniform control strain of *Candida* between experiments to make the results from different centers or experiments globally comparable. With the advances in genomics, molecular typing methods have become the most precise method of identifying a yeast isolate. Therefore, the use of one or more genotyped reference *Candida* strains in future animal studies would greatly facilitate the comparison of global data from different groups of researchers or different experiments within the same research group.

Notwithstanding these drawbacks of the studies described above, the following could be deciphered from the available data. (i) The primate model appears ideal for experimental investigations of *Candida*-associated denture stomatitis, mainly due to the need for fabrication of a close-fitting acrylic appliance analogous to human denture prostheses. Both erythematous candidiasis and pseudomembranous

candidiasis have been produced in monkeys fitted with such acrylic plates. However, monkeys are difficult to obtain and expensive to maintain, and hence their use is highly resource sensitive. (ii) The rat model (both SD and Wistar) is the most well proven for observing clinical oral candidiasis in vivo, especially because of its relatively large oral compartment, ease of breeding and handling, and ready availability. Consequently, rats are the animals of choice for studying long-term candidal colonization and chronic infection. (iii) The mouse model and its variants with immune abnormalities are undoubtedly suited to evaluate humoral and cellular immune response in oral candidiasis. Many well-characterized variants of the mouse model with baseline information on their immunologic and genetic constitution are available, including gnotobiotics, athymic (*nu/nu*), euthymic (*nu/+*), beige (*bg/bg*), black (*bg/+*), beige athymic (*bg/bg nu/nu*), beige euthymic (*bg/bg nu/+*), and SCID mice, as well as the recently developed MAIDS model. Furthermore, mice are also widely available, easy to handle, and relatively inexpensive. Hence, it is not surprising that the mouse model has proved popular, especially for short-term studies of the humoral and cellular immune response in oral candidiasis. (iv) The hamster cheek pouch epithelium, although used as a model for oral infection, is not a good surrogate for the human oral mucosa due to the absence of a salivary component, the artificiality of tying off cheek pouches to initiate the lesion, and the unique oral anatomy.

A number of other general conclusions can be drawn from these studies. (i) In almost all models, the infection could be initiated with antibiotic and/ or dietary supplements. (ii) The yeasts preferentially colonize different sites of the oral cavity (dorsal surface of tongue, buccal mucosa, and gingival mucosa), but the mid-posterior lingual dorsum appears to be the most favored site of infestation, as in humans. (iii) There is inter- and intraspecies variations in the infectivity of *Candida* in animal models, although this area has been very poorly researched. (iv) The histopathologic changes of the mucosa are highly consistent with those of human lesions. Nonetheless, an ideal model which is relatively inexpensive and representative of the human oral environment in ecological and microbiological terms has yet to be described.

Our knowledge of the pathogenesis and management of oral candidiasis has been considerably expanded by the use of these various animal models. However, there is much more to be elucidated. For instance, the chronic recurrences of oral *Candida* infection in HIV-infected patients and the increasingly frequent emergence of *Candida* resistance to the newer antifungals pose new challenges, which may be resolved by animal experimentation. Unfortunately, the enthusiasm and interest shown in animal experiments in the 1970s and 1980s is gradually diminishing, with only a handful of investigations conducted during the last decade. Although the reasons for this may be obscure, the time-consuming and intrinsic difficulties associated with animal experimentation, which is not always rewarding, may have played a contributory role; the politics of vivisection may be another relevant factor. Notwithstanding these drawbacks, animal experiments play a definitive role in our understanding and the management of this all too common mucosal disease. Prudent choices of models to suit the investigational aims, as reviewed above, and appropriate standardization of experimental protocols to obtain broadly comparable and meaningful data, which need to be analyzed and interpreted cautiously, should be rewarding for researchers who venture into this arena.

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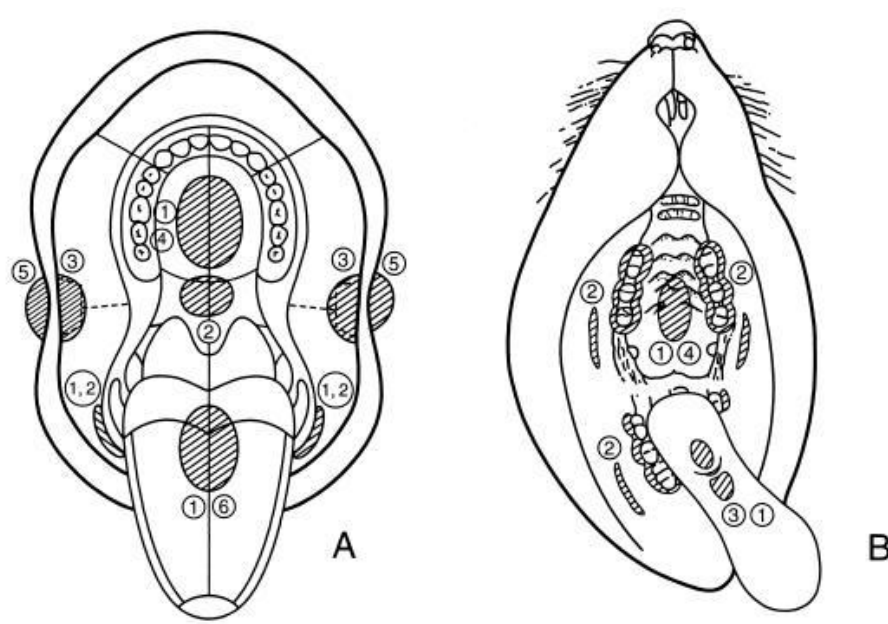
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FIGURES AND TABLES

FIG. 1

Simplified schematic diagram comparing the topographic distribution of common lesional sites in human oral candidiasis (A) and experimental candidal infection in an animal model (rat/mouse/hamster) (B). The most common sites of infection are shaded. The clinical variants of the disease and their preponderant sites are as follows: 1, erythematous candidiasis; 2, pseudomembranous candidiasis; 3, hyperplastic candidiasis; 4, *Candida*-associated denture stomatitis; 5, *Candida*-associated angular cheilitis; 6, *Candida*-associated median rhomboid glossitis.

