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INVESTIGATION OF ADHERENCE OF *CANDIDA ALBICANS*, OTHER *CANDIDA* SPP., AND *TORULOPSIS GLABRATA* TO HUMAN BUCCAL EPITHELIAL CELLS *IN VITRO*

By

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ABSTRACT

INVESTIGATION OF ADHERENCE OF *CANDIDA ALBICANS*, OTHER *CANDIDA* SPP., AND *TORULOPSIS GLABRATA* TO HUMAN BUCCAL EPITHELIAL CELLS *IN VITRO*

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Adherence of *Candida albicans* to human buccal epithelial cells was proportional to germ tube formation capability, and greater than *Torulopsis glabrata*, *C. tropicalis*, *C. pseudotropicalis*, *C. parapsilosis*, and *C. stellatoidea*. Adherence of formalized and viable yeast for *Candida* spp. and *T. glabrata* isolates was similar. Inhibition of adherence was greatest for FHA treatment of organisms, although citric acid, con-A, and α-D-methyl-mannopyranoside treatment decreased adherence equally. Greatest decreases in adherence were seen for *C. albicans* isolates, although changes in adherence did not correlate with germ tube formation capability. Compared to *C. albicans*, variations in adherence with treatment inhibition methods for non-*C. albicans* organisms were consistent, suggesting that differences in cell wall structure, mannan concentration, and adhesin characteristics exist. Little variation in adherence to individual or pooled human buccal epithelial cells for treated or viable organisms was seen. This is the first comparison of inhibition of adherence in non-*C. albicans* *Candida* spp. and *T. glabrata* isolates.

This thesis is dedicated to my father,

Melvin C. Hanselman,

whose love, determination to live,
and extraordinary courage
have given me inspiration,
compassion, and strength
of spirit to endure.

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It may be we shall touch
     happy isles,
And see the great Achilles,
     whom we knew,
And though much is taken,
     much abides:

We are not now that strength
     which in olden days moved
     earth and heaven,
But that which we are, we are . . . .

We are one equal temper of
     heroic hearts, made weary
     by time and fate,
But strong in will to strive,
     to seek, and to find,
But NEVER to yield.

Closing stanza of
Tennyson's poem
"Ulysses."

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INTRODUCTION

Microbial infections have plagued humankind throughout history (Sherris, 1984). Adhesion of microorganisms to epithelial and mucosal surfaces is considered to be an initial and essential stage in the colonization and infection of the host (Beachey et al., 1981). The adherence mechanism utilized by various bacteria in causing disease has been determined, but for other pathogenic microorganisms such as Candida albicans and other Candida sp., the precise mechanism of adherence has not been established. Candidiasis is a primary or secondary infection involving a member of the genus Candida and is considered to be opportunistic since these organisms are usually saprophytic endogenous species within the human host (Odds, 1979). Superficial candidiasis is among the most common of all infections with oral and vaginal candidiasis as its most prevalent forms (Hurley, 1980). Although adherence of C. albicans to mucosal surfaces is neither necessary for its survival or for its growth (Odds, 1979), it represents an initial step of the more complex phenomenon of colonization of tissue surfaces for which effective mechanisms of cellular attachment have been developed (Tronchin et al., 1984). Recent research suggests that the mannan component of the cell wall of C. albicans is primarily responsible for the adherence of this organism to epithelial cell surfaces of mucous membranes

(Poulain et al., 1978), although the adherence mechanism of other Candida sp. is not known. Understanding the adherence mechanism utilized by these organisms in the establishment of disease should lead to a better understanding of candidiasis and to possible prevention of infection.

The objectives of the following experiments were to investigate the differences in adhesion ability of C. albicans isolates of differing germ tube formation capability to human buccal epithelial cells, to determine the differences in adherence of different species of Candida and Torulopsis glabrata to human buccal epithelial cells using viable yeast, to determine the effect of formalization of yeast cells on adherence, to compare the influence of two mannan inhibitors on adhesion of these organisms to epithelial cells, to determine the effect of decreased cell wall mannan on adherence ability, and to determine the effect of a complementary sugar of mannan on adhesion in the adherence assay. Experiments were designed to compare the adherence of various Candida sp. to buccal mucosal cells in a group of normal or candidal free individuals using viable and formalized yeast cell preparations . Other experiments involved the use of methods designed to competitively inhibit adherence of Candida sp. and T. glabrata to buccal epithelial cells,

based on the concept of mannan-mediated adherence. In these studies, yeast cells were subjected to treatment methods which permitted binding of the cell wall mannan ligands through the use of the mannose-specific lectin concanavalin-A (Sandin and Rogers, 1982), incubation of the adherence mixtures with α-D-methylmannopyranoside (Sandin et al., 1982), and treatment of the yeast cells with filamentous hemagglutinin (FHA), a protein adhesin of Bordetella pertussis which preferentially binds mannan (Sato and Sato, 1984). In another experiment, cell wall mannan of yeast isolates was decreased using a citric acid extraction technique (Hamada et al., 1981). These organisms were then used in adherence assays to determine the effect of decreased cell wall mannan on adherence of different Candida sp. and T. glabrata to buccal epithelial cells. In addition, the relationship of germ tube formation to percent adherence of C. albicans yeast isolates to buccal epithelial cells was examined.

LITERATURE REVIEW

Candidiasis is one of the oldest diseases known to humankind. Thrush, an oral Candida infection, was described in debilitated patients by Hippocrates in his Epidemics (Odds, 1979; Rippon, 1982). Candidiasis is a primary or secondary infection caused by members of the genus Candida, asporogenous yeasts that reproduce by forming blastoconidia and do not possess a capsule. They may form hyphae or pseudohyphae, and belong to the form-class Deuteromycetes and the form-family Cryptococcaceae (Rippon, 1982). Within the genus Candida, C. albicans is the most pathogenic member, although other members of the genus can cause disease (Hurley, 1980). The seven most virulent species of Candida include C. albicans, C. tropicalis, C. stellatoidea, Torulopsis (Candida) glabrata, C. krusei, and C. parapsilosis (Hurley, 1980). In a study done by Klotz et al., 1983, adherence to vascular endothelium was greatest with C. albicans and C. tropicalis, less with C. krusei, and least with C. parapsilosis, C. pseudotropicalis, and T. glabrata. In in vitro studies, adherence of C. albicans and C. tropicalis to mucosal epithelial cells exceeded that of other Candida sp. (Rotrosen et al., 1986). In another study these two organisms together caused approximately 80% of the candidiasis cases seen clinically while C. parapsilosis and T. glabrata comprised 10-15% of isolates (Hopfer, 1985).

These studies reflect known virulence of different Candida sp. in vivo.

Candida albicans is considered to be normal flora on alimentary tract mucosal surfaces (Odds, 1979; Rippon, 1982). It is an opportunistic yeast that leads a saprophytic existence in the gastrointestinal and urogenital tracts of 30-50% of normal humans (Miles et al., 1977). It is believed that this organism never leaves the host spontaneously during life once it becomes part of the fecal flora (Miles et al., 1977). Thirty to fifty percent of normal humans harbor C. albicans in their gut (Miles et al., 1977), 39% of normal females have it in their vagina (Carter et al., 1959), 30% have it in their oral cavity (Baum et al., 1960) and in 46% of normal individuals, it is present on perianal skin (Recio and DeLeon, 1957). In addition, recovery of C. albicans from the human body increases two-fold in hospital patient populations as compared to healthy individuals, with the exception of skin, which remains the same regardless of the health of the individual (Odds, 1979).

Since C. albicans is normal flora in the human host, any invasion is considered to be opportunistic. Candida albicans differs from other Candida sp. in that it is rarely found outside the natural animal host (Drake and Maiback, 1973) or as a normal resident of skin flora

(Greshem and Whittle, 1961). Factors predisposing an individual to candidiasis include extremes in age, physiological changes such as endocrine dysfunction or diabetes, prolonged administration of antibiotics, immunosuppressive agents or corticosteroids, generalized debility such as in end-stage terminal cancer or immunodeficiency states, and iatrogenic mechanical or trauma induced barrier breaks (Rippon, 1982).

Candidiasis presents clinically as a wide spectrum of disease states including superficial colonization, mucocutaneous involvement, systemic infections, and allergies (Rippon, 1982), with the most common manifestations including oral and vaginal candidiasis (Hurley, 1980). Oral candidiasis is produced by overgrowth and colonization of the tongue, soft palate, buccal mucosa or other oral surfaces with C. albicans or other Candida sp. Its distribution is discrete, confluent, or patchy and presents as a white to gray pseudomembrane covering the area. Oral candidiasis is a common problem that presents as a chronic recurring infection which can be a reservoir for severe, spreading, localized, or systemic disease in the compromised host (Epstein et al., 1984). Oral candidiasis of the neonate has been reported as high as 18%, but the average is approximately 4%, with infection resulting from passage through the birth canal (Rippon, 1982). In

newborns, a small amount of the organism in the oral cavity is a prelude to clinical thrush (Rippon, 1982). Since the normal resident flora of the gastrointestinal tract is unestablished at birth, if C. albicans is not present by the third day of life, clinical thrush rarely developes (Rippon, 1982). Studies have shown that antepartum treatment with clotrimazole lowers the vaginal Candida contamination and consequent thrush in newborn infants (Rippon, 1982).

Oral candidiasis can be found in patients of all ages (Rippon, 1982), and in a study of adherence to human buccal epithelial cells, it was found that adherence was the same in normal adults and children, suggesting that a stable cell receptor system is present which is not age dependent (Cox, 1983). In addition, adherence of C. albicans to buccal epithelial cells from full-term infants and healthy school age children was lower than adherence to epithelial cells from premature infants until 5 days of age (Cox, 1986).

In adults, predisposing factors to oral candidiasis include avitaminosis (riboflavin deficiency), diabetes complications, polyendocrine disturbance, neoplasia, steroids, antibiotics, and other drugs (Rippon, 1982). For example, chronic oropharyngeal candidiasis is recognized as a complication of inhaling steroids such as beclomethasone

diproprionate for treatment of respiratory disease (Rippon, 1982). Increased candidal adherence to buccal epithelial cells during antibiotic therapy is considered to be an important factor explaining the increased incidence of Candida colonization in patients receiving antibiotics and the persistence of the organism which then permits disease development (Cox, 1983).

Denture stomatitis is recognized as the most common form of oral candidiasis (Odds, 1979) and is characterized by erosive and painful lesions of the oropharynx (Rippon, 1982). Acrylic is considered to be a possible reservoir for C. albicans in denture stomatitis (McCourtie and Douglas, 1981), and the presence of glucose and other sugars besides sucrose increases adherence of C. albicans to acrylic (McCourtie et al., 1981). This is important because there is increased salivary glucose in diabetic patients and those undergoing antibiotic or steroid therapy (Knight and Fletcher, 1971), predisposing these patients to candidiasis (Odds, 1979). In addition, dental plaque is a contributing factor in denture stomatitis and is known to be increased by high dietary sucrose (Hamada and Slade, 1980). Although Streptococcus mutans, the major component of dental plaque (Hamada and Slade, 1980) has no significant effect on candidal adhesion to epithelial cells and HeLa cells when incubated in adherence assays,

Streptococcus salivarius and Streptococcus mitior, constituents of normal oral flora, decrease candidal adhesion, and incubation with saliva increases candidal adhesion to epithelial cells and HeLa cells, suggesting that multiple factors are involved in the pathogenesis of denture stomatitis (Samaranayake and MacFarlane, 1982).

Vaginal candidiasis is also a common disease caused by Candida sp. and presents as a thick, yellow, milky discharge with patches of a gray-white pseudomembrane on the vaginal mucosa (Rippon,1982). Conditions which predispose the patient to vaginal candidiasis include diabetes, antibiotic therapy, oral contraceptives and pregnancy, especially during the third trimester (Rippon, 1982). Infections do occur in nonpregnant patients prior to menstruation (Rippon, 1982). For example, in one study 30% of vaginitis in pregnant females and 18% in nonpregnant females with vaginal discharge was caused by C. albicans (Holti, 1966). In in vitro studies, researchers found an increase in C. albicans adherence in situations where there was an increase in the number of intermediate epithelial cells. In addition, adherence of C. albicans isolates from patients with vaginitis was significantly higher than that of isolates of asymptomatic carriers (Segal, et al., 1984). Scanning electron microscopy studies have shown non-uniform distribution of adhering

microorganisms with diminished adherence in areas of active mitosis and proliferation, and increased adherence to mature flat cells often in the process of desquamation (Sobel, et al., 1982). Local defense factors present in the vaginal area include a preventative coating of vaginal cells with lactobacilli which decrease the pH of the environment and decrease the number of adhering C. albicans (Sobel et al., 1981). The pH of the vagina is increased and is optimum for candidal vaginitis during and after menses because lactobacilli are less prevalent in the vagina at that time (Mead, 1974). In addition, recurrent vaginitis is a common problem in patients and it is postulated that the continuous presence of C. albicans in the gastrointestinal tract of the host represents a reservoir from which this yeast can reinfect the vagina in cases of recurrent vaginitis (Miles et al., 1977). Enhanced susceptibility to adherence does not appear to be a factor responsible for recurrent vulvovaginal candidiasis patients who lack recognized predisposing factors, based on comparison to normal healthy volunteers (Trumbore and Sobel, 1986).

