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Adherence of *Candida albicans* to Human Buccal Epithelial Cells

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The adherence of *Candida albicans* to human buccal epithelial cells after 2 h at 37°C was significantly greater in human saliva than in phosphate-buffered saline. In saliva, viable fungi adhered much better than did nonviable fungi, and this adherence was greater at 37 than at 25°C. Viable yeasts, preincubated in saliva for 90 min at 37°C before being washed and mixed with epithelial cells in phosphate-buffered saline, adhered better than nonviable yeasts or yeasts preincubated in phosphate-buffered saline. Enhanced adherence in saliva appeared to be associated with germination of the yeast cells. Conditions permitting germination (growth in tissue culture medium 199 at 37°C but not at 25°C) also supported enhanced adherence. After germination had occurred, the fungi could be killed with Formalin without interfering with their rapid and efficient adherence to epithelial cells. These data indicate that the enhanced adherence of *C. albicans* observed after incubation in saliva is related to changes in the fungi, rather than to a requirement for prolonged interaction between fungi and epithelial cells.

For successful colonization and infection by various bacteria, selective adherence to host mucosal surfaces is a necessity (5). The significance of adherence as an ecological determinant is suggested by the relationship between in vivo adherence of oral bacteria to different surfaces and their proportional distribution in the mouth (4, 5). Strains of *Neisseria gonorrhoeae* that adhere strongly to human buccal epithelial cells (12), human erythrocytes (1), spermatozoa (6), and vaginal epithelial cells (10) are more virulent than strains that adhere less well. In *Proteus mirabilis* also, the ability of these microorganisms to adhere to oral and bladder mucosal cells is associated with their capacity to initiate retrograde pyelonephritis in experimental animals (14). In addition, pathogenic bacteria of the gastrointestinal tract have been shown to adhere to mucosal surfaces of the gut (3, 7, 11).

Reports on the adherence of fungi to mucosal surfaces are few; most studies involved the opportunistic fungus *Candida albicans*. Liljemark and Gibbons observed attachment of *C. albicans* to rat tongue and cheek cells and found that *C. albicans* adhered in lower numbers to epithelial cells from conventional rats than to those from germfree rats, suggesting that indigenous oral flora may interfere with attachment and colonization by candida (9). In addition, King et al. reported that *C. albicans* adheres to human vaginal epithelial cells and appears to adhere better than other species of *Candida* (R. D. King, A. L. Morris, R. L. Taylor, and E. E. M. Moody, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, F23, p. 122). The experiments we report here were designed to determine if *C. albicans* also adheres to human oral epithelial cells and to examine the effects of saliva on the interactions between *C. albicans* and the human host.

MATERIALS AND METHODS

Adherence media. Adherence tests were carried out in 0.01 M phosphate-buffered saline (PBS; isotonic buffer containing sodium phosphate and sodium chloride at pH 7.0), whole, clarified human saliva, or tissue culture medium 199 (TC 199). Unstimulated whole human saliva was collected in sterile tubes kept on ice and was clarified by centrifugation at 10,000 × *g* at 4°C for 30 min. The supernatant was used undiluted in the adherence tests within 2 to 3 h after collection. Salivary anti-*Candida* antibodies were measured by indirect immunofluorescence as described by Lehner (8), and only saliva samples without detectable anti-*Candida* antibodies were used in these experiments. TC 199 (Grand Island Biological Co., Santa Clara, Calif.) was adjusted to pH 7.0 with sodium bicarbonate and did not contain serum or antibiotics.

Epithelial cells. Buccal epithelial cells were collected from healthy human subjects by gently rubbing the inside of the cheeks with sterile swabs which were then agitated in 12 ml of PBS. Epithelial cells were washed twice in PBS to remove unattached microorganisms and were resuspended in PBS, whole saliva, or TC 199 at 2×10^5 cells per ml. Epithelial cells from

several donors were pooled for each adherence test, because similar results were obtained when epithelial cells collected from different donors were tested separately. Washed epithelial cells usually had no attached yeasts before the adherence test.