TABLE 1

Experimental studies on oral candidiasis in animal models

Model, yr (reference)	Authors	No. of animals/age /sex/avg wt ^a	Diet ^a	Other conditions ^a	<i>Candida</i> strain, inoculum size ^a	Length of study	<i>Candida</i> carriage ^a
Monkey							
1971 (26)	Budtz- Jørgensen	6/adult/m and f/NR	NR	T onto acrylic plate weekly/biweekly	CA type A (Hasenclever)/SDA/48 h/37°C; inoculum, 100 mg (wet wt) of CA growth	12 wk	NR
1973 (27)	Budtz- Jørgensen	14/adult/m and f/NR	NR	Animals were treated with AZA	CA type A; inoculum: 100 mg (wet wt) of CA growth	8 mo	<i>Candida</i> blastospores and hyphae observed
1975 (28)	Budtz- Jørgensen	13/adult/m and f/2.5–5.4 kg	NR	Animals were treated with TRI	CA type A (Hasenclever)/SDA/48 h/37°C; inoculum: 100 mg (wet wt) of CA growth	5 mo	Control: mucosal smears showed <i>Candida</i> in small numbers; test: large numbers of <i>Candida</i> and hyphae were seen in mucosal smear;
1977 (133)	Olsen and Haanaes	10/NR/m and f/NR	CMF	OXY was given to suppress saliva flow	CA type A (Hasenclever)/SDA/48 h/37°C; inoculum: 100 mg (wet wt) of CA growth	14 wk	Control: few yeast were seen in palatal-smears; test: blastospores and hyphae were abundant

Model, yr (reference)	Authors	No. of animals/age /sex/avg wt ^a	Diet ^a	Other conditions ^a	<i>Candida</i> strain, inoculum size ^a	Length of study	<i>Candida</i> carriage ^a
Wistar rat							
1970 (78)	Jones and Adams	26/NR/m and f/350 g	Food and water ad libitum	HH injected	CA type A/SDA/48 h/37°C; inoculum: 0.25 ml of 10 ⁸ cells/ml in saline	10 days	CA found in the mouths of animal; hyoscine did not change the frequency of recovery of CA
1971 (1)	Adams and Jones	42/NR/NR/NR	NR		CA type A/SDA/48 h/37°C; inoculum: 0.25 ml of 10 ⁸ cells/ml in saline	6 wk	NR
1978 (132)	Olsen and Bondevik	38/NR/m/NR	SRP and T-water		CA from denture stomatitis patient/SDA/48 h/37°C; inoculum: 30 mg	2 wk	NR
1981 (179)	Shakir et al.	77/NR/m/350 g	Food and water ad libitum		CA 3091/ serotype A/SDA/36 h/30°C; inoculum: 30 mg (wet wt) of CA suspension	6 wk	NR
1982 (54)	Fisker et al.	50/5–8 mo/m and f/200–370 g	SRP and T-water		CA Hasenclever strain A/SDA/20 h/35°C; inoculum: 0.1 ml of 6 × 10 ⁸ cells in saline	6 wk	100% of swabs were +ve for <i>Candida</i> , wk 1; 33% Ca +ve, wk 5
1983 (180)	Shakir et al.	35/NR/NR/NR	Food and water ad libitum		CA (serotypes A and B), CT, CG	2 wk	NR
1983 (97)	Lamb and Martin	10/NR/m/350 g	Food and water ad libitum	Autopolymerizing acrylic plates used	CA 30911	8 wk	<i>Candida</i> was observed in animals fitted with acrylic plates unsupplemented

Model, yr (reference)	Authors	No. of animals/age /sex/avg wt ^a	Diet ^a	Other conditions ^a	<i>Candida</i> strain, inoculum size ^a	Length of study	<i>Candida</i> carriage ^a
							with chlorhexidine
1984 (111)	Martin et al.	24/NR/m/350 g	Food and water ad libitum		CA (GT +ve and -ve strains), CA 3091 serotype A/SDA/24 h/30°C; inoculum: 30 mg (wet wt) of CA suspension	2 wk	CA was recovered from all animals
1985 (126)	Norris et al.	Expt 1, 26/NR/m/324 g; expt 2, 37/NR/m/315 g	Food and water ad libitum	Autopolymerizing acrylic resin was used	CA 3091/SDA/24 h/37°C; inoculum: 30 mg (wet wt) of CA suspension	Expt 1, 4 wk; expt 2, 8 wk	Swabs taken from animals wearing MS-treated plates were -ve for CA
1986 (181)	Shakir et al.	20/NR/NR/NR	Food and water ad libitum		CA 3091/serotype A; inoculum: 30 mg (wet wt) of CA suspension	6 wk	CA was recovered from inoculated animals
1986 (182)	Shakir et al.	35/NR/m/NR	Food and water ad libitum	The rats were sequentially exposed to 12-h dark and 12-h light periods to stimulate diurnal variation	CA 3091/ serotype A; inoculum: 30 mg (wet wt) of CA suspension	4 wk	NR