Other less common forms of candidiasis include endocarditis, pulmonary candidiasis, mucocutaneous candidiasis and esophageal candidiasis. The most common predisposing factors include generalized debility and other

conditions in which the host is immunocompromised in some way (Rippon, 1982). For example, esophageal candidiasis is one of the most common initial presenting conditions in patients with acquired immunodeficiency syndrome (AIDS) (Conant, 1987). Esophageal or oral candidiasis are conditions often present in patients with terminal neoplastic disease, or those who are being treated with antibacterial agents, corticosteroids or antimetabolic drugs (Braunswald et al., 1987). Prompt, aggressive treatment is a necessity to not only prevent systemic dissemination of the candidiasis, but also to prevent further deterioration of the patients immune status (Braunswald et al., 1987).

HOST DEFENSE

Host defense mechanisms against C. albicans involve both cell mediated and humoral immunity as well as non-specific immune mechanisms (Rogers and Balish, 1980). Antibodies to C. albicans are found in most individuals without a known prior history of Candida infection (Gough, 1984). It is postulated that the persistence of C. albicans in the gastrointestinal tract generates an antibody response in most individuals (Lehnmann and Reiss, 1980), even though other microorgansism that also reside in the gastrointestinal tract in higher numbers than C. albicans do not invoke a similar immune response (Berg and

Savage, 1972). The density of C. albicans in the gastrointestinal tract is affected by the intestinal flora and diet (Rippon, 1982). Certain piliated strains of bacteria such as Klebsiella can enhance the attachment of C. albicans to epithelial cells (Centeno et al., 1983), while increased Candida adherence in the presence of Escherichia coli is mediated by the presence of fimbriae on the bacterial cells (Makrides and MacFarlane, 1983).

Specific anti-candida antibodies are present in non-colonized patients, infected carriers of C. albicans without evidence of candidiasis, subjects with acute candidiasis and subjects with chronic candidiasis (Epstein et al., 1982). The titers of these antibodies reflect the degree of antigenic stimulation, and are significantly higher in candidiasis than in controls or colonized carriers (Epstein et al., 1982). Other researchers have found that titers from patients with Candida infections are often within the same range as those individuals without a Candida infection (Gough et al., 1984). In in vitro studies, Scheld et al., 1983, found that humoral antibody protected against C. albicans endocarditis through inhibition of adhesion. In patients with mucocutaneous candidiasis with a primary macrophage dysfunction leading to impairment of specific cellular immune responsiveness, carbohydrate antigens, essentially mannan, persisted in the

patients serum and inhibited the specific Candida-induced proliferation of lymphocytes and antibody (Fischer et al., 1982).

Bodily secretions have also been found to contain anti-candida antibody. Vaginal secretions contain IgE and IgA antibody classes to C. albicans (Gough, 1984), and lack of IgA in cervicovaginal secretions has been found to be related to the increased adherence capacity of C. albicans to vaginal epithelial cells in vitro (Romero-Piffiguer et al., 1985). In a study by Vudhichamnong et al., 1982, secretory IgA isolated from human breast milk inhibited the adherence of C. albicans to human oral epithelial cells by blocking surface sites on C. albicans, depending on the content and concentration of specific candidal antibody. This activity however could not be attributed solely to specific agglutination properties of the surface IgA. Conversely, nonspecificically bound surface IgA enhanced adherence of the yeast and impaired the immune disposal of C. albicans. In another study, a significant inverse correlation was found between salivary IgA anti-candida antibody and the adherence of C. albicans to buccal epithelial cells, suggesting that IgA antibody can inhibit adherence of Candida to oral mucosa (Epstein et al., 1982).

Polymorphonucleocytes (PMN'S) (Diamond, 1981) and macrophages (Lehrer and Fleishman, 1982) destroy C.

albicans as part of their nonspecific immune function against microinvasion of the host. In a study by Bistoni et al., 1986, pretreatment of mice with a strain of C. albicans incapable of yeast-mycelial conversion conferred protection against subsequent challenge with pathogenic C. albicans strains. This was accompanied by an increase in PMN's in peripheral blood and activation of splenic cells with highly candidacidal reactivity. Adoptive transfer of plastic adherent cells from pretreated mice to histocompatible recipients conferred protection against subsequent pathogenic strain challenge. In another study, PMN's from patients suffering from nodular lepromatous leprosy who were deficient in cellular immunity and from healthy controls had the same capacity to phagocytize and destroy C. albicans, indicating that other defense mechanisms are operational in dealing with infectious agents in patients with depressed cellular immunity (Hobers et al., 1986). Baccarini et al., 1983, also found that phagocytic killing involved multiple mechanisms such that candidacidal activity was found early in inflamation and was due to PMN's, adherent spleen cell populations, and macrophages to a limited extent. In another study, antigen specific activation of human T-cells using a C. albicans polysaccharide extract required both antigenic presentation and interleukin-I production (Lombardi et al.,

1984). In the absence of functional T-cells and viable bacterial flora, athymic and heterozygous littermate mice (adult and neonatal BALB-C) colonized with pure cultures of C. albicans manifested resistance to extensive mucocutaneous and systemic candidiasis (Balish et al., 1984). In a clinical study by Gardner et al., 1984, phagocytic cell function in the elderly did not decline at the same rate as the specific antibody immune response, indicating that the increased incidence of candidiasis with increasing age is associated with multiple host defense factors.

VIRULENCE

Candida albicans and other Candida sp. are 5-7 μm in size and grow as creamy smooth colonies although older C. albicans colonies can be wrinkled and folded in appearance (Beneke and Rogers, 1980). Candida species form mycelium and pseudomycelium under specific environmental conditions and when grown on specific media (Beneke and Rogers, 1980). Pseudomycelium is composed of elongated, undetached spores that may have clusters of blastoconidia at constrictions (Beneke and Rogers, 1980). The presence of mycelial forms in tissue connotes that colonization of the host has occurred (Rippon, 1982).

The mycelial form is considered to be the pathogenic or parasitic stage (Rippon, 1982). In one study epithelial

membranes were penetrated by hyphae but not blastoconidia (Howlett and Squier, 1980), although in tissue both forms are found (Rippon, 1982). Studies of oral candidiasis show that the filamentous form of C. albicans is necessary to penetrate epithelial cells (Martin et al., 1984; Montes and Wilborn, 1968). In a study using a rabbit model of renal candidiasis, the pseudohyphal form of C. albicans was necessary to cause a fatal infection (Whittle and Gresham, 1960), while in another study of renal candidiasis caused by C. albicans, growth of a germ tube into renal tubules provided an advantage for amplification of the organism (Barnes et al., 1983). Some investigators have found that the ability to become filamentous allows C. albicans to escape destruction in professional phagocytes (Arai et al., 1977). Others have found that the formation of germ tubes by C. albicans is a mechanism by which this organism escapes from phagocytic cells by conferring greater resistance to destruction by neutrophils and macrophages, as compared to the blastoconidial form (Culter and Poor, 1981). Other researchers believe that both yeast and mycelial forms of C. albicans can adhere, invade and proliferate in an infected host (Shepherd, 1985) and that the capacity of C. albicans to produce hyphae appears to be an important but nonessential virulence factor in the pathogenesis of candidal vaginitis (Sobel et al., 1984).

In addition, a recent study showed that C. albicans and C. tropicalis were capable of initiating tissue invasion before germ tubes had the opportunity to form and participate in the invasive process (Klotz et al., 1983).

Candida sp. and Torulopsis glabrata, as well as other pathogenic yeasts, can be cultured from clinical specimens using Sabourauds Dextrose Agar as an isolation medium (Beneke and Rogers, 1980). Identification of these yeasts involves demonstration of sugar assimilation patterns or secretion of enzymes specific for the organism in question (Beneke and Rogers, 1980; Sherris et al., 1984). Commercial yeast identification systems such as "API" or "Yeast Ident" are used in clinical microbiology laboratories for the identification of these organisms. In addition, C. albicans is the only organism which forms germ tubes when incubated in rich media or serum for one hour at 37^{o}C (Beneke and Rogers, 1980), and thus can be identified with this technique.

The virulence of Candida sp. in causing disease is determined by many factors including the immunological state of the host and characteristics of the organisms, including the ability to produce extracellular enzymes which aid in attachment and penetration of epithelial surfaces (Rippon, 1982). Although C. albicans produces several proteinases (Ruchel, 1984), there is evidence that

only one of these enzymes is a virulence factor (MacDonald and Odds, 1983). C. albicans produces phospholipases (Banno et al., 1985; Sammaranayake et al., 1984) which aid in the penetration of hyphae into epithelial cells (Howlett and Squier, 1980). Only C. albicans produces phospholipase activity while C. tropicalis, T. glabrata, and C. parapsilosis do not (Ruchel,1984). Banno et al., 1985, demonstrated that the mycelial form of C. albicans produces twice as much phospholipase activity as the yeast form, supporting the concept that the mycelial form of C. albicans is the most pathogenic form. In one study antibody to proteinase was detected in serum and in infected tissue with indirect immunofluorescence (MacDonald and Odds, 1980). In a study by Ghanndum and Elteen, 1986, all C. albicans isolates were found to secrete an inducible proteinase and C. albicans isolates which adhered most strongly to buccal epithelial cells had the highest relative proteinase activity and were most pathogenic. Other researchers have suggested that carbohydrases may also play a role in pathogenesis (Elinov, 1984), and that keratolytic proteinase activity in C. albicans is important in the pathogenicity of the organism in vivo (Hattori et al., 1984).

CELL WALL STRUCTURE

The cell wall of fungi is very important in protecting the organism from the environment and from host defense mechanisms (Rippon, 1982). Fungi can be subdivided into two categories according to cell wall composition, including chitin-glucan and chitin-mannan-glucan components (SanBlas, 1982). Other chemical components such as protein are present as well. Candida species are included in the chitin-mannan-glucan group along with other members of the Cryptococcaceae and the Saccharomycetaceae (SanBlas, 1982). Five to eight layers of the cell wall of C. albicans have been identified depending on growth conditions or cytochemical techniques utilized (Poulain et al, 1978). Chitin has been identified as the compound composing the bulk of the most electron transparent layer in transmission electron microscopy studies of germ tube formation (Cassone et al., 1973). The mycelial form of C. albicans has three times as much chitin and only one third as much protein as the blastoconidial form, which may explain the greater resistance of the mycelial form to host defense mechanisms that allows for the persistence of the mycelial form in vivo (Chattaway et al., 1968).

The localization of mannan in the cell wall of C. albicans has been investigated using a variety of techniques including lectins such as concanavalin-A,

antibodies to mannan, and biochemical and histochemical methods. Mannan is a highly branched sugar molecule with short chains of α-1,2 linked D-mannopyranosyl residues joined by α-1,6 linkages (Bishop et al., 1960). Histochemically, the localization of mannan in the outer cell wall of C. albicans has been confirmed by Djaczenko and Cassone, 1971; Poulain et al., 1978; and Evron and Drewe, 1984. Cassone et al., 1978, found an outer capsule-like component of spikey fibrillar protrusions of mannan, a homopolymer of mannose. Mannan-protein complexes span the entire cell wall of C. albicans and reach into the inner matrix, a bulky, rigid framework of glucan and chitin (Cassone et al., 1978; Chattaway et al., 1976). Other studies have shown that during the regeneration of protoplasts of C. albicans, radioactively labelled mannan is not associated with the protoplast indicating that mannan is not a structural polysaccharide of the cell wall as chitin and glucan are (Elorza et al., 1983).

The outer mannan layer is apparently easily shed (Montes and Wilborn, 1968; Poulain et al., 1978). Old cultures of C. albicans lose their outer cell wall layers which contain mannan (Poulain et al., 1978). Diedrich et al., 1984, and Diamond et al., 1980, isolated a glycoprotein that contained mannose in culture filtrates. In a study by Brawner and Cutler, 1986, two different cell

surface carbohydrate determinates both containing mannose and glucose were not expressed on very young cells and disappeared from the cell surface of most C. albicans strains with age. Poulain et al., 1978, postulated that this loss of outer cell wall mannan in older cells could account for the mannan antigenemia seen in patients with invasive and disseminated candidiasis (Weiner and Yount, 1976) and the suppression of the immune response experienced by these patients (Weiner and Coats-Stephens, 1979). In addition, antibody to mannan can be found in the serum of patients with candidiasis, in experimental infections in animals (Weiner and Coats-Stephens, 1979) and in normal uninfected individuals (Lehnman and Reiss, 1980). Circulating antigen-antibody complexes have been detected in the serum of patients with candidiasis (Burges et al., 1983) and immune complexes can be generated by adding mannan to human serum (Reiss et al., 1981).

BACTERIAL ADHERENCE

Adherence of microorganisms to biological surfaces has been recognized as the initial step in colonization and tissue invasion of mammalian membranes (Gibbons and VanHonte, 1975; Reed and Williams, 1978). It is also a mechanism by which sloughing of the organisms is prevented during the secretion of bodily fluids, food movement, or defecation (King et al., 1980). Specific mechanisms of

adherence between organisms and interface exist which utilize complementary molecular structures (Marshall, 1976). The most common mechanism of adherence based on this principle involves lectin-carbohydrate interaction (Dazzo, 1980; Sharon and Lis, 1972), while other mechanisms such as enzymatic interaction are recognized (Gibbons, 1977).