Yeast cell suspensions. Recent isolates of *C. albicans* from the oral cavity of a healthy human subject were identified by standard techniques (13) and maintained on Sabouraud glucose agar slants stored at 4°C. The strain designated N-1-5 was used for most of these studies. For the adherence tests, yeasts were grown for 18 to 24 h at 37°C on a Sabouraud glucose agar slant, transferred to Sabouraud glucose broth, and incubated for 18 to 24 h at 37°C. The fungi, which were virtually all in the budding yeast phase, were washed twice in PBS and resuspended in PBS, whole saliva, or TC 199 at 10^8 cells per ml. In some experiments, yeasts were first killed by heating at 63°C for 80 to 120 min or by overnight incubation at 4°C in 0.5% formaldehyde in normal saline. These fungi were washed twice in PBS before being tested.

Adherence assay. The adherence method of Gibbons and van Houte (4) was used with a few modifications. For the assay, 0.25 ml of epithelial cells and 0.25 ml of fungi were mixed in tubes and incubated on a rocker at 37°C for 1 to 3 h. Control tubes contained epithelial cells and PBS or saliva. The epithelial cells were collected on polycarbonate 12-μm-pore size filters (Nuclepore Corp., Pleasanton, Calif.) and washed with 70 ml of PBS to remove unattached fungi. The washed epithelial cells on the filters were air dried, fixed with a few drops of absolute methanol, and stained with Gram crystal violet. At least 100 epithelial cells on the filters were counted, and the numbers of fungi adhering per epithelial cell were determined. All filters were coded and read "blind." The presence of germ tubes was assessed visually.

RESULTS

Effect of yeast cell concentration on adherence in PBS and saliva. Yeasts at concentrations ranging from 10^3 to 10^9 organisms per ml were incubated for 2 h with buccal epithelial cells in either PBS or saliva. The ratios of yeast cells to epithelial cells ranged from 1:100 to 10,000:1. Figure 1 illustrates the results of one of three such experiments, all of which gave similar curves. Adherence in both diluents was detectable at yeast cell concentrations above 10^4 per ml. Attachment of *C. albicans* to buccal epithelial cells was significantly greater ($P < 0.05$) in saliva than in PBS at 37°C at yeast concentrations from 10^5 to 10^8 cells per ml. At 10^9 yeast cells per ml, adherence could only be determined in PBS and not in saliva because of the high numbers of unattached yeasts, which could not be removed by the filtration procedure. A final concentration of 5×10^7 fungi per ml of the incubation mixture was selected for the adherence assays, giving a yeast-to-epithelial cell ratio of 500:1.

Effect of temperature on adherence in PBS and saliva. Figure 2 shows results of one