Model, yr (reference)	Authors	No. of animals/age /sex/avg wt ^a	Diet ^a	Other conditions ^a	<i>Candida</i> strain, inoculum size ^a	Length of study	<i>Candida</i> carriage ^a
1987 (110)	Martin et al.	34/NR/m/NR; expt 1, 18; expt 2, 16	SERD and water ad libitum		CA NCPF 3091 serotype A/SDA/48 h/37°C; inoculum: 30 mg (wet wt) of CA suspension	Expt 1, 10 wk; expt 2, 4 wk	NR
1987 (51)	Dourov and Coremans- Pelseneer	48/NR/m and f/300 g		Diabetes mellitus was induced in rats	CA strain 4019; inoculum: 12×10^6 cells/ml in H ₂ O	10 mo	Candidal carriage was +ve for diabetic rats after mycotic infection
1989 (108)	Martin	224/NR /m/230–290 g	NR	FL and KE used	CA NCPF 3091/SDA/48 h/37°C; inoculum: 30 mg (wet wt) of CA suspension	42 days	<i>Candida</i> was recovered from ra treated with >7.0 mg of KE kg ⁻¹ and >0.5 mg of FL kg ⁻¹
1993 (83)	Jorge et al.	20/NR/m/170– 200 g		Major salivary glands surgically removed	CA/SDA/24 h/37°C; inoculum: 0.2 ml of 10^8 CFU/ml in saline	32 wk	<i>Candida</i> was observed in 40% c control animals and 100% of xerostomic anima
1993 (84)	Jorge et al.	12/NR/m/170– 200 g	Food and T-water ad libitum	T reduced to 0.001 mg/ml	CA isolated from patient with chronic oral candidiasis; inoculum: 0.2 ml of 10^8 CFU/ml in saline	18 wk	Numbers of CA isolates were significantly large in sialoadenectomize rats ($P < 0.05$)

Model, yr (reference)	Authors	No. of animals/age /sex/avg wt ^a	Diet ^a	Other conditions ^a	<i>Candida</i> strain, inoculum size ^a	Length of study	<i>Candida</i> carriage ^a	
Sprague- Dawley rat							than in normal controls	
	1973 (154)	Russell and Jones	Expt 1, 60; Expt 2, 20	CRD, SMP, and water	Mycelial and yeast forms were used	CA; inoculum: 0.1 ml of 6 × 10 ⁸ cells/ml in saline	Expt 1, 36 days; expt 2, 39 days	Expt 1: candidal carriage was greater in the group given CRD
	1973 (155)	Russell and Jones	60/NR/m and f/200g	CRD, SMP, and T-water		CA isolated from human carrier/SDA /35°C/48 h; inoculum: 0.1 ml of 6 × 10 ⁸ cells/ml in saline	34 days	NSD between normal and CRD (yeast/hyphal phase) CA cells
	1973 (79)	Jones and Russell	36/12 days/NR/NR/	NR	Mycelial and yeast forms were used	CA; inoculum: 0.1 ml of 10 ⁸ cells/ml in saline	15 days	Rats harbored the mycelial form when CA was inoculated
	1975 (156)	Russell and Jones	60/NR/m and f/NR	SRD and T-water		CA; inoculum: 0.1 ml of 6 × 10 ⁷ cells/ml in saline	12 mo	Much variation in candidal carriage; the rat seems to become adapted to the presence of the yeast
	1975 (157)	Russell et al.	120/NR/m and f/200 g	SRD and T-water		CA; inoculum: 0.1 ml of 5 × 10 ⁷ cells/ml in saline	22 wk	Initial short-term or long-term T treatment did not affect colonization by CA
	1976 (82)	Jones et al.	Expt 1, 80/NR/NR /80–100 g; Expt 2, 39/NR/NR/NR	Oxoid irradiated diet, vitamin K with or without T-water		CA; inoculum: 0.1 ml of 6 × 10 ⁸ cells/ml in saline	Expt 1, 9 wk; expt 2, 27 wk	Antibiotics and GI state favored oral candidal carriage
1982 (6)	Allen et al.	10/NR/f/200 g	SLC and T-water		CA/mycological agar; inoculum: 0.1 ml of 5 × 10 ⁸ cells/ml in saline	40 wk	Variation in candidal carriage	