Bacterial attachment to epithelial membranes rarely occurs by direct contact between the bacteria and the epithelial cell membrane (Fletcher and Floodgate, 1973). These organisms utilize components of their capsules, fimbriae, or pili to complete the linkage to the host cell (Fletcher and Floodgate, 1973). For example, Escherichia coli (Johnson et al., 1979; Ofek and Beachey, 1978; Ofek et al., 1977), Klebsiella pneumoniae (Fader et al., 1979), Pseudomonas aeruginosa (Ramphal et al., 1980; Woods et al., 1980), Salmonella typhimuriam (Berkeley et al., 1981), Proteus (Silverblatt, 1974), and Shigella (Duguid et al., 1976) are type-1 piliated strains of bacteria which attach to epithelial cells via surface pili or fimbriae. They adhere in greater numbers to epithelial cells than non-piliated organisms, and utilize a mannose specific lectin that recognizes mannose moieties on the surface of epithelial cells (Ofek and Beachey, 1978; Ofek et al., 1977). Non-type-1 piliated strains of bacteria possess

capsular K antigens which resemble pili morphologically but are protein in nature which mediate adherence to epithelial cell surfaces (Savage, 1980). K-88 (Jones and Rutter, 1972; Berkeley et al., 1981) and K-99 (Moon et al., 1977) antigens mediate the adherence of enterotoxigenic Escherichia coli to neonatal swine small intestinal epithelial cells. The epithelial cell receptors to these antigens are terminal α-D-galactosyl residues of membrane glycoproteins (Gibbons and VanHonte, 1975; Gibbons, 1977). Other mediators of adherence in bacteria include mannose-resistant hemagglutinin for strains of Salamonella typhi (Berkeley, et al.,1981), pili for Neisseria sp.(Gubish et al., 1979), lipoteichoic acid for alpha Streptococci (Bartlett and Duncan, 1978; Ofek et al., 1975), and extracellular polymers attached to the cell surface such as with Streptococcus mutans (Kelstrup and Funder-Nielson, 1974).

ADHERENCE OF CANDIDA SPECIES

Adherence of Candida sp. to biological surfaces is important in establishing colonization and infection in the host. It has been shown that C. albicans adheres to buccal cells (Kimura and Pearsall, 1978,1980; Sandin et al., 1982), fibrin platelet matrixes formed in vitro (Maisch and Calderone, 1980, 1981), acrylic surfaces (McCourtie and Douglas, 1981), PMN's (Diamond et al., 1978), and other

reticuloendothelial system cells (Warr, 1980; Stahl et al., 1978). C. albicans has been found to adhere in greater numbers than C. stellatoidea, C. tropicalis, and other Candida sp. to vaginal cells (King et al., 1980), buccal cells (Kimura and Pearsall, 1980; King et al., 1980; Rotresen et al., 1986) and to fibrin platelet matrixes formed in vitro (Rotresen et al., 1985). Candida albicans also adheres more strongly to epithelial cells than fungal cells of other Candida sp. (Macura 1985).

It is generally agreed that there is great variation in the number of receptor sites for C. albicans on epithelial cells collected from different individuals (King et al., 1980; Sandin et al., 1987), and that day to day variations in adherence exist in swabbings from a single subject (King et al., 1980). Researchers have hypothesized that indigenous flora could suppress adherence of C. albicans by competing with it for receptor sites on epithelial cells, modifying these sites to hamper candidal adherence, or enzymatically altering the yeast surface (Liljemark and Gibbons, 1973). For example, the observation that females are more prone to suffer candidal vaginitis during and after menses (Mead, 1974) when lactobacilli are less prevalent in the vagina and pH is increased (Saigh et al., 1978) correlates with the findings that C. albicans adheres better to vaginal cells at a pH 6

rather than pH 3-4 in *in vitro* experiments (Sobel et al., 1981). Other researchers have found increased candidal adherence to small intestine cells due to the presence of *Escherichia coli* which is mediated by the presence of fimbriae on the bacterial cells and mannose-like receptors on both the surface of *C. albicans* and epithelial cells (Makrides and MacFarlane, 1983). Centeno et al., 1983 found that *Klebsiella* could also enhance the attachment of of *C. albicans* to intestinal epithelial cells.

Nonspecific host factors which affect candidal adherence to biological surfaces include local defense mechanisms and environmental conditions. Oral factors such as the epithelial barrier, salivary flow, microbial interactions, antimicrobial constituents of saliva, lysozyme, lactoferrin and lactoperoxidase systems, iron levels and salivary glycoproteins have been found to affect adherence (Epstein et al., 1984). Tear proteins including albumin, lactoferrin, lysozyme, and fibronectin enhance yeast adherence to both hard and soft contact lenses (Butrus and Klotz, 1986). Samaranayake and MacFarlane, 1982, found that exogenous sugar sources affected oral and vaginal carriage of *C. albicans* by modifying adherence properties.

The study of the adherence of *C. albicans* to biological surfaces has included both viable and formalized

cells in different growth stages. Many studies have shown that the germinated form of C. albicans adheres to epithelial cells in greater numbers than non-germinated forms (Kimura and Pearsall, 1978, 1980; King et al., 1980; Sandin et al., 1982; Rotresen et al., 1986), although both blastoconidial and mycelial forms of C. albicans can adhere, invade, and proliferate in an infected host (Shepherd, 1985). Another study showed that the capacity of C. albicans to produce hyphae appeared to be an important but nonessential virulence factor in the pathogenesis of candidal vaginitis (Sobel et al., 1984). Preincubation of C. albicans isolates in tissue-culture medium M-199 prior to adherence favors germ tube formation (Kimura and Pearsall, 1978, 1980; Sandin et al., 1982). Once germinated, the cells can be killed in formalin with no significant decrease in adherence, while treatment before germination decreases adherence (Kimura and Pearsall, 1978; Sandin et al., 1982). In a study of attachment to endothelial cells, viable or killed Candida organisms were enveloped by membrane processes from endothelial cell surfaces and were incorporated into the endothelial cells in phagosomes (Rotresen et al., 1985). Researchers have postulated that changes in the cell wall of C. albicans as it germinates could be responsible for the increased adherence seen (Kimura and Pearsall, 1978;

Sobel et al., 1981). In addition, the stationary phase of growth of C. albicans has shown greater adherence values than cells at the logarhythmic stage (King et al., 1980).

Candida albicans produces several extracellular enzymes which aid in adherence to epithelial cells and that contribute to the virulence of this organism. In a study by Ghanndum and Elteen, 1986, C. albicans isolates, especially strain type-C, adhered most strongly to buccal epithelial cells, had the highest proteinase activity and were most pathogenic. Candida albicans isolates secreted an inducible proteinase and showed a tendency toward greater adherence ability. In another study, C. albicans isolates which adhered most strongly to buccal epithelial cells were most pathogenic in mice and had the highest phospholipase activity (Barrett et al., 1985).

Adherence of C. albicans to polymeric surfaces has been studied and Minagi et al., 1985, found that the closer the surface free energy of the substrate surface and the microorganism, the higher the probability of adherence. Forces responsible for adherence of C. albicans and other Candida sp. to inert polymeric surfaces are London-Van der Waal forces (hydrophobic forces) and electrostatic forces, and in this study the hydrophobicity of the yeasts correlated with the tendency of yeast to adhere to polystyrene (Klotz et al., 1985). Another study suggests

that several factors are involved in the adhesion of C. albicans to plastic, and indicates that surface hydrophobicity is of minor importance in direct adhesion to epithelial cells but that it may contribute to indirect attachent to epithelial cells by promoting yeast coadhesion (Kennedy et al., 1988).

As stated previously, the mycelial form of C. albicans is postulated to be the pathogenic stage (Kimura and Pearsall, 1980), although other studies have shown that all stages of the organism can adhere and invade to cause infection in the host (Shepherd, 1985). In scanning electron microscopy studies, adherence of C. albicans to mouse vagina showed that six hours after infection short projections of the yeast were attaching to mucosal cell surfaces, and that later mycelium grew from the yeast (Campisi et al., 1986). In another study Barnes et al., 1983, found that attachment of C. albicans to endothelium within the capsule of the renal cortex was a key event in the disease process, and that growth of a germ tube into the renal tubules provided an advantage for amplification of C. albicans. In another study, Klotz et al., 1983, found that adherence and penetration of epithelial cells in the initiation of tissue invasion occurred before germ tubes had the opportunity to form and take part in the invasive process.

Characterization of the adhesin which mediates adherence of C. albicans to biological surfaces has been studied extensively. Kimura and Pearsall, 1978, found that enhanced adherence of C. albicans after incubation in saliva was related to changes in the fungus itself. In a study by Sobel et al., 1981, adherence of C. albicans was enhanced by a surface component of the germinated yeast, postulated to be a glycoprotein. In scanning electron microscopy studies yeast were associated with a mucus layer on epithelial surfaces throughout the gastrointestinal tract (Pope and Cole, 1981). Another study showed that attachment of C. albicans to buccal epithelial cells appeared to involve spatial rearrangement of their cell wall surface. Adhering yeast developed a fibrogranular surface layer which was detected by using specific carbohydrate staining techniques and concanavalin-A binding that appeared to mediate attachment of yeast to epithelial cells by a mannose-mediated receptor (Tronchin et al., 1984). Cassone et al, 1978, found that mannan is the major constituent of the fibrillar-floccular layer of the cell wall of C. albicans using con-A.

A variety of methods have been used to study the inhibition of adherence of C. albicans to different epithelial surfaces. Pretreatment of C. albicans with trypsin, chymotrypsin or proteinase decreased adherence

significantly (King et al., 1980; Maisch and Calderone, 1980; Silverblatt, 1974). Other researchers using an alkali treatment method determined that the extract was predominantly mannose (Maisch and Calderone, 1981). Loss of adherence after treatment of yeast cells with α-mannosidase or papain suggests that the cell wall mannoprotein is an essential component of the C. albicans adhesin (Lee and King, 1983). In a study by Sandin and Rogers, 1982, con-A pretreatment of the yeast inhibited adherence, while preincubation of the lectin with d-mannose restored adherence during the adherence assay. In addition, addition of α-D-methylmannopyranoside in the incubation medium during the adherence assay between yeast and buccal epithelial cells inhibited adherence (Sandin et al., 1982). In competitive inhibition studies using crude and purified cell wall products, blocking antibody, lectins, and controlled degradation of cell surface of C. albicans, mannans and mannoproteins appeared to be important constituents of the adhesin mediating adherence (Rotresen et al., 1986).

Chitin has also been found to inhibit adherence of C. albicans via N-acetylglucosamine to human vaginal epithelial cells, leading the authors to conclude that amino groups of sugars are responsible for inhibition of adherence of C. albicans to epithelial cells (Segal et al.,

1982). In another study, inhibition of adherence was produced by aminosugars including mannosamine, glucosamine and galactosamine (Collins-Lech et al., 1984). In addition, in experimental murine vaginitis, pretreatment of animals with chitin derivatives blocked the attachment of yeast to vaginal mucosal surfaces and led to the prevention of vaginal infection (Lehrer et al., 1983).

Scanning electron microscopy studies have also shown non-uniform distribution of adhering microorganisms with diminished adherence in areas of active mitosis and proliferation, and increased adherence to mature flat cells often in the process of desquamation (Sobel et al., 1982). Other studies have shown increased C. albicans adherence in situations where there is an increase in the number of intermediate epithelial cells (Segal et al., 1984), such as during pregnancy, and the first and fourth weeks of the menstrual cycle (Robbins et al., 1982), indicating that epithelial cell characteristics also affect adherence.

Elucidation of the adherence mechanisms utilized by C. albicans and other Candida sp. in causing disease is important in research and development of new treatment methods. Drugs used in the treatment of candidiasis or experimentally have been used to study adherence of C. albicans. Amphotericin B is a fungal cell membrane synthesis inhibitor which is used in the treatment of

candidiasis (Katzung, 1985). When used in adherence inhibition studies, amphotericin B inhibited adherence after 3-72 hours of exposure time at MIC or sub-MIC values. In comparison, 5-fluorocytosine, nystatin, miconazole and ketoconazole which all are drugs used in the treatment of candidiasis (Katzung, 1985), interfered with adherence only after 72 hours and at levels greater than MIC (Brenciaglia et al., 1986). Other studies with amphotericin-B showed a protective effect in mice receiving a single intraperitoneal injection which was dependent on dose, time of drug administration and size of yeast innoculum. These mice also showed resistance to subsequent challenge with C. albicans. Effector cells and macrophages from animals exposed to amphotericin-B show strong candidacidal reactivity (Bistoni et al., 1985).

Other drugs have also been used to study candidal adherence. For example, chlorhexidine is another antifungal agent which in one study decreased adherence by 19-86% in treatment of saliva or serum coated acrylic persisting up to 19 days after exposure. Adherence was also significantly decreased by pretreatment with chlorhexidine under conditions which enhanced adherence including stationary phase of growth, high galactose, and high sucrose (McCourtie et al., 1986). Noxythiolin, another antifungal agent, decreased adherence in both

exponential and stationary growth phases of both the blastoconidial and pseudohyphal forms, as well as when only the epithelial cells were treated with the drug (Gorman et al., 1986). In a clinical study of the use of nystatin, patients receiving two weeks of therapy had marked improvement in the signs and symptoms of candidiasis as well as a significant reduction in the number of candidal organisms present although the condition recurred rapidly following cessation of treatment (Epstein et al., 1981).