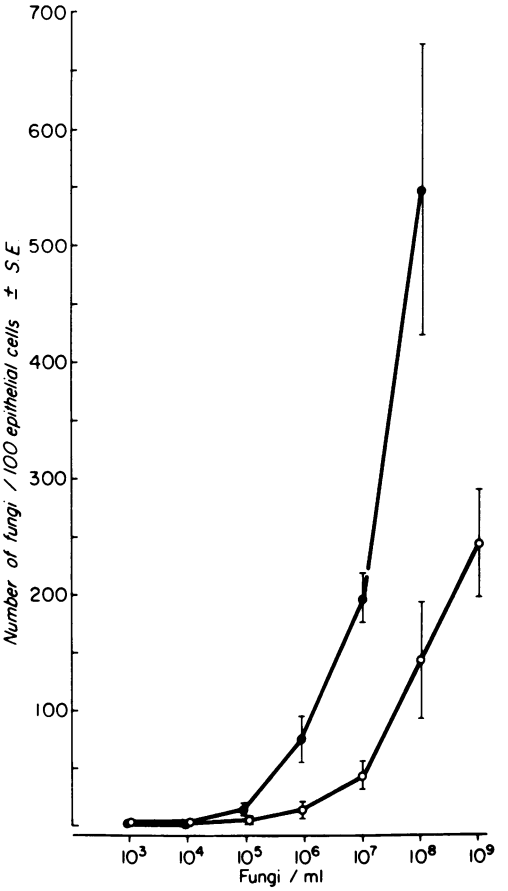

FIG. 1. *Effect of yeast concentration on adherence in saliva and PBS at 37°C for 2 h. Symbols:* (○) *PBS;* (●) *saliva.*

of two similar experiments. In whole saliva, attachment of *C. albicans* to buccal epithelial cells was significantly greater ($P < 0.05$) at 37 than at 25°C in saliva and greater than adherence at either temperature in PBS at 3 h ($P < 0.05$). In PBS, attachment was not significantly different at 37 and 25°C. After 2 h in saliva at 37°C, the numbers of yeasts adhering to epithelial cells increased remarkably. This increase in adherence in saliva was not the result of an increase in numbers of yeasts in these mixtures; direct counts of yeasts incubated in saliva at a concentration of 5×10^7 per ml from 1 to 3 h revealed no detectable increase in numbers of organisms.

Comparison of adherence in PBS and saliva at 37°C. Data taken from 18 different experiments demonstrated the increased adherence in saliva as compared with PBS at 37°C at periods of incubation over 2 h, as indicated in the typical experiment described by Fig. 2.

Effect of yeast cell viability on adherence. Fungi killed by treatment with Formalin

or heat, as described previously, were unable to adhere to epithelial cells incubated in saliva at 37°C as well as did viable yeasts (Table 1). When viable yeasts were incubated in saliva for 90 min at 37°C and then washed twice with PBS and mixed with epithelial cells in PBS, the adherence was greater than that obtained when viable yeasts were pretreated with PBS (Table 2). When yeasts were killed by heating or Formalin before pretreatment, the amount of adherence of saliva-pretreated and PBS-pretreated yeasts was about the same. These results suggest that viable yeasts incubated in saliva undergo changes which enhance their ability to adhere to epithelial cells.

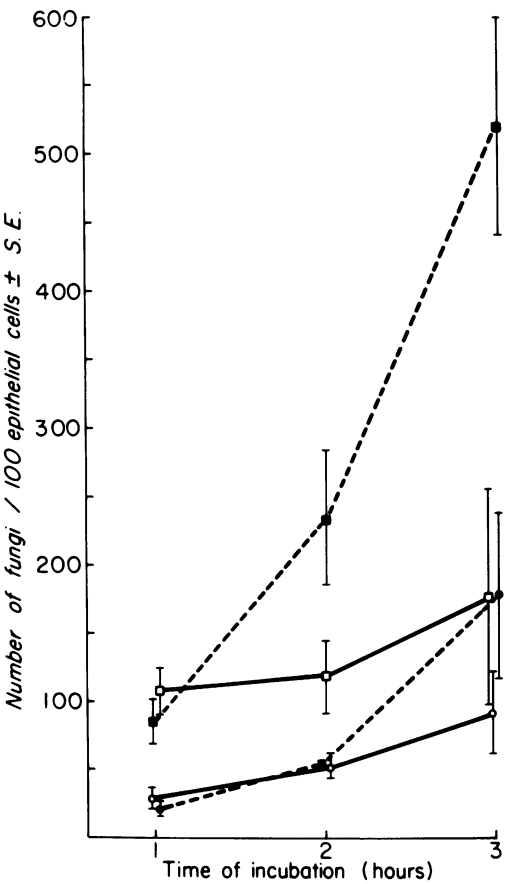

FIG. 2. *Effect of temperature on adherence in PBS and saliva. Symbols:* (○) *PBS, 25°C* (□) *PBS, 37°C;* (●) *saliva, 25°C;* (■) *saliva, 37°C.*