Model, yr (reference)	Authors	No. of animals/age /sex/avg wt ^a	Diet ^a	Other conditions ^a	<i>Candida</i> strain, inoculum size ^a	Length of study	<i>Candida</i> carriage ^a
					10 ⁷ cells/ml in saline		was evident within rats in a particular week and in successive culture from a particular rat
1982 (55)	Fisker et al.	104/6 wk/m and f/120–150 g	SRP and T-water	SPF rats	CA-Hasenclever strain A; inoculum: 0.1 ml of 6 × 10 ⁸ cells/ml in saline	34 wk	50% of animals were +ve for <i>Candida</i> during 3 wk 25% of animal showed hyphal penetration
1983 (3)	Allen and Beck	50/NR/f/200 g	SLC and T-water		Four strains of CA from clinical lesions; inoculum: 0.1 ml of 5 × 10 ⁷ cells/ml in saline	25 wk	Three of the four CA strains were recovered on culture of swabs
1983 (147)	Rennie et al.	62/NR/m/NR	Normal rat diet and T	Fe-free vitamin-rich diet	CA MRL 3153/SDA/48 h/37°C; inoculum: 0.2 ml of 10 ⁷ –10 ⁸ CFU/ml in saline	14 wk	T was effective in reducing oral candidal carriage
1985 (66)	Hassan et al.	120/35 days/m and f/100–110 g	SRP and T-water; CRD and T-water		CA isolated from human carrier/SDA /35°C/48 h; inoculum: 0.1 ml of 6 × 10 ⁸ cells/ml in saline	14 wk	T and CRD enhanced candida carriage regardless of being inoculate once or on several occasions
1985 (5)	Allen et al.	40/NR/f/200 g	SLC; group 1, T-water; group 2, DDDH		CA/SDA/23°C/72 h; inoculum: 0.1 ml of 5 × 10 ⁷ cells/ml in saline	20 wk	No difference in candidal carriage between the 2 groups
1986 (abstract)	Walrath and Blozis	107/NR/f/NR	LRF and T-water	Food pellets soaked in an ethanol/ clotrimazole solution and	CA	8 mo	

Model, yr (reference)	Authors	No. of animals/age /sex/avg wt ^a	Diet ^a	Other conditions ^a	<i>Candida</i> strain, inoculum size ^a	Length of study	<i>Candida</i> carriage ^a
				dried			
1987 (4)	Allen and Beck	320/NR/f/150– 175 g	SLC and T-water	CA isolates were from different patients	16 isolates of CA/SDA /23°C/72 h; inoculum: 0.1 ml of 5×10^7 cells/ml in saline	16 wk	Variable recovery rates were noted for the 16 isolates
1987 (199)	Van Wyk et al.	46/ 5–8 wk/NR/NR			CA/BHI broth/37°C/96 h; inoculum: above suspension in 250 ml of drinking water (10^6 CFU/ml)	36 days	<i>Candida</i> organism appeared in the oral epithelium after 48 h and colonization became extensive after 3–7 days; yeasts were eliminated after 8 days
1988 (8)	Allen et al.	60/NR/f/200 g	SLC and water	Inoculated rats were treated with KE	CA; inoculum: 0.1 ml of 5×10^7 CFU/ml	27 wk	
1989 (7)	Allen et al.	210/NR/f/175 g	SLC and tap water	SEM was conducted	CA/SDA/25°C/48 h; inoculum: 0.1 ml of 3×10^8 yeasts/ml	20 wk	NR

Model, yr (reference)	Authors	No. of animals/age /sex/avg wt ^a	Diet ^a	Other conditions ^a	<i>Candida</i> strain, inoculum size ^a	Length of study	<i>Candida</i> carriage ^a
1990 (145)	Reed et al.	80/NR/m/150– 250 g	RRD	CA was grown in chemically defined medium	CA/SDA/37°C/24 h; inoculum: 5- and 23-h CA culture supernatants	31 h	NR
1990 (119)	Meitner et al.	Expt 1, 18/27 days/NR/NR; expt 2, 40/26 days/NR/NR; expt 3, 40/26 days/NR/NR	Diet 2000 (56% sucrose) and sucrose in water	PSG ligated; SM and SL glands surgically removed	CA strains 613 and 623-ml (a colony morphology mutant)	3–4 wk	CA carriage of HS were 30 fold more than normal rats
1993 (131)	O'Grady and Reade	63/NR/f/NR	SRD and T-water	Trauma was induced by applying heat on the tongue	CA/SDA; inoculum: 0.1 ml of 6×10^8 yeasts/ml	35 days	NR
1994 (9)	Allen et al.	79/NR/f/200 g	SLC and water	Cyclosporin was given to some rats	Two isolates of CA (lesion-inducing and non-lesion-inducing isolates); inoculum: 0.1 ml of 10^8 yeasts/ml	8 wk	

Model, yr (reference)	Authors	No. of animals/age /sex/avg wt ^a	Diet ^a	Other conditions ^a	<i>Candida</i> strain, inoculum size ^a	Length of study	<i>Candida</i> carriage ^a
1998 (172)	Samaranayake et al.	15/4 wk/m/200 g	CRD, SRP, and T-water	Rats were given CY	CA/2 CK isolates/SDA /37°C/24 h; inoculum: 0.1 ml of 10 ⁸ yeast/ml	29 wk	CA demonstrated higher oral carriage rate in comparison to the 2 CK isolate
Mouse							
1982 (185)	Sofaer et al.	150/NR/m/NR	NR		CA/MB/37°C/48 h; Inoculum: expt 1, a drop of a 10 ⁸ -CFU/ml yeast suspension; expt 2, 5 × 10 ⁴ CFU/ml added to drinking water	23 days	Expt 1: approximate comparison of CF was made; expt 2: greater numbers of <i>Candida</i> were observed
1983 (72)	Holbrook et al.	80/NR/NR/NR	NR		Virulent strain 19321 and attenuated strain 22114; inoculum: 10 ⁴ CFU/ml added to drinking water	3 wk	Approximate colony counts were taken
1984 (15)	Balish et al.	NR/NR/NR/NR	NR		CA B311 (type A) SDA/37°C/24 h; inoculum: 10 ⁵ cells/ml in drinking water for 2–24 h		GF mice were colonized with CA