MATERIALS AND METHODS

BUCCAL MUCOSAL CELLS

Buccal cells were collected by gently rubbing the inside cheek area of six healthy adult volunteers with sterile swabs and swirling the swabs in phosphate buffered saline (PBS), pH 7.2 (Sandin et al., 1982). Donors had no signs or symptoms of oral thrush, were culture-negative for yeast (pharyngeal specimen) and were not taking antibacterial or antifungal agents at the time. The individual specimens were washed three times in PBS and then separately suspended to concentrations of 2×10^5 viable cells per ml of PBS, as determined by methylene blue staining and hemocytometer count.

ORGANISMS

Organisms used included clinical *Candida albicans* isolates MSU-1, 1840, UCLA, DA-05900, OHC-1134, 10-16-35F, *C. parapsilosis*, *C. tropicalis*, *C. pseudotropicalis*, *C. stellatoidea*, *Torulopsis glabrata*-1, and *T. glabrata*-3 (See Appendix A-I for clinical source of isolates). Each organism was grown on Sabauraud's Dextrose Agar (BBL, Cockeysville, MD, USA) slants at 37°C for 24-48 hours. A loop of cells was transferred to 100 ml of Trypticase Soy Broth (BBL Microbiology Systems, Cockeysville, MD) plus 4% glucose and incubated at 37°C on a rotary shaker (180 rpm) for approximately 15 hours to develop the stationary phase

of growth. The cells were then washed three times with PBS. Candida albicans isolates were resuspended in tissus-culture medium M199 (Gibco Laboratories, Santa Clara, CA) adjusted to pH of 7.2. These suspensions were incubated for one hour at 37°C for the developement of germ tubes (Sandin et al., 1982). Yeast with and without germ tubes were counted to determine the percentage of cells which had germinated. The organisms were then washed three times in PBS. For all organisms, approximately seven eighths of the yeast (germinated for Candida albicans isolates) were retained for viable cell studies and for cell treatment methods to be used in adherence studies. The remaining cells were resuspended in 0.5% formaldehyde in PBS for 30 min. at 4°C to kill the cells. After removal of the formaldehyde the yeast were washed three times in PBS. Viable and formalized preparations of the organisms were resuspended at 10^6 viable cells per ml of PBS, as determined by methylene blue staining and hemocytometer count. See Appendix A-II for diagram of organism preparation.

ADHERENCE ASSAY

In the adherence assay 0.2 ml samples of buccal and yeast cells were pipetted into 12 x 75 mm test tubes and incubated on a shaker at 180 rpm for 1 hr. at 37°C. Three tubes were used for each control (buccal cells only in

tube) and for each experimental test. Polycarbonate filters (12 μm pore size; Nucleopore Corp., Pleasanton, CA) were used for collection of the adherence mixture from each tube and washed with 100 ml PBS under continual agitation. These filters were used because the pore size allows only non-adhering yeast to go through, while buccal cells with adhering yeast remain. The filters were stained with Gram's Crystal Violet and the number of yeasts adhering to 100 buccal cells was determined by light microscopy at 430X. Double-blind conditions were used in all studies (Sandin et al., 1982). (See Appendix A-III for diagram of adherence procedure.)

TREATMENT METHODS (See Appendix A-IV for diagram of treatment method protocols.)

CONCANAVALIN-A: Concanavalin-A (Con-A) was used at a concentration of 10 μg/ml of PBS containing added cations (0.002 M Magnesium, Calcium, and Manganese salts), (Sandin et al., 1982). Con-A was obtained from ICN Pharmaceuticals, Cleveland, OH. A 5.0 ml sample of the germinated yeast (Candida albicans isolates) or viable yeast were pelleted and suspended in 15.0 ml of Con-A solution and incubated at room temperature on a shaker for 45 minutes. The cells were washed and resuspended in PBS to 5 ml and mixed with buccal cells for the adherence

assay. All steps were carried out under conditions of continual agitation to prevent agglutination.

α-D-METHYLMANNOPYRANOSIDE: 200 mg of α-D-methylmannopyranoside (A-DM) was dissolved in 0.5 ml PBS and added to adherence test tubes that already contained a total of 0.4 ml of the standardized yeast and buccal cell suspensions, or to the control tubes. The contents of each tube were mixed on a Vortex mixer and were immediately set on a shaker at 180 rpm to proceed with the adherence test.

CITRIC ACID: A 5.0 ml sample of the germinated or viable yeast was pelleted and resuspended in 15.0 ml of a 0.1 M, pH 7.0) solution of citric acid in PBS, autoclaved for 1 hr. at 120°C, washed three times in PBS, and resuspended to 5.0 ml in PBS (Tronchin et al., 1984). These cells were then mixed with buccal cells for the adherence assay.

FILAMENTOUS HEMAGGLUTININ: A 5.0 ml sample of the germinated or viable yeast preparation was pelleted and resuspended in 15.0 ml of a 40 μg/ml solution of filamentous hemagglutinin (FHA) (courtesy of Dr. Larry Winberry, Michigan Department of Public Health, Biologic Products Division) and incubated for 45 min. on a shaker at room temperature. The cells were washed three times in PBS and resuspended to 5.0 ml in PBS. They were then mixed with the buccal cells for the adherence assay. All steps

were carried out under conditions of continual agitation to prevent agglutination.

STATISTICS

The results were analyzed using a two way analysis of variance where two factors are considered simultaneously and the two sources of variance are of equal rank. (Robert, et al., 1981). Mean and standard deviation calculations were also completed where appropriate.

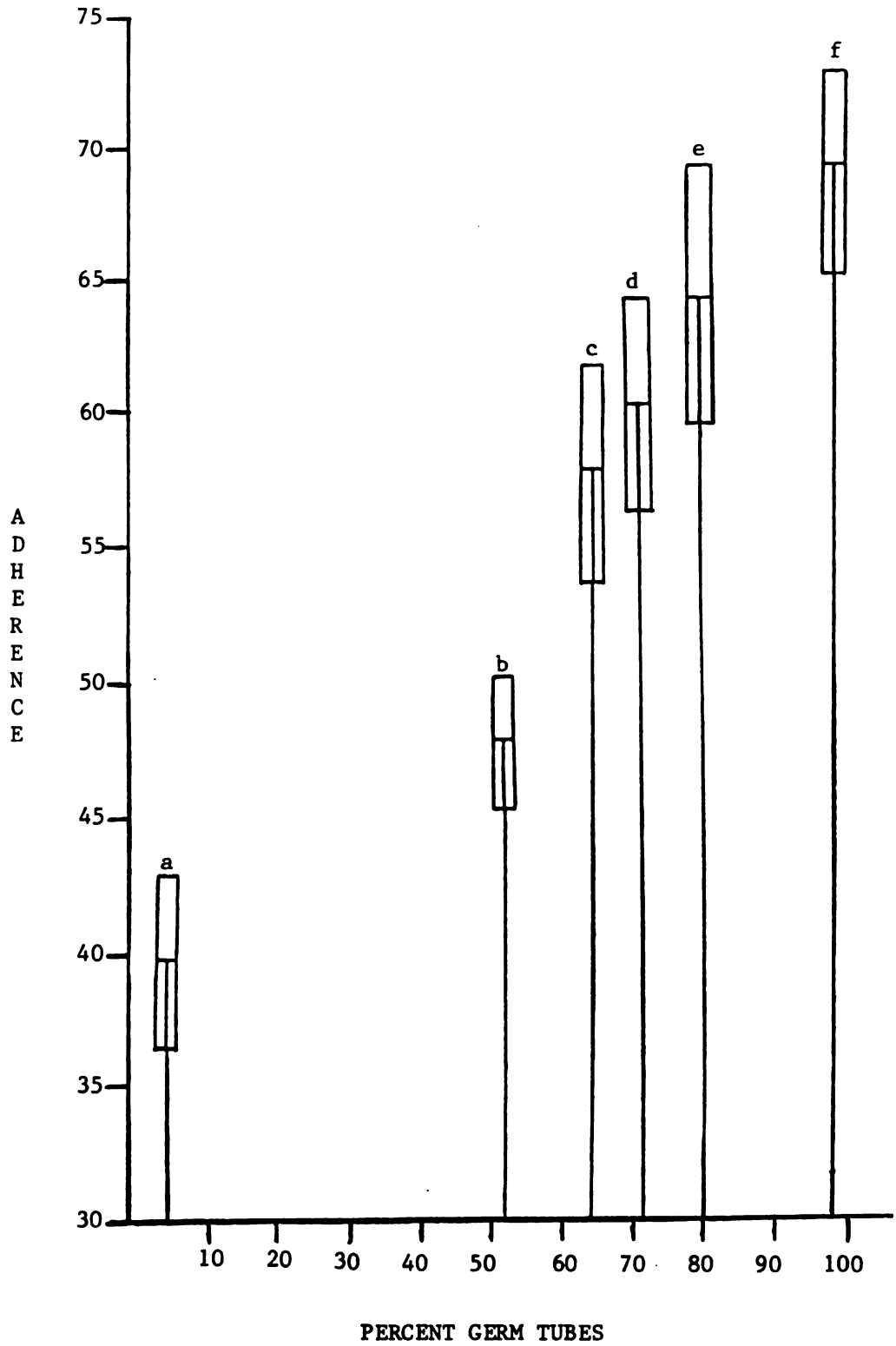
RESULTS

Candida albicans isolates were subjected to conditions which permitted germ tube formation and used in the adherence assay. Adherence represents the average number of yeast adhering to 100 buccal epithelial cells in six different human buccal epithelial cell donor samples, in triplicate. C. albicans isolate MSU-1 (99% germ tubes) had an average of approximately 69 yeast cells adhering to 100 buccal cells in each donor sample, while isolate 10-16-35F (80% germ tubes) had an average of 64 adhering yeast per 100 bucccal cells. Isolate ONC-1134 (71% germ tubes) had approximately 60 adhering yeast, DA-05900 had an average of 57 yeast adhering, UCLA had approximately 47 yeast adhering, and 1840 had an average of 40 adhering yeast per 100 buccal cells (Figure 1). As germ tube formation capability increased for C. albicans isolates, adherence increased. The C. albicans strains used were clinical isolates (Appendix A-I).

Adherence of Candida spp. and Torulopsis glabrata isolates to human buccal epithelial cells was examined using germinated C. albicans and non-germinated alternate Candida spp. and T. glabrata isolates. (The number in parenthesis after each isolate represents the range of the average number of yeast adhering to one humdred buccal epithelial cells in six different human buccal epithelial cell samples tested.) C. albicans (69) adhered to a

FIGURE 1. Comparison of adherence of Candida albicans isolates of differing germ tube formation capability to human buccal epithelial cells. (Percent adherence for each organism determined by averaging percent adherence of formalized and viable yeast to epithelial cells.) Organisms include:

a. 1840, 5% germ tubes
b. UCLA, 52% germ tubes
c. DA-05900, 65% germ tubes
d. ONC-1134, 71% germ tubes
e. 10-16-35F, 80% germ tubes
f. MSU-I, 99% germ tubes


75
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65
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50
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35
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A
D
H
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R
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N
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b
c
d
e
f
10
20
30
40
50
60
70
80
90
100
PERCENT GERM TUBES

greater extent than T. glabrata isolates (30-33) or other Candida spp. (15-25) to human buccal epithelial cells, with T. glabrata isolates (30-33) adhering greater than (in order of decreasing adherence) C. tropicalis (25), C. pseudotropicalis (22), C. parapsilosis (19), or C. stellatoidea (15) (Figure 2). Viable organisms (germinated C. albicans) were used in the adherence assay. Organisms used in this study were clinical isolates (see Appendix A-I) and were chosen based on frequency of isolation in patient populations with yeast colonization and infection.

Figures 3-14 demonstrate the effect of various treatment methods on adherence of six C. albicans isolates, two T. glabrata strains, C. stellatoidea, C. parapsilosis, C. tropicalis, and C. pseudotropicalis to six different human buccal epithelial cell samples. Treatment methods included formalization (FY) which killed the organisms, treatment with citric acid (CY) which decreased cell wall mannan, filamentous hemagglutinin treatment (FHA) which bound cell wall mannan and functioned as a mannan inhibitor, concanavalin-A treatment (CON-A) which also bound cell wall mannan and also functioned as a mannan inhibitor, and treatment of the adherence mixture with α-D-methylmanno pyranoside (A-DM) which as a complementary sugar, inhibited mannan. Viable yeast were used for all

FIGURE 2. Comparison of adherence of clinical isolates of *Candida* sp. and *Torulopsis* glabrata to human buccal epithelial cells.