Adherence in TC 199. After 2 h of incubation in saliva at 37°C, about 1 to 5% of the yeast cells began to form germ tubes and to attach to epithelial cells. No germ tubes were formed when yeasts were incubated in saliva at 25°C or in PBS at either temperature for up to 3 h. To determine whether the enhanced adherence in saliva was associated with germination, we assayed adherence in TC 199, a medium which effectively induces germ tube formation in *C.*

TABLE 1. *Adherence of viable, Formalin-treated, and heat-killed C. albicans to buccal epithelial cells in saliva*

Yeasts	No. of yeasts attached per 100 epithelial cells ± SE[a] at:			P^b
	1 h	2 h	3 h	
Viable	179 ± 54	493 ± 45	611 ± 43	<0.001[c]
Formalin treated	9 ± 4	53 ± 21	34 ± 19	<0.001[d]
Heat killed	68 ± 23	115 ± 6	50 ± 8	

[a] Mean of triplicate determinations. SE, Standard error of the mean.

[b] *P* values for adherence at 3 h as determined by the standard *t* test.

[c] Significance of difference between viable and Formalin-treated yeasts.

[d] Significance of difference between viable and heat-killed yeasts.

TABLE 2. *Adherence to buccal epithelial cells of C. albicans pretreated with either saliva or PBS*

Expt	Yeast	Pretreatment[a]	No. of yeasts attached per 100 epithelial cells ± SE[b]	P^c	P^d
1	Viable	PBS	142 ± 27	NS	<0.001
	Heat killed	PBS	63 ± 24		
	Viable	Saliva	640 ± 77	<0.001	
	Heat killed	Saliva	75 ± 17		
2	Viable	PBS	209 ± 22	NS	<0.005
	Formalin treated	PBS	188 ± 60		
	Viable	Saliva	387 ± 24	<0.005	
	Formalin treated	Saliva	213 ± 25		

[a] Either viable or nonviable yeasts were incubated in PBS or saliva for 90 min at 37°C, washed twice with PBS, and mixed with epithelial cells.

[b] Mean of quadruplicate determinations. SE, Standard error of the mean. The adherence assay was done entirely in PBS for 45 min at 37°C.

[c] Significance of differences between viable and nonviable yeasts. NS, Not significant.

[d] Significance of differences between PBS and saliva pretreatment of viable yeasts.

albicans at 37°C but not at 25°C (2). When our strain of *C. albicans* was incubated in TC 199, about 40 to 50% of the yeast cells formed germ tubes by 2 h at 37°C, and none of the yeasts formed germ tubes at 25°C, even after 3 h. Adherence of *C. albicans* at 37°C in TC 199 was significantly greater ($P < 0.01$) than adherence at 25°C, after 1 and 2 h (Table 3). When four other strains of *C. albicans* were tested, adherence was greater in TC 199 at 37 than at 25°C, after 90 min (Fig. 3). Yeasts of all strains began to form germ tubes by 90 min at 37°C but not at 25°C.

To determine whether adherence was rapid once germination had occurred, yeasts were first treated with TC 199 at 25 or 37°C for 2 h, washed, and tested for adherence to epithelial cells in PBS at 37°C for only 30 min. Yeasts incubated in TC 199 at 37°C formed germ tubes and adhered much better ($P < 0.001$) than did yeasts incubated at 25°C. Once germ tube formation had occurred, the fungi could be killed with Formalin without interfering with rapid and efficient adherence (Table 4).

TABLE 3. *Adherence of viable C. albicans to epithelial cells in TC 199*

Incubation temp (°C)	No. of attached fungi per 100 epithelial cells ± SE[a] at:			
	1 h	(P)[b]	2 h	(P)[b]
25	208 ± 13	(<0.01)	514 ± 91	(<0.001)
37	766 ± 100		2,472 ± 34	

[a] The values given are the means of triplicate determinations. SE, Standard error of the mean.