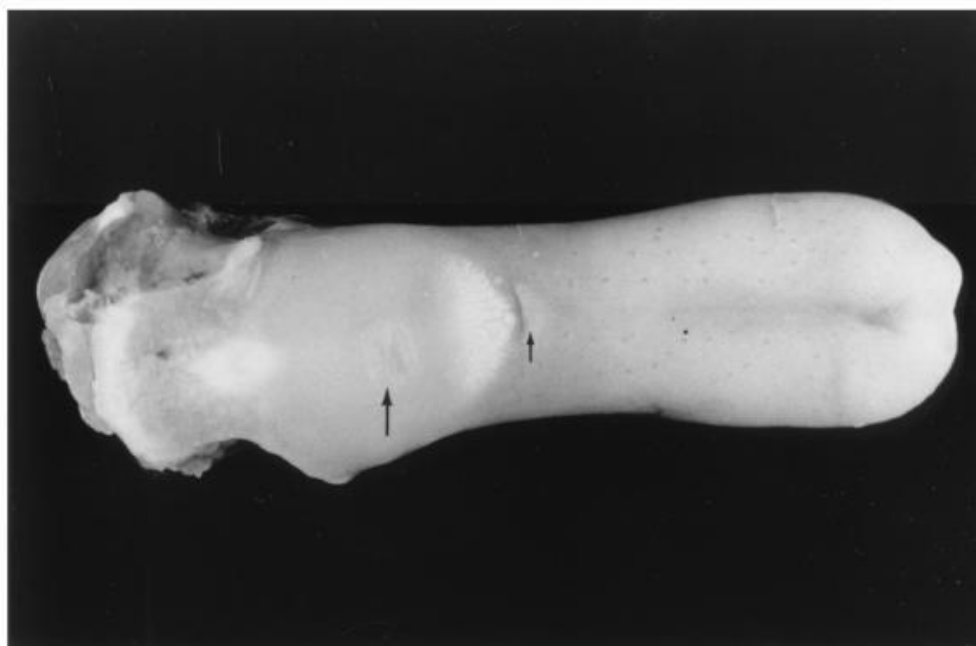
Model, yr (reference)	Authors	No. of animals/age /sex/avg wt ^a	Diet ^a	Other conditions ^a	<i>Candida</i> strain, inoculum size ^a	Length of study	<i>Candida</i> carriage ^a
1989 (92)	Krause and Schaffner	NR/NR/NR/NR	Rat pellets and acidified water ad libitum	Cyclosporin A was given	CA #1; inoculum: 10 ⁶ cells in saline	15 days	NR
1990 (16)	Balish et al.	NR/6–8 wk/NR/NR	NR		CA B311 type A/SDA/37°C/24 h; inoculum: dipping a swab into an inoculum, 10 ⁶ cells/ml in water	24 wk	CA invaded the dorsal tongues of both <i>nu/nu</i> and <i>nu/+</i> mice; infection persisted for 24 wk in <i>nu/nu</i> mice but resolved in <i>nu/+</i> mice by 14 wk
1990 (96)	Lacasse et al.	NR/17–23 wk/m/NR	NR		CA/Lee's medium/25°C/18 h; inoculum size: 50 µl (10 ⁸ cells/ml in PBS)	13 days	<i>Candida</i> recovered during 1–7 days postinoculation from oral mucosa and saliva sample
1991 (32)	Cantoma and Balish	NR/NR/NR/NR	NR		CA B311 type A/SDA/37°C/24 h; inoculum: 10 ⁵ cells/ml mixed in drinking water	20 wk	Yeasts and hyphae were observed on the tongue surface
1993 (95)	Lacasse et al.	NR/17–23 wk/m/NR	NR		CA/Lee's medium/25°C/18 h; inoculum: 10 ⁸ cells/ml in saline	33–92 days	Following primary inoculation, <i>Candida</i> showed peak CFU on days 3–4; these counts declined after a second inoculation

Model, yr (reference)	Authors	No. of animals/age /sex/avg wt ^a	Diet ^a	Other conditions ^a	<i>Candida</i> strain, inoculum size ^a	Length of study	<i>Candida</i> carriage ^a
1993 (17)	Balish et al.	NR/NR/NR/NR	NR	CY given	CA B311 type A/SDA/37°C/24 h; inoculum: 10 ⁵ cells/ml mixed in drinking water	16 wk	NR
1994 (37)	Chakir et al.	NR/8–10 wk/m/NR			<i>C. albicans</i> (LAM-1)/IMDA; inoculum: 10 ⁸ cells/ml in saline	25 days	At 5 h postinoculation, CA was seen in digested mucosal tissue of both BALB/c and DBA/2 mice; a carrier state of the yeast was maintained following resolution of candidal infection; statistical analysis indicated that the viable <i>Candida</i> carrier pattern for DBA/2 was significantly different from the BALB/c pattern o days 3–6.
1995 (47)	Deslauriers et al.	75/8–10 wk/m/NR	NR	Topical application of corticosteroid	CA (LAM-1) IMDM/27°C/48 h; inoculum: 10 ⁸ cells/ml in saline		<i>Candida</i> carrier state was established within 10 days and persisted for at least 3 mo <i>Candida</i> carrier state was established in control mice in <1 days and remained stable at <100 CFU for more than 6 mo; similar colonization patterns was seen for 70% of retrovirus-infected mice on day 10 after <i>C. albicans</i> inoculation; the carrier state fluctuated in 30% of infected mice
1997 (46)	Deslauriers et al.	6/adult/f/3–4 wk	NR	Mice were injected with the Du5H(G ₆ T ₂) virus mixture of murine leukemia viruses to induce MAIDS	<i>C. albicans</i> (LAM-1); inoculum: 10 ⁸ cells/ml in saline	210 days	