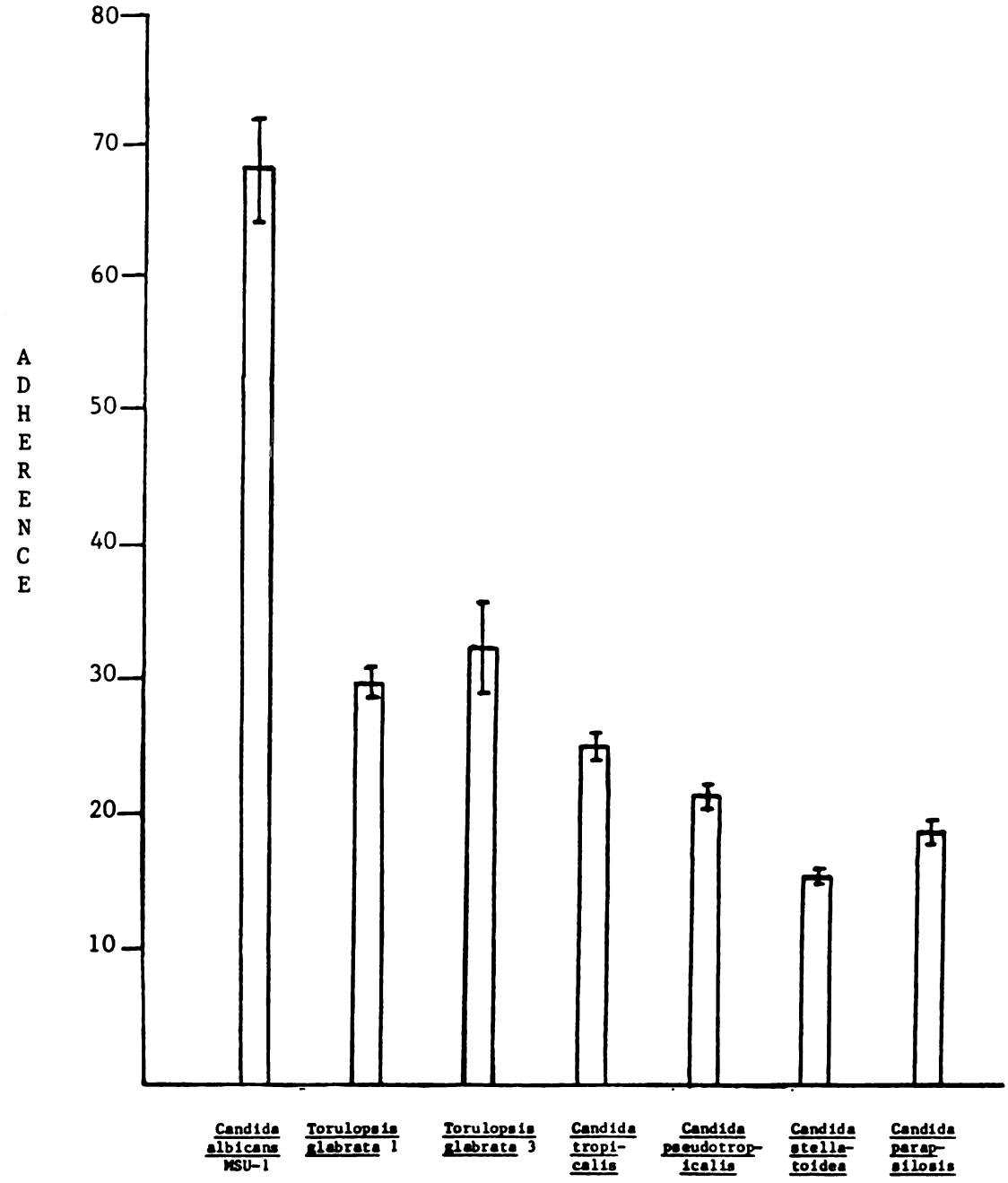
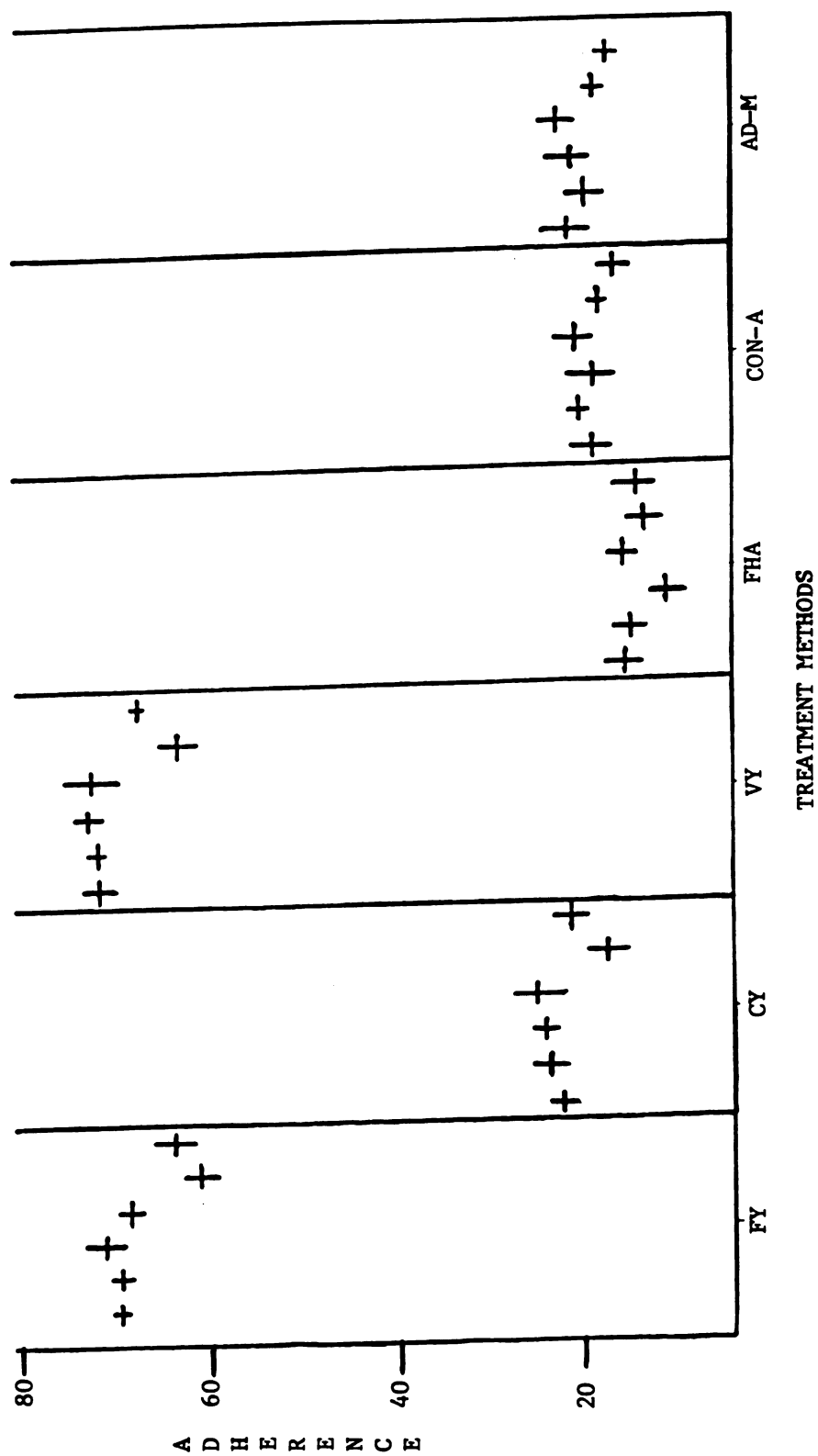

80
70
60
50
40
30
20
10
ADHERENCE
Candida albicans MSU-1
Torulopsis glabrata 1
Torulopsis glabrata 3
Candida tropicalis
Candida pseudotropicalis
Candida stellatoidea
Candida parapsilosis

FIGURE 3. Comparison of the effect of different treatment methods on adherence of Candida albicans isolate MSU-I to six different human buccal epithelial cell samples.* Treatment methods include:

- FY: formalin treated yeast (kills yeast)
- CY: yeast treated with citric acid (decreases cell wall mannan)
- VY: viable yeast
- FHA: filamentous hemagglutinin treated yeast (binds mannan resulting in inhibition)
- Con-A: concanavalin-A treated yeast (binds mannan resulting in inhibition)
- A-DM: α-D-methylmannopyranoside treated adherence mixtures (complementary sugar mannan inhibition).

* The vertical error bars indicate the standard deviation within individual buccal cell samples.


80
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40
20
ADHERENCE
FY
CY
VY
FHA
CON-A
AD-M
TREATMENT METHODS

FIGURE 4. Comparison of the effect of different treatment methods on adherence of Candida albicans isolate 10-16-35F to six different human buccal epithelial cell samples.* Treatment methods include:

FY: formalin treated yeast (kills yeast)
CY: yeast treated with citric acid (decreases cell wall mannan)
VY: viable yeast
FHA: filamentous hemagglutinin treated yeast (binds mannan resulting in inhibition)
Con-A: concanavalin-A treated yeast (binds mannan resulting in inhibition)
A-DM: α-D-methylmannopyranoside treated adherence mixtures (complementary sugar mannan inhibition).

* The vertical error bars indicate the standard deviation within individual buccal cell samples.

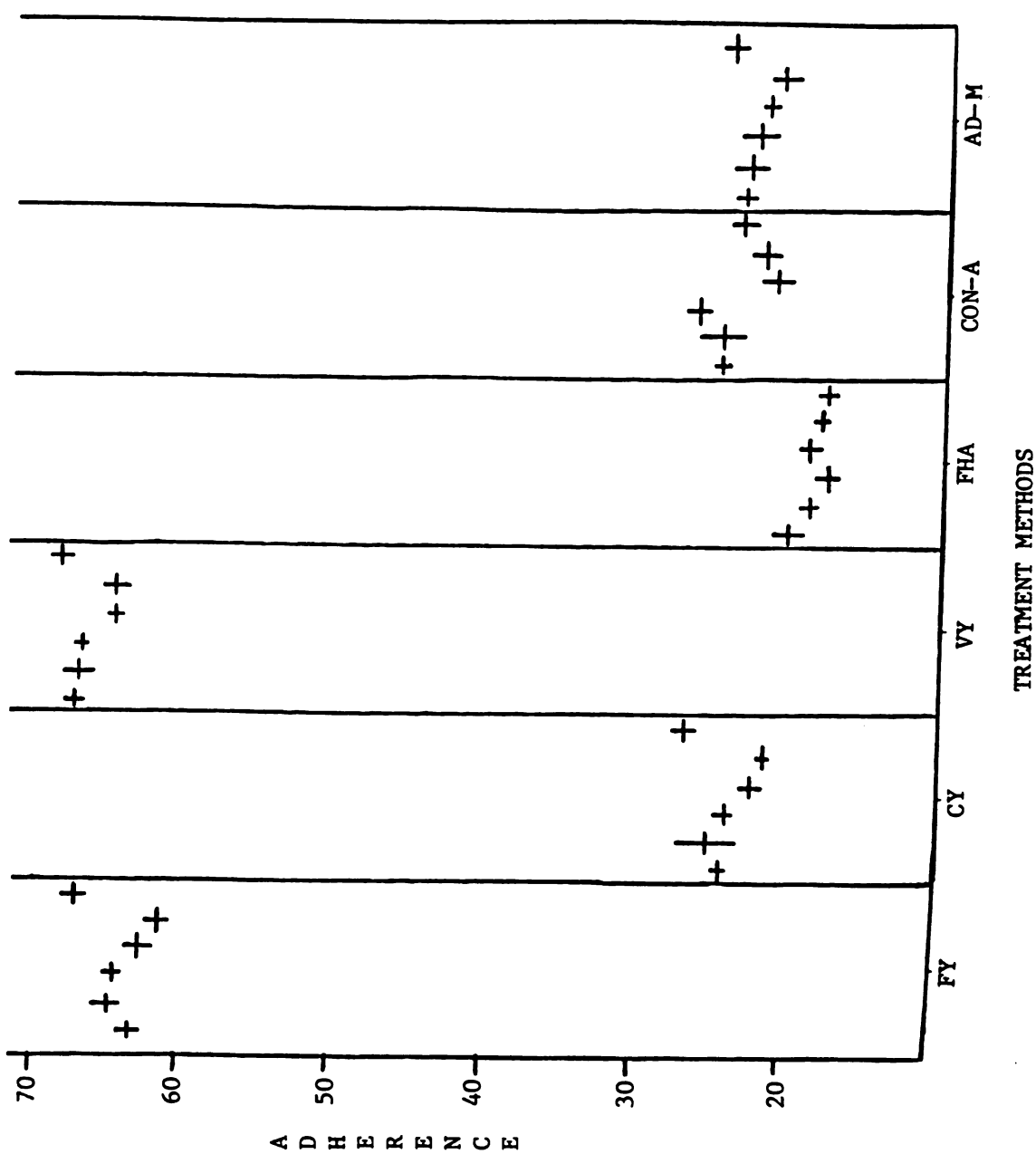
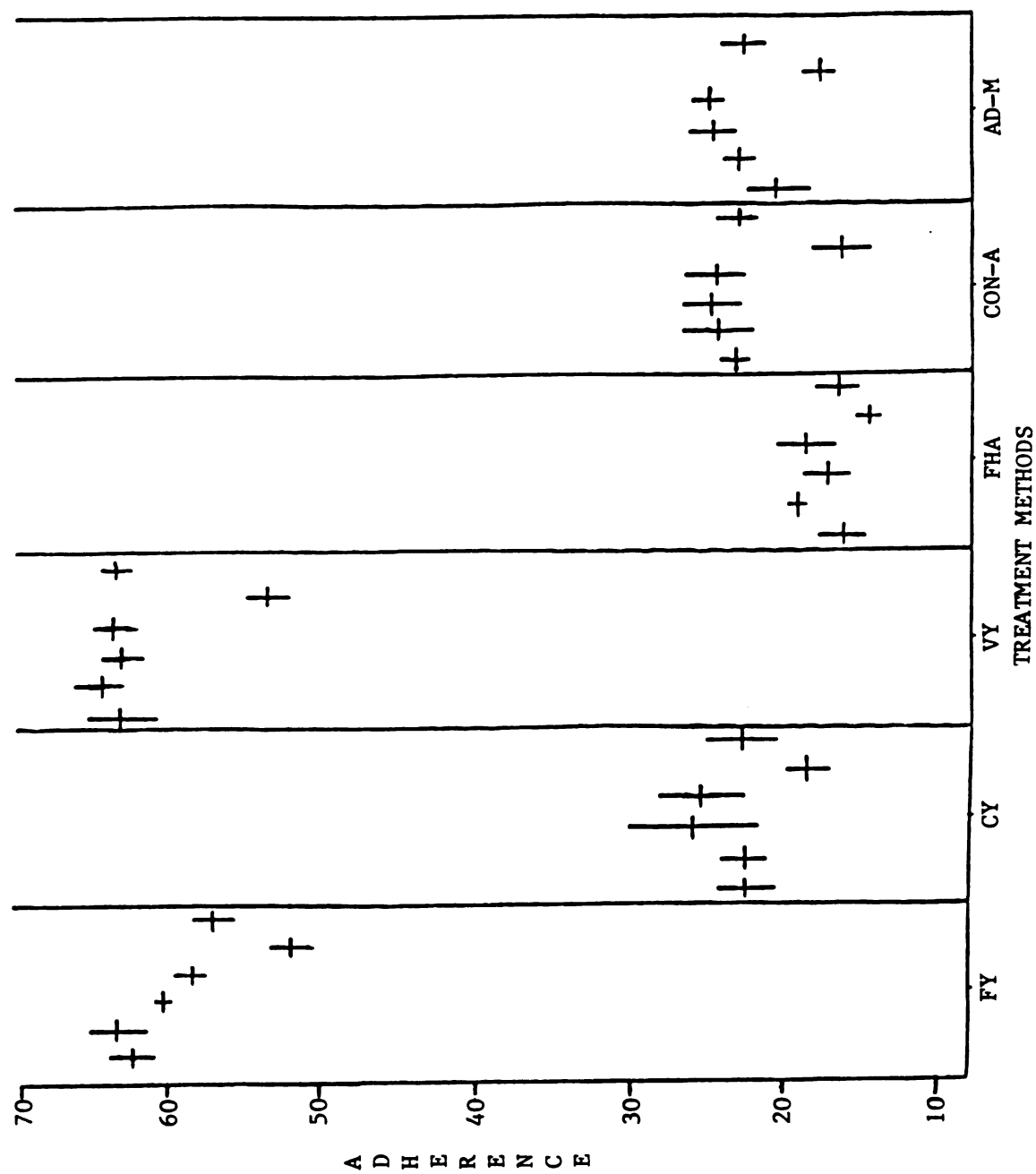