[b] Statistical significance of the differences between adherence at 25 and 37°C.

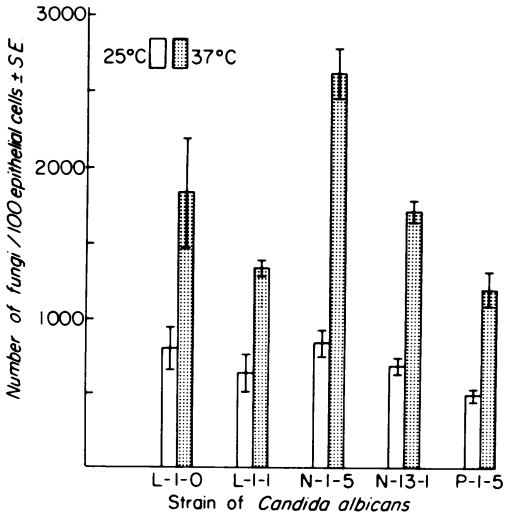

FIG. 3. *Adherence of five different strains of viable C. albicans to buccal epithelial cells in TC 199 at 90 min at 25 and 37°C. Germ tube formation occurred with all strains at 37°C but not at 25°C.*

DISCUSSION

C. albicans must be able to survive the antimicrobial and flushing actions of saliva if the fungi are to colonize and, ultimately, to infect the oral mucosa. Bacteria of many kinds persist in the oral cavity and in analogous environments by virtue of selective adherence to surface structures such as epithelial cells; the results reported here indicate that the fungus, *C. albicans*, also can adhere to oral epithelial cells. It is reasonable to assume that this adherence is an essential, initial step in the establishment of candida infections in human beings.

Adherence of *C. albicans* was dependent upon yeast cell concentration and was detectable in vitro above 10^4 yeast cells per ml of incubation mixture (at a ratio of 1 yeast cell to 10 epithelial cells). In this respect, the *C. albicans* adherence rate was similar to that of bacteria. It has been reported that *Streptococcus sanguis* can only be recovered from teeth when the concentration of bacteria equals or exceeds 10^3 to 10^4 organisms per ml of saliva (15).

Adherence of *C. albicans* to buccal epithelial cells differed from that of bacterial adherence in that attachment was greater when the tests were done in saliva than when they were done in PBS. Gibbons and van Houte (4) reported that the adherence of oral streptococci to buccal epithelial cells in vitro was similar in PBS and in saliva after 30 min. Substantial attachment of *C. albicans* to epithelial cells in saliva occurred only after 2 or more hours of incubation at 37°C. In

TABLE 4. *Adherence to buccal epithelial cells of C. albicans incubated in TC 199 before assay*[a]

Incubation in TC 199 before assay[b]	Germ tubes	No. of attached fungi per 100 epithelial cells ±SE[c]	P[d]
25°C	Absent	404 ± 62	<0.001
37°C	Present	1,770 ± 195	
25°C, Formalin[e]	Absent	574 ± 34	<0.001
37°C, Formalin[e]	Present	1,697 ± 191	

[a] The adherence assay was done entirely in PBS for 30 min at 37°C.

[b] Viable yeasts were incubated in TC 199 for 2 h at either temperature, washed twice, and resuspended in PBS before the adherence test.

[c] SE, Standard error of the mean.

[d] P values for the differences between fungi incubated in TC 199 at 25 or 37°C.

[e] After incubation in TC 199, these fungi were kept overnight in 0.5% Formalin-saline, washed twice, and resuspended in PBS before the adherence test.

vivo, substances in saliva may inhibit the attachment of bacteria to buccal cells, as evidenced by the small numbers of bacteria attached to cells in vivo compared with the numbers which attach to buccal cells in PBS in vitro (5).