Model, yr (reference)	Authors	No. of animals/age /sex/avg wt ^a	Diet ^a	Other conditions ^a	<i>Candida</i> strain, inoculum size ^a	Length of study	<i>Candida</i> carriage ^a
Hamster							from day 100 postinoculation, with high levels of <i>Candida</i> proliferation for 2–3-wk episodes, separated by transient recoveries to the carrier state
1985 (116)	McMillan and Cowell	64/NR/NR/NR	NR		CA (ATCC 10261), CA (UOI), CT (3100)/MEA; inoculum: 1 ml of 10 ⁷ cells/ml in water	6 wk	No hyphal invasio of the superficial epithelium
1986 (58)	Franklin and Martin	35/4–6 wk/m/NR	Food and water ad libitum	Epithelial hyperplasia induced with TLP50	CA NCPF 3091 serotype A/SDA/36 h/37°C; inoculum: 60 mg of aqueous suspension of CA	20 wk	6 animals retain CA for 3 wk
1992 (117)	McMillan and Cowell	80/adult/m/NR	NR		CA (ATCC 10261), CA (UOI)/MEA; inoculum: 1 ml of 10 ⁷ cells in water		CA in the yeast form was seen scattered on the entire mucosa; no epithelial invasior by CA was found

^aAbbreviations: aw, average weight; AZA, azathioprine; BHI, brain heart infusion; CA, *Candida albicans*; CG, *Candida glabrata*; CK, *Candida krusei*; CT, *Candida tropicalis*; CMF, commercial monkey fodder; CY, cyclophosphamide; DDDH, double-distilled demineralized water; ERC, Epol rat cubes; f, female; FL, fluconazole; GF, germ free; GT, germ tubes; HH, hyoscine hydrobromide; HSR, hyposalivatory rats; IC, immunocompetent mice; IMDM, Iscove's modified Dulbecco's medium; KE, ketoconazole; LRF, laboratory rat food; m, male; MB, malt broth; MS, miconazole supplemented; NR, not recorded; NSD, no significant difference; OXY, oxyphencyclimine; RRD, routine rat diet; SDA, Sabouraud's dextrose agar; SERD; Sprotts expanded rodent diet, SG, salivary glands; SL, sublingual; SLC, standard laboratory chow; SM, submandibular; SMP, standard mouse pellets; SPF, specific pathogen free; SRD, standard rat diet;

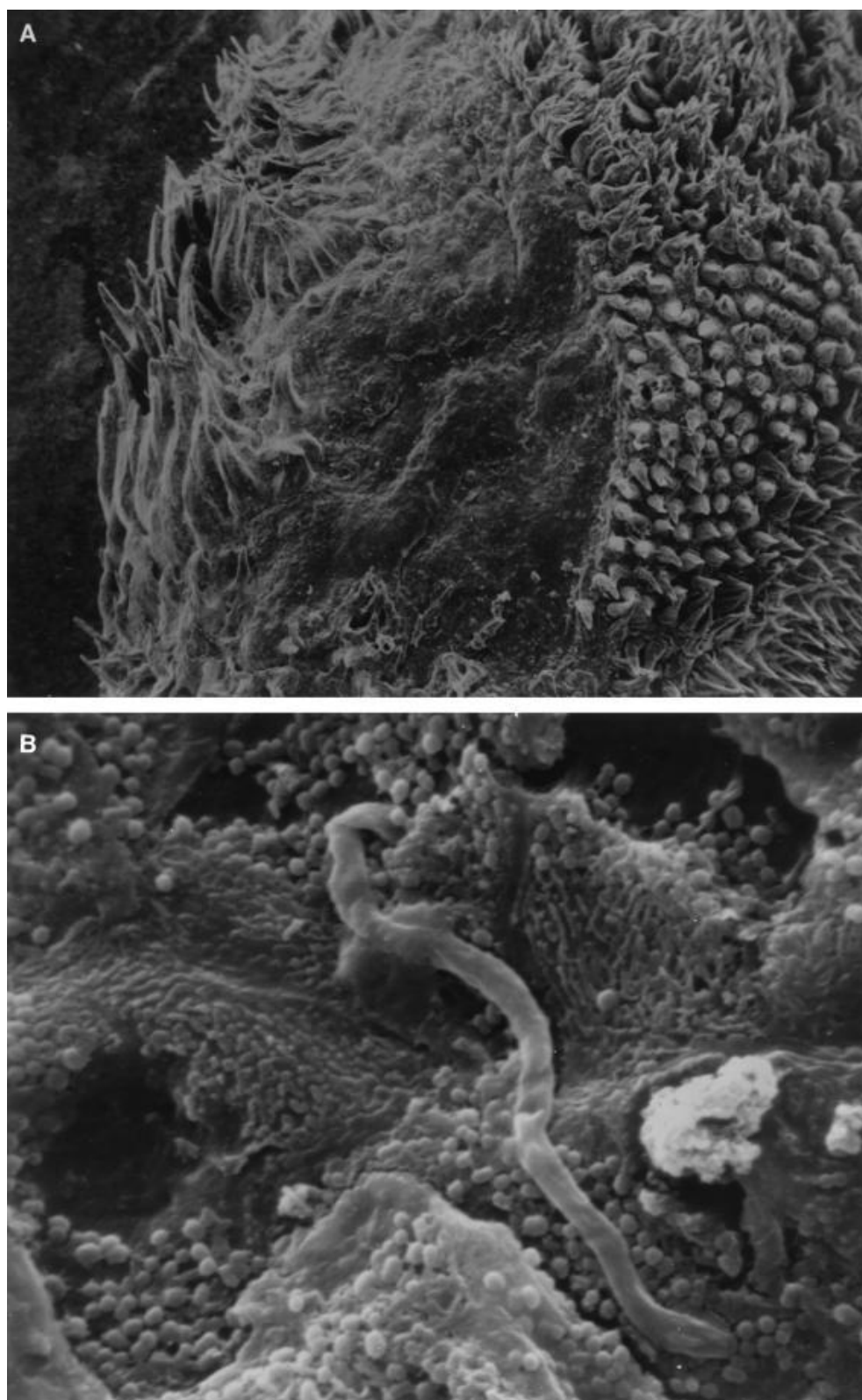
SRP, standard rat pellets; T, tetracycline; TRI, triamcinalone acetonide; TSB, tryptic soy broth; TLP50, 50% turpentine and liquid paraffin.