ADHERENCE
70
60
50
40
30
20
FY
CY
VY
FHA
CON-A
AD-M
TREATMENT METHODS

FIGURE 5. Comparison of the effect of different treatment methods on adherence of Candida albicans isolate ONC-1134 to six different human buccal epithelial cell samples.* Treatment methods include:

FY: formalin treated yeast (kills yeast)
CY: yeast treated with citric acid (decreases cell wall mannan)
VY: viable yeast
FHA: filamentous hemagglutinin treated yeast (binds mannan resulting in inhibition)
Con-A: concanavalin-A treated yeast (binds mannan resulting in inhibition)
A-DM: α-D-methylmannopyranoside treated adherence mixtures (complementary sugar mannan inhibition).

* The vertical error bars indicate the standard deviation within individual buccal cell samples.


70
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20
10
ADHERENCE
FY
CY
VY
FHA
CON-A
AD-M
TREATMENT METHODS

FIGURE 6. Comparison of the effect of different treatment methods on adherence of Candida albicans isolate DA-05900 to six different human buccal epithelial cell samples.* Treatment methods include:

FY: formalin treated yeast (kills yeast)
CY: yeast treated with citric acid (decreases cell wall mannan)
VY: viable yeast
FHA: filamentous hemagglutinin treated yeast (binds mannan resulting in inhibition)
Con-A: concanavalin-A treated yeast (binds mannan resulting in inhibition)
A-DM: α-D-methylmannopyranoside treated adherence mixtures (complementary sugar mannan inhibition).

* The vertical error bars indicate the standard deviation within individual buccal cell samples.

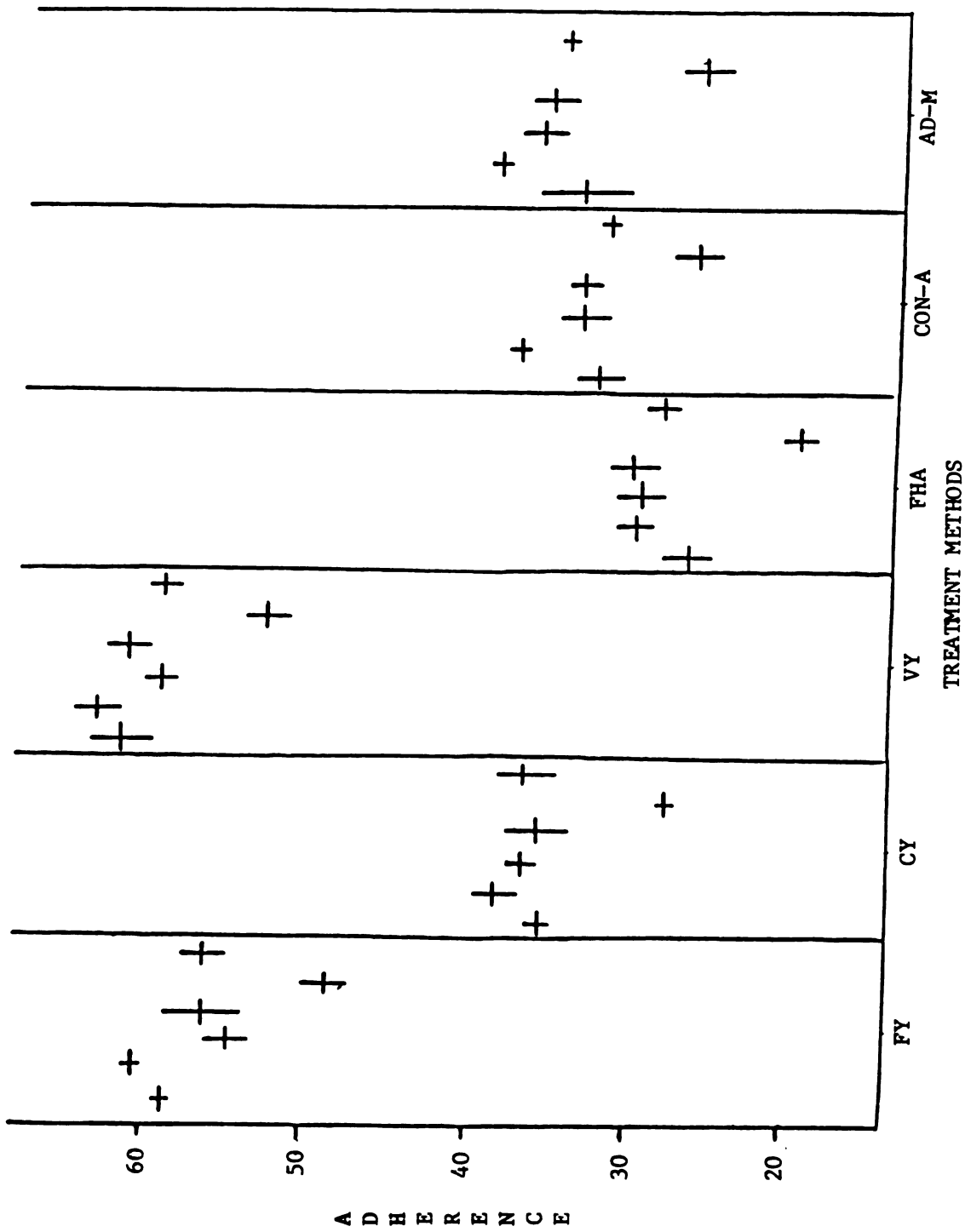

ADHERENCE
60
50
40
30
20
FY
CY
VY
FHA
CON-A
AD-M
TREATMENT METHODS

FIGURE 7. Comparison of the effect of different treatment methods on adherence of Candida albicans isolate UCLA to six different human buccal epithelial cell samples.* Treatment methods include:

FY: formalin treated yeast (kills yeast)
CY: yeast treated with citric acid (decreases cell wall mannan)
VY: viable yeast
FHA: filamentous hemagglutinin treated yeast (binds mannan resulting in inhiition)
Con-A: concanavalin-A treated yeast (binds mannan resulting in inhibition)
A-DM: α-D-methylmannopyranoside treated adherence mixtures (complementary sugar mannan inhibition).

* The vertical error bars indicate the standard deviation within individual buccal cell samples.

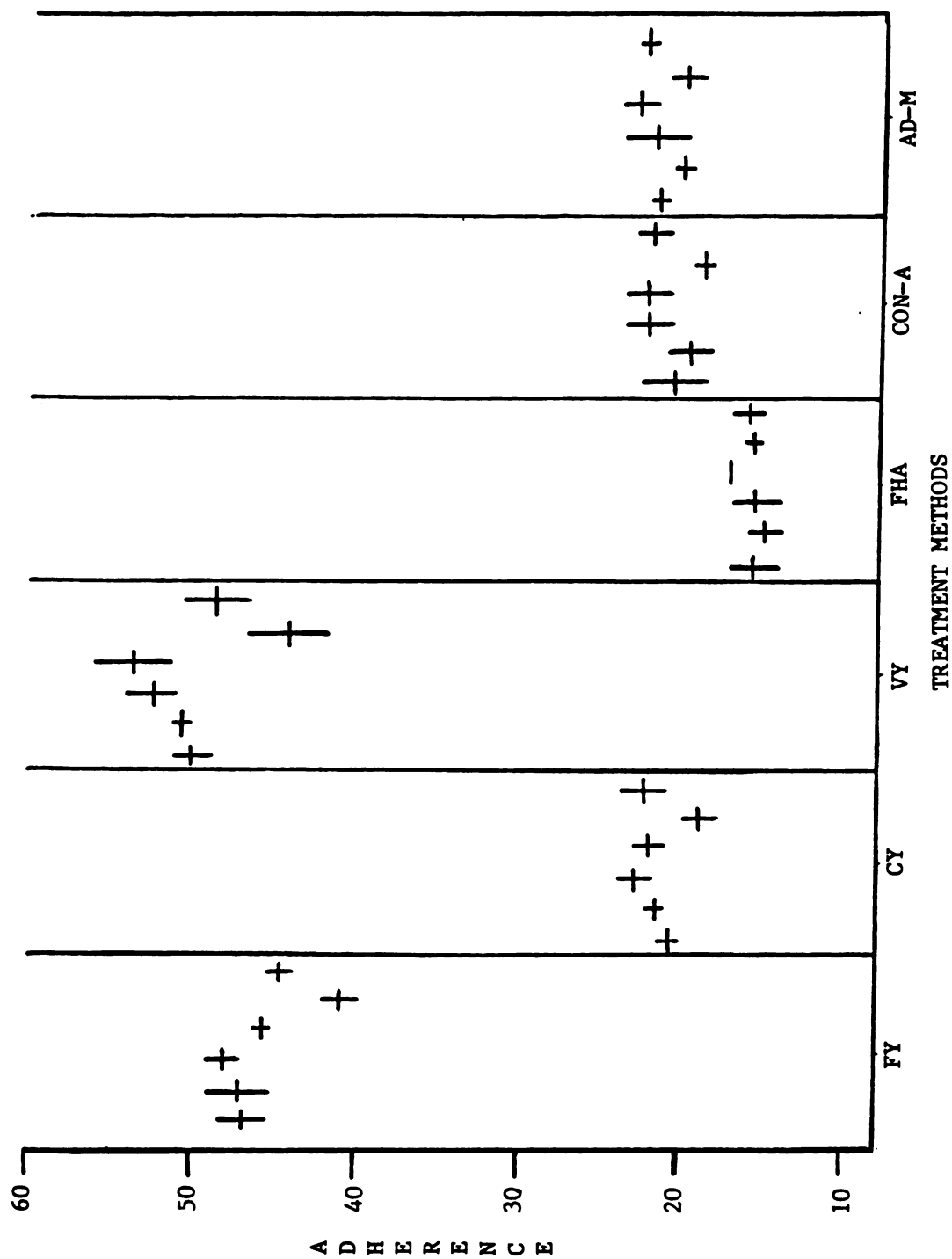

ADHERENCE
60
50
40
30
20
10
FY
CY
VY
FHA
CON-A
AD-M
TREATMENT METHODS

FIGURE 8. Comparison of the effect of different treatment methods on adherence of Candida albicans isolate 1840 to six different human buccal epithelial cell samples.* Treatment methods include:

- FY: formalin treated yeast (kills yeast)
- CY: yeast treated with citric acid (decreases cell wall mannan)
- VY: viable yeast
- FHA: filamentous hemagglutinin treated yeast (binds mannan resulting in inhibition)
- Con-A: concanavalin-A treated yeast (binds mannan resulting in inhibition)
- A-DM: α-D-methylmannopyranoside treated adherence mixtures (complementary sugar mannan inhibition).

* The vertical error bars indicate the standard deviation within individual buccal cell samples.