The data show that *C. albicans* adhered to human buccal epithelial cells better under conditions conducive to germ tube formation than when germ tubes were not formed. The fungi adhered to a small extent in PBS and to a significantly greater extent in human saliva after 2 h at 37°C. In saliva, viable candida adhered much better than did the nonviable fungi, and adherence was better at 37 than at 25°C.

It is possible that during the transition of *C. albicans* from yeast form to filamentous form, changes in surface components occur that could account for the increased adherence. The results shown in Table 4 suggest that preincubation under conditions that permit germ tube formation leads to rapid adherence within 30 min after pretreated yeasts are mixed with epithelial cells in PBS. Thus, the increase in adherence of candida over a 2- or 3-h period of incubation in saliva appears to be related to changes in the fungi, rather than to a requirement for prolonged interaction between fungi and epithelial cells.

Studies are in progress to investigate further the changes in the fungi that cause enhanced adherence. Salivary anti-*Candida* antibodies were not demonstrable in any of the studies reported here; however, our preliminary data (unpublished) suggest that such antibodies can inhibit adherence.

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LITERATURE CITED

1. **Buchanan, T. M., and W. A. Pearce.** 1976. Pili as a mediator of the attachment of gonococci to human erythrocytes. Infect. Immun. **13**:1483–1489.
2. **Dabrowa, N., D. H. Howard, J. W. Landau, and Y. Shecter.** 1970. Synthesis of nucleic acids and proteins in the dimorphic forms of *Candida albicans*. Sabouraudia **8**:163–169.
3. **Freter, R., and G. W. Jones.** 1976. Adhesive properties of *Vibrio cholerae*: nature of the interaction with intact mucosal surfaces. Infect. Immun. **14**:246–256.
4. **Gibbons, R. J., and J. van Houte.** 1971. Selective bacterial adherence to oral epithelial surfaces and its role as an ecological determinant. Infect. Immun. **3**:567–573.
5. **Gibbons, R. J., and J. van Houte.** 1975. Bacterial adherence in oral microbial ecology. Annu. Rev. Microbiol. **29**:19–44.
6. **Holmquest, A. N. J., J. Swanson, T. M. Buchanan, R. D. Wende, and R. P. Williams.** 1974. Differential attachment by piliated and nonpiliated *Neisseria gonorrhoeae* to human sperm. Infect. Immun. **9**:897–902.
7. **Jones, G. W., and J. M. Rutter.** 1972. Role of the K88 antigen in the pathogenesis of neonatal diarrhea caused by *Escherichia coli* in piglets. Infect. Immun. **6**:918–927.
8. **Lehner, T.** 1966. Immunofluorescence study of *Candida albicans* in candidiasis, carriers and controls. J. Pathol. Bacteriol. **91**:97–104.
9. **Liljemark, W. F., and R. J. Gibbons.** 1973. Suppression of *Candida albicans* by human oral streptococci in gnotobiotic mice. Infect. Immun. **8**:846–849.
10. **Mårdh, P.-A., and L. Weström.** 1976. Adherence of bacteria to vaginal epithelial cells. Infect. Immun. **13**:661–666.
11. **Nelson, E. T., J. D. Clements, and R. A. Finkelstein.** 1976. *Vibrio cholerae* adherence and colonization in experimental cholera: electron microscopic studies. Infect. Immun. **14**:527–547.
12. **Punsalang, A. P., and W. D. Sawyer.** 1973. Role of pili in the virulence of *Neisseria gonorrhoeae*. Infect. Immun. **8**:255–263.
13. **Silva-Hutner, M., and B. H. Cooper.** 1974. Medically important yeasts, p. 491–507. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
14. **Silverblatt, F. J.** 1974. Host parasite interaction in the rat renal pelvis, a possible role for pili in the pathogenesis of pyelonephritis. J. Exp. Med. **140**:1696–1711.
15. **van Houte, J., and D. B. Green.** 1974. Relationship between the concentration of bacteria in saliva and the colonization of teeth in humans. Infect. Immun. **9**:624–630.