FIG. 2

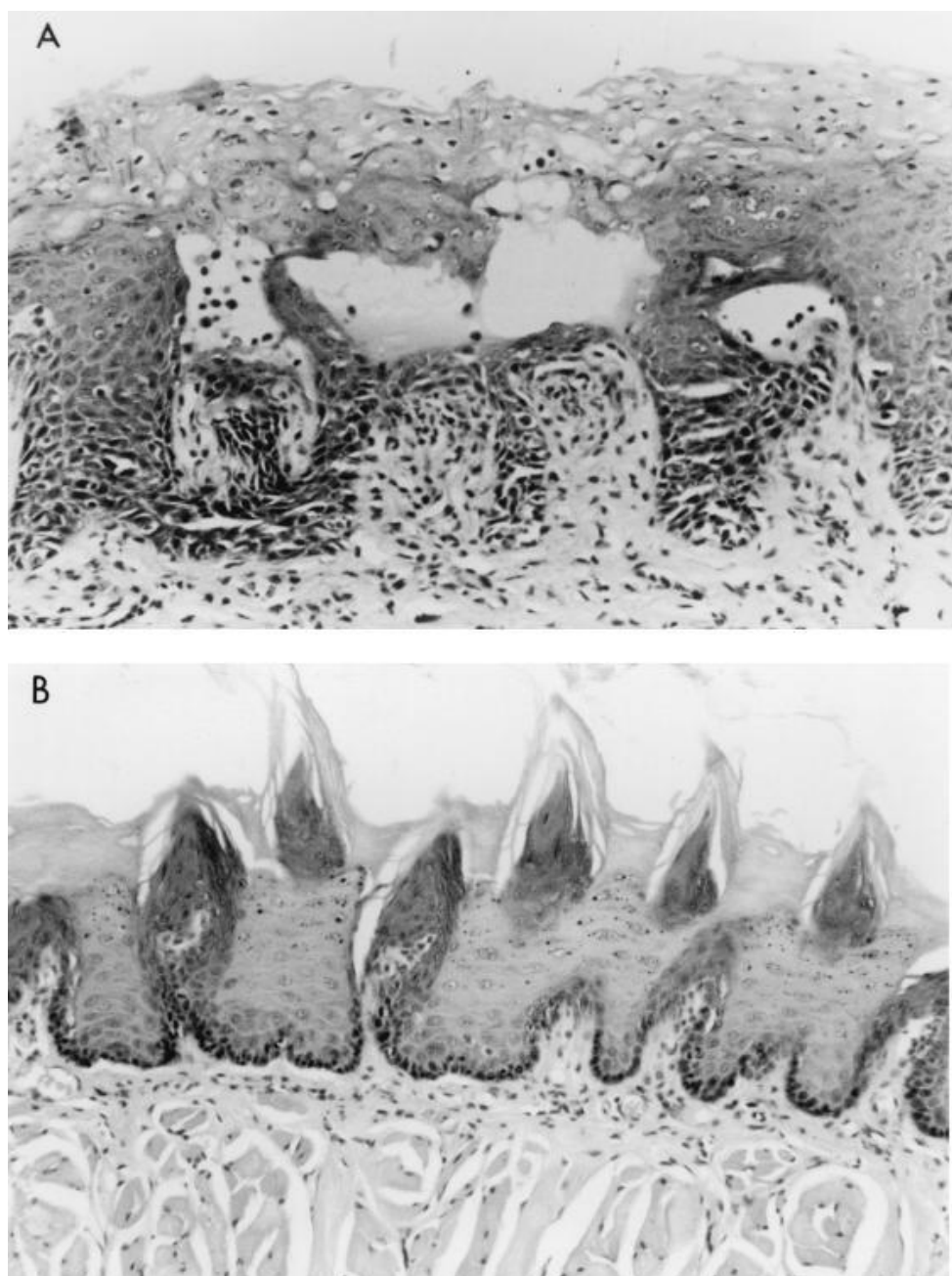
Macroscopic appearance of typical lesions observed on the dorsal surface of SD rat tongue infected with *C. albicans* after tetracycline and cyclophosphamide administration. Note the areas of hyperplasia or leukoplakia (arrows) and the conical papillae appearing as a crescent in between.