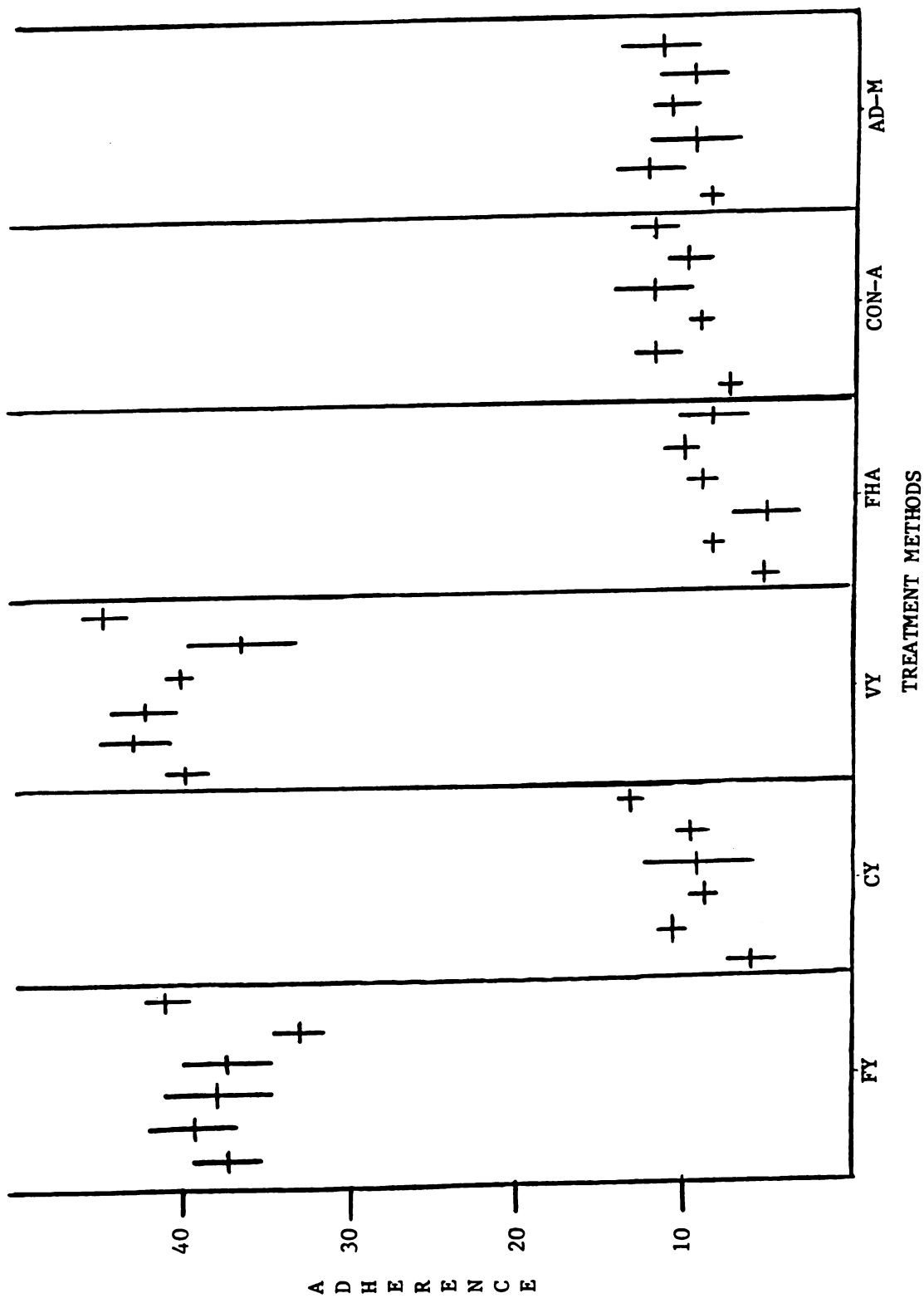

ADHERENCE
40
30
20
10
FY
CY
VY
FHA
CON-A
AD-M
TREATMENT METHODS

FIGURE 9. Comparison of the effect of different treatment methods on adherence of Torulopsis glabrata isolate #1 to six different human buccal epithelial cell samples.* Treatment methods include:

FY: formalin treated yeast (kills yeast)
CY: yeast treated with citric acid (decreases cell wall mannan)
VY: viable yeast
FHA: filamentous hemagglutinin treated yeast (binds mannan resulting in inhibition)
Con-A: concanavalin-A treated yeast (binds mannan resulting in inhibition)
A-DM: α-D-methylmannopyranoside treated adherence mixtures (complementary sugar mannan inhibition).

* The vertical error bars indicate the standard deviation within individual buccal cell samples.

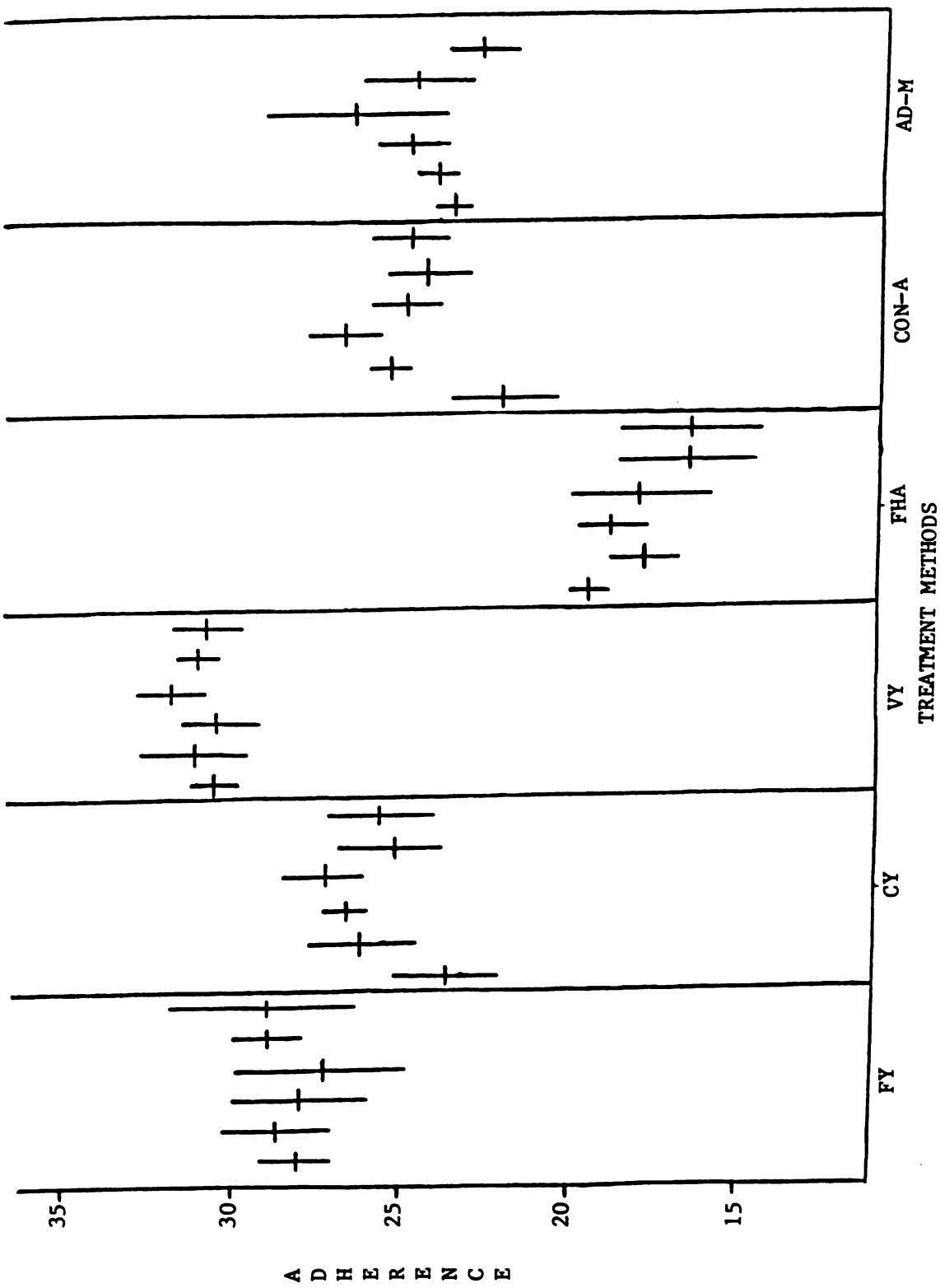

ADHERENCE
35
30
25
20
15
FY
CY
VY
FHA
CON-A
AD-M
TREATMENT METHODS

FIGURE 10. Comparison of the effect of different treatment methods on adherence of Torulopsis glabrata isolate #3 to six different human buccal epithelial cell samples.* Treatment methods include:

FY: formalin treated yeast (kills yeast)
CY: yeast treated with citric acid (decreases cell wall mannan)
VY: viable yeast
FHA: filamentous hemagglutinin treated yeast (binds mannan resulting in inhibition)
Con-A: concanavalin-A treated yeast (binds mannan resulting in inhibition)
A-DM: α-D-methylmannopyranoside treated adherence mixtures (complementary sugar mannan inhibition).

* The vertical error bars indicate the standard deviation within individual buccal cell samples.

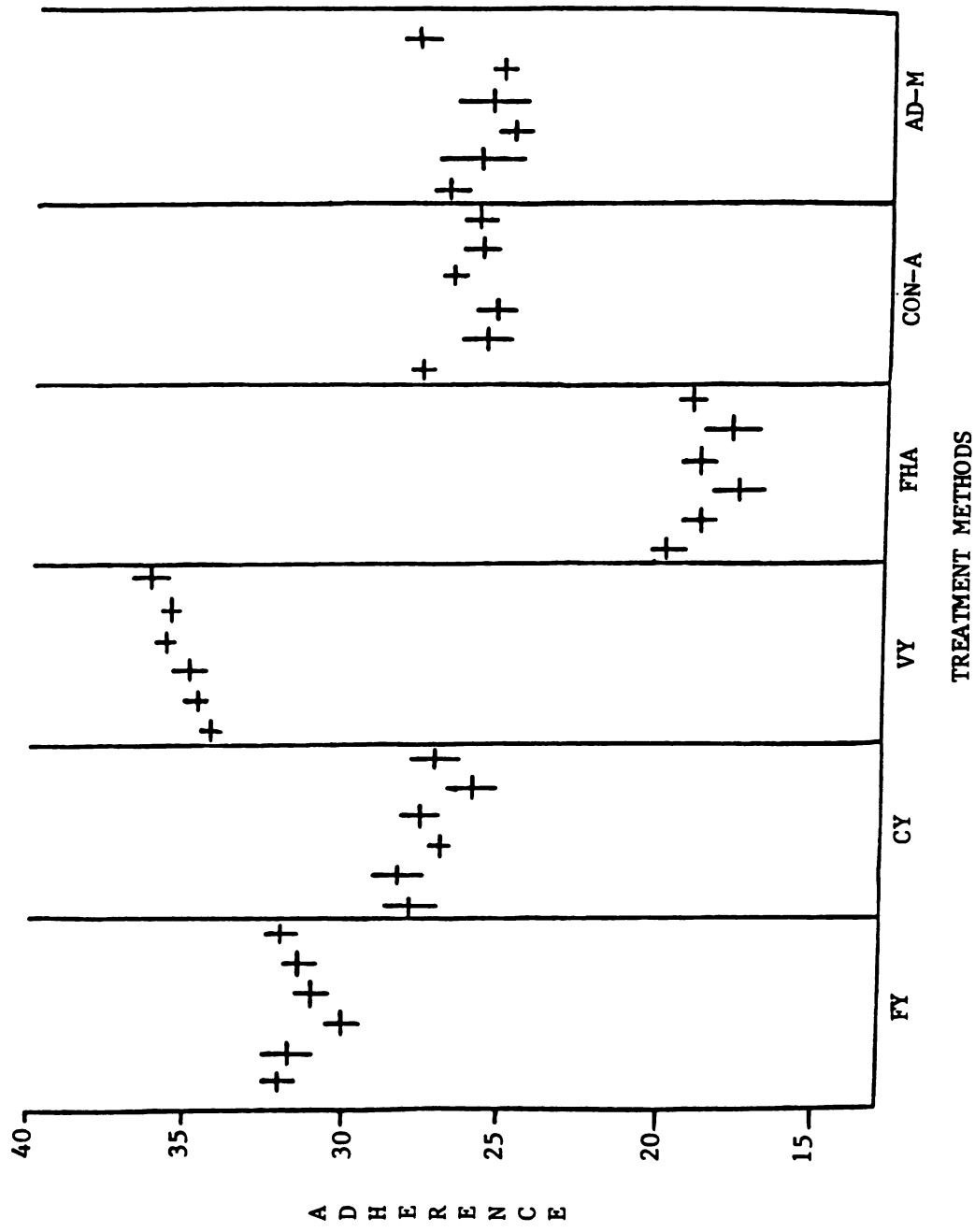

ADHERENCE
40
35
30
25
20
15
FY
CY
VY
FHA
CON-A
AD-M
TREATMENT METHODS

FIGURE 11. Comparison of the effect of different treatment methods on adherence of Candida stellatoidea to four different human buccal epithelial cell samples.* Treatment methods include:

FY: formalin treated yeast (kills yeast)
CY: yeast treated with citric acid (decreases cell wall mannan)
VY: viable yeast
FHA: filamentous hemagglutinin treated yeast (binds mannan resulting in inhibition)
Con-A: concanavalin-A treated yeast (binds mannan resulting in inhibition)
A-DM: α-D-methylmannopyranoside treated adherence mixtures (complementary sugar mannan inhibition).

* The vertical error bars indicate the standard deviation within individual buccal cell samples.

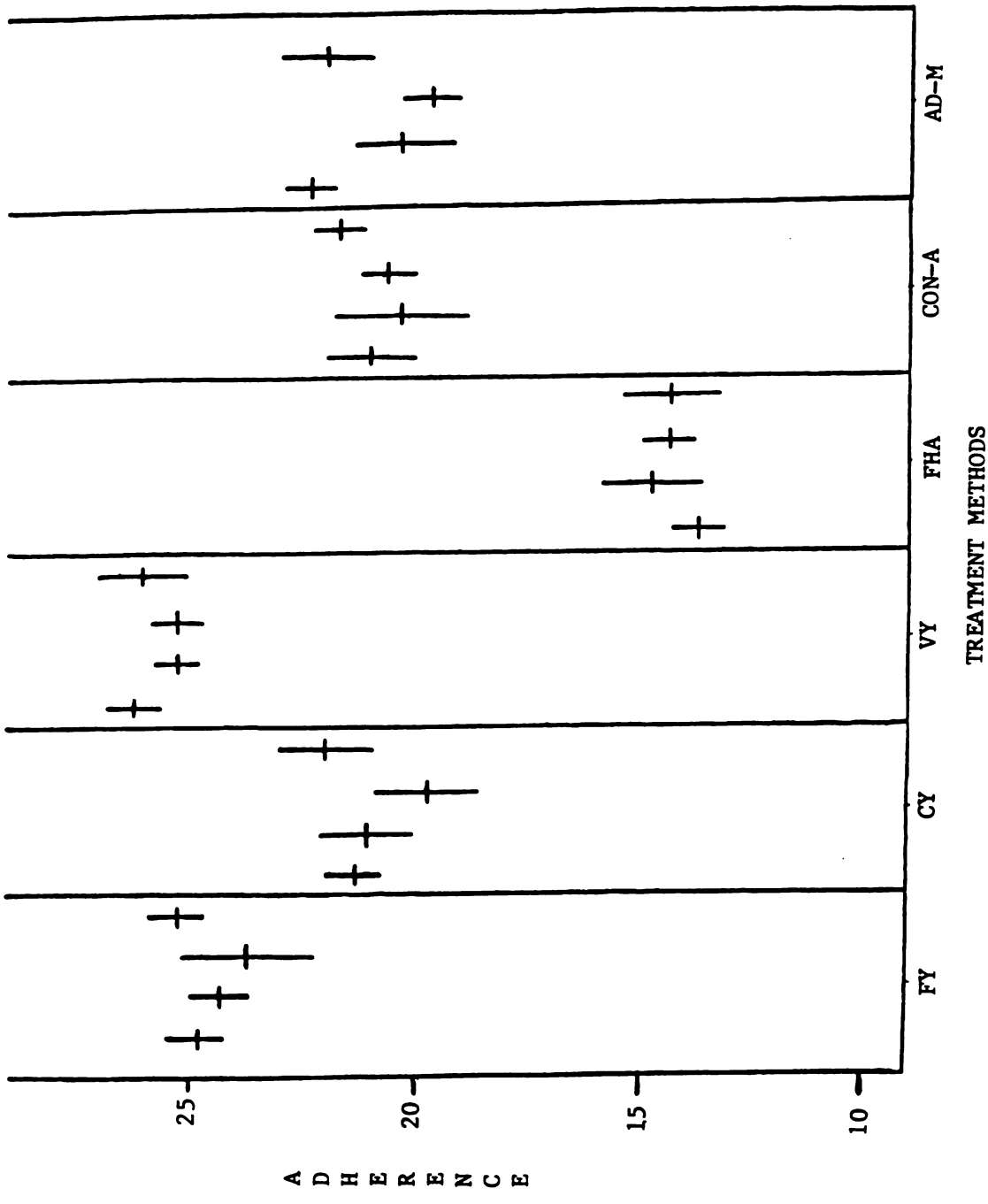

ADHERENCE
25
20
15
10
FY
CY
VY
FHA
CON-A
AD-M
TREATMENT METHODS

FIGURE 12. Comparison of the effect of different treatment methods on adherence of Candida parapsilosis to four different human buccal epithelial cell samples.* Treatment methods include:

FY: formalin treated yeast (kills yeast)
CY: yeast treated with citric acid (decreases cell wall mannan)
VY: viable yeast
FHA: filamentous hemagglutinin treated yeast (binds mannan resulting in inhibition)
Con-A: concanavalin-A treated yeast (binds mannan resulting in inhibition)
A-DM: α-D-methylmannopyranoside treated adherence mixtures (complementary sugar mannan inhibition).

* The vertical error bars indicate the standard deviation within individual buccal cell samples.

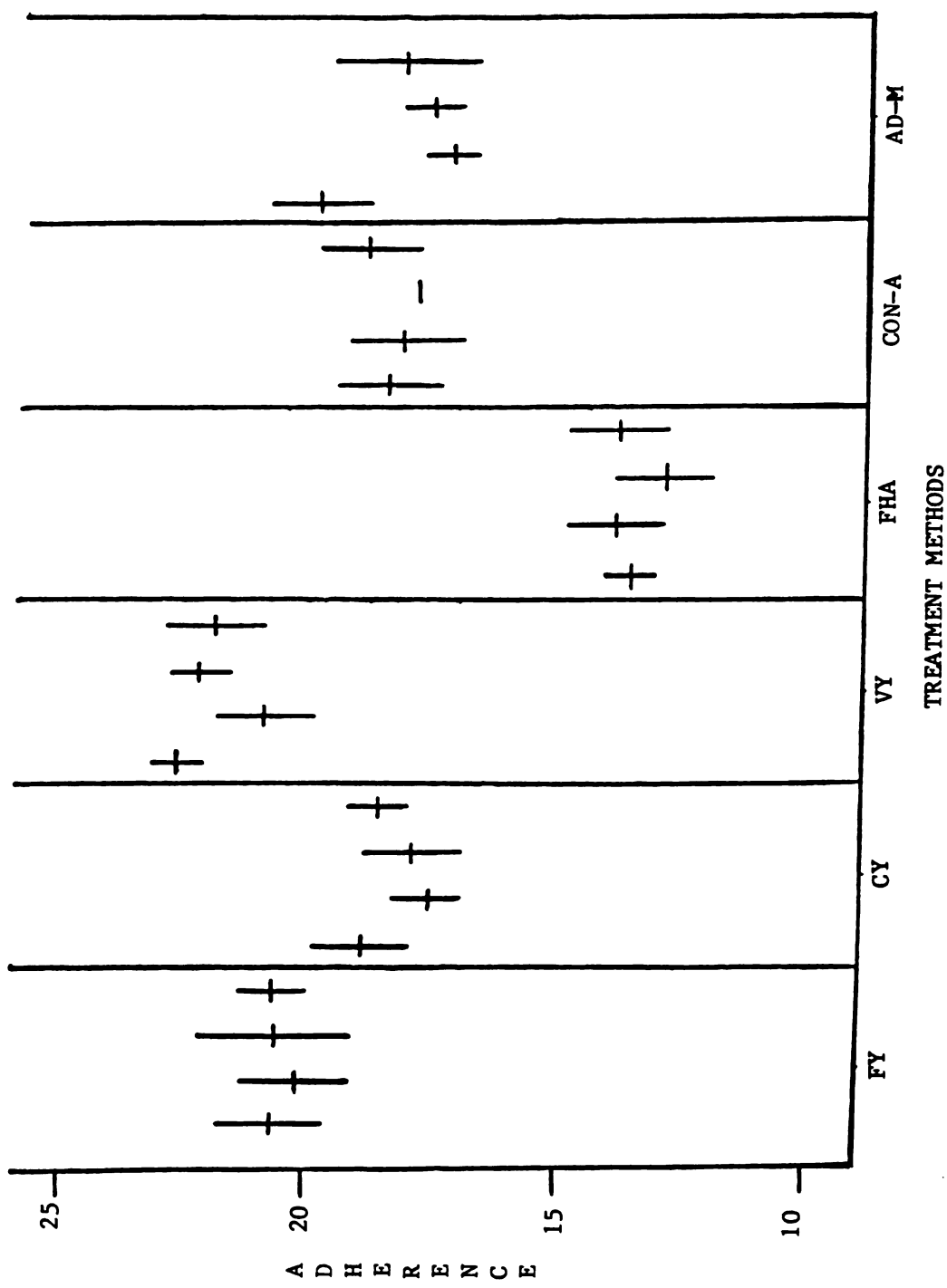

25
20
15
10
ADHERENCE
FY
CY
VY
FHA
CON-A
AD-M
TREATMENT METHODS

FIGURE 13. Comparison of the effect of different treatment methods on adherence of Candida tropicalis to four different human buccal epithelial cell samples.* Treatment methods include:

FY: formalin treated yeast (kills yeast)
CY: yeast treated with citric acid (decreases cell wall mannan)
VY: viable yeast
FHA: filamentous hemagglutinin treated yeast (binds mannan resulting in inhibition)
Con-A: concanavalin-A treated yeast (binds mannan resulting in inhibition)
A-DM: α-D-methylmannopyranoside treated adherence mixtures (complementary sugar mannan inhibition).

* The vertical error bars indicate the standard deviation within individual buccal cell samples.

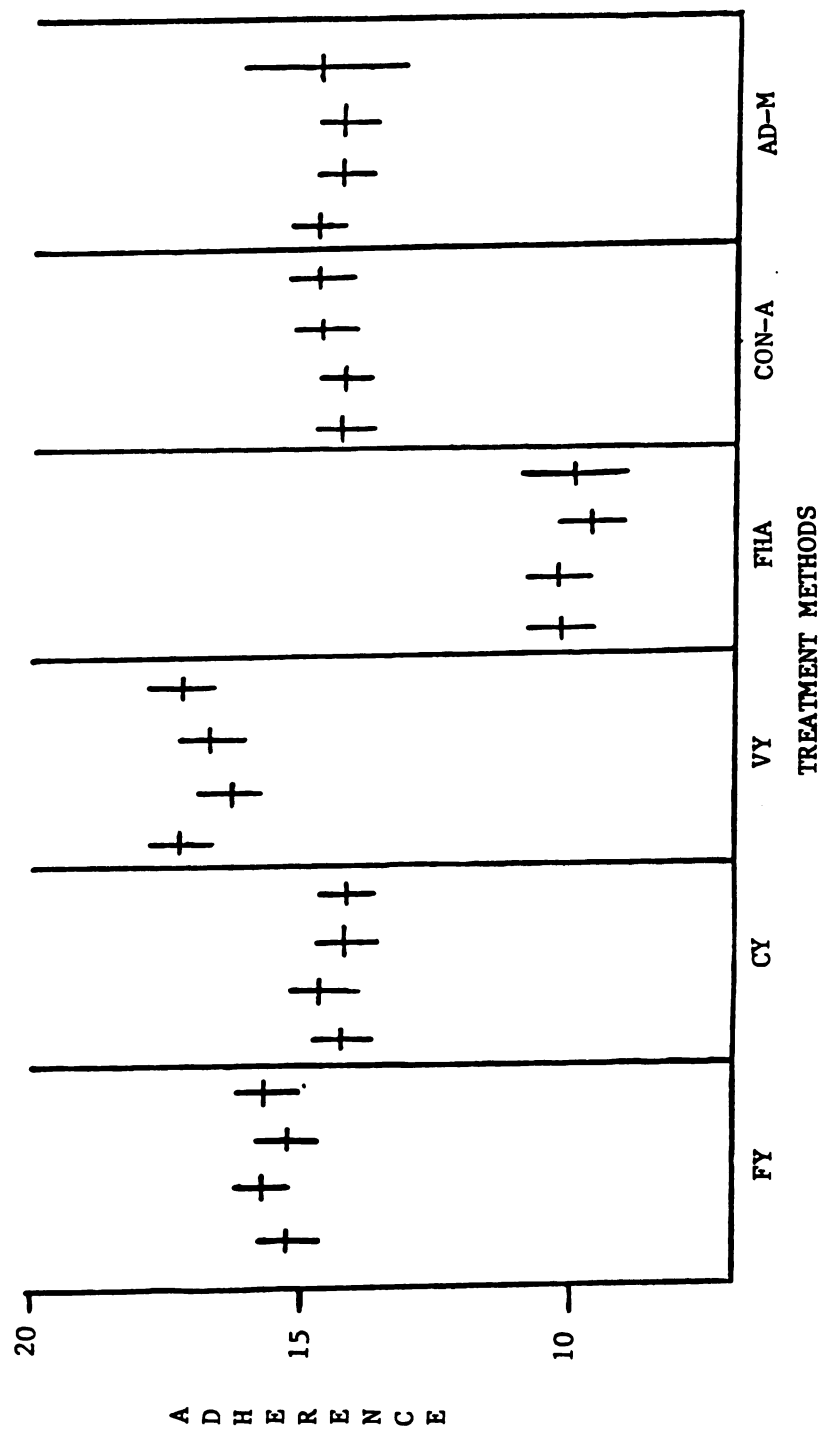