FIG. 3

Photomicrograph of a biopsy specimen illustrating the hyperplastic epithelial response to candidal invasion of the rat oral mucosa. Note the hyphal elements in the superficial layers. Periodic acid-Schiff stain. Magnification, $\times 40$.

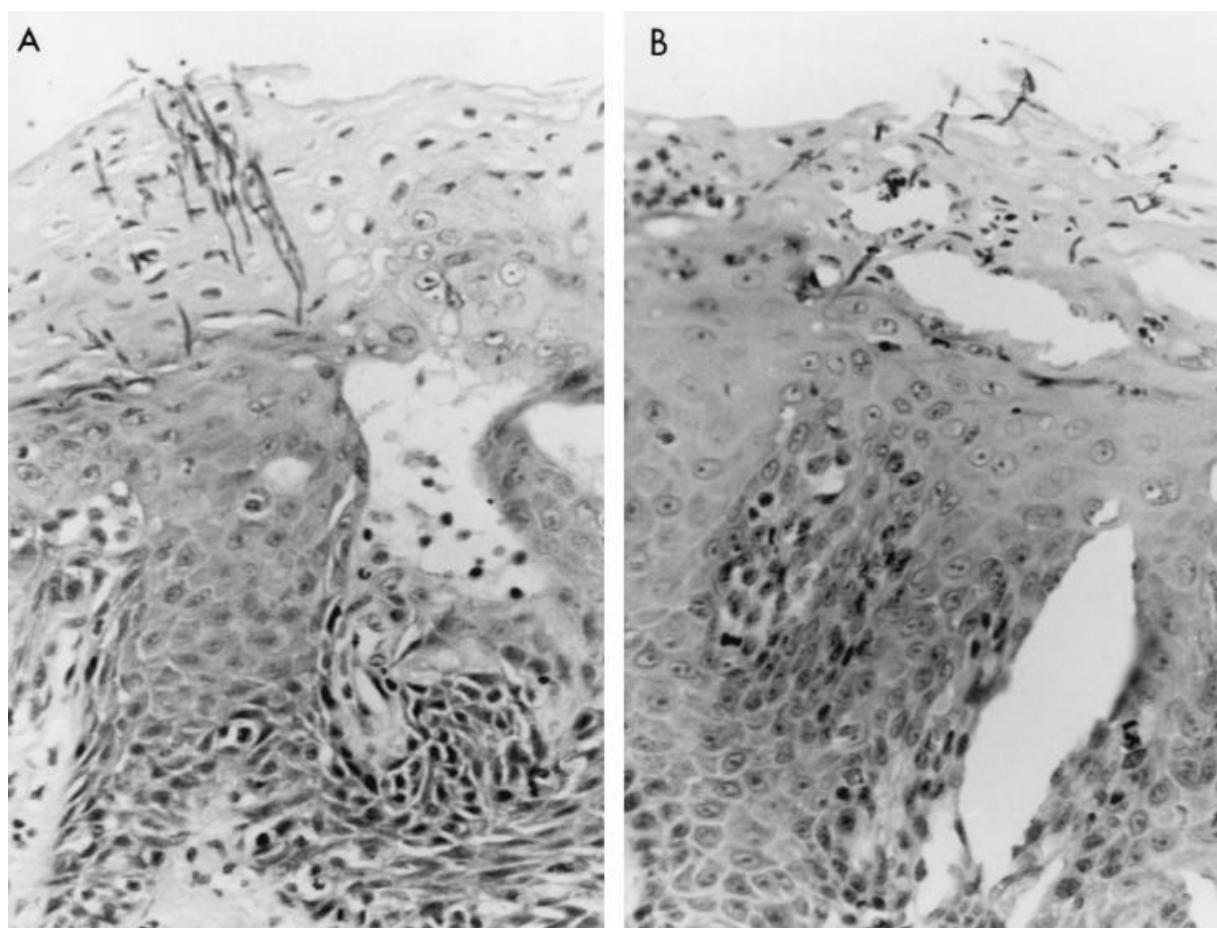
FIG. 4

Scanning electron micrographs of a depapillated lingual lesion observed in experimental candidiasis in a rat, resembling erythematous candidiasis of humans (magnification, $\times 38$) (A) and the lesion showing a hyphal element of *C. albicans* penetrating the epithelium, together with a multitude of commensal bacteria (magnification, $\times 2,400$) (B). Photographs provided courtesy of Carl M. Allen, College of Dentistry, Ohio State University.

FIG. 5

(A) Histopathologic section of leukoplakia on the dorsal tongue of an SD rat infected with *C. albicans*. Note the loss of filiform papillae and the flat-surfaced, parakeratotic, edematous and acanthotic lingual epithelium. (B) Photomicrograph of the lingual mucosa from the posterior dorsal tongue of an SD rat demonstrating uninfected epithelium. Note the normal filiform papillae covered by a layer of thick acellular orthokeratin. Hematoxylin and eosin stain. Magnifications, $\times 200$.

FIG. 6



Histopathologic sections of depapillated areas on the dorsal tongue surface of two SD rats, showing epithelial invasion by *C. albicans* (5.00 to 17.71 μm) (A) and *C. krusei* (5.01 to 8.34 μm) (B) hyphal elements. Periodic acid-Schiff stain. Magnifications, $\times 400$. Note the longer hyphal elements of *C. albicans* compared with *C. krusei*.

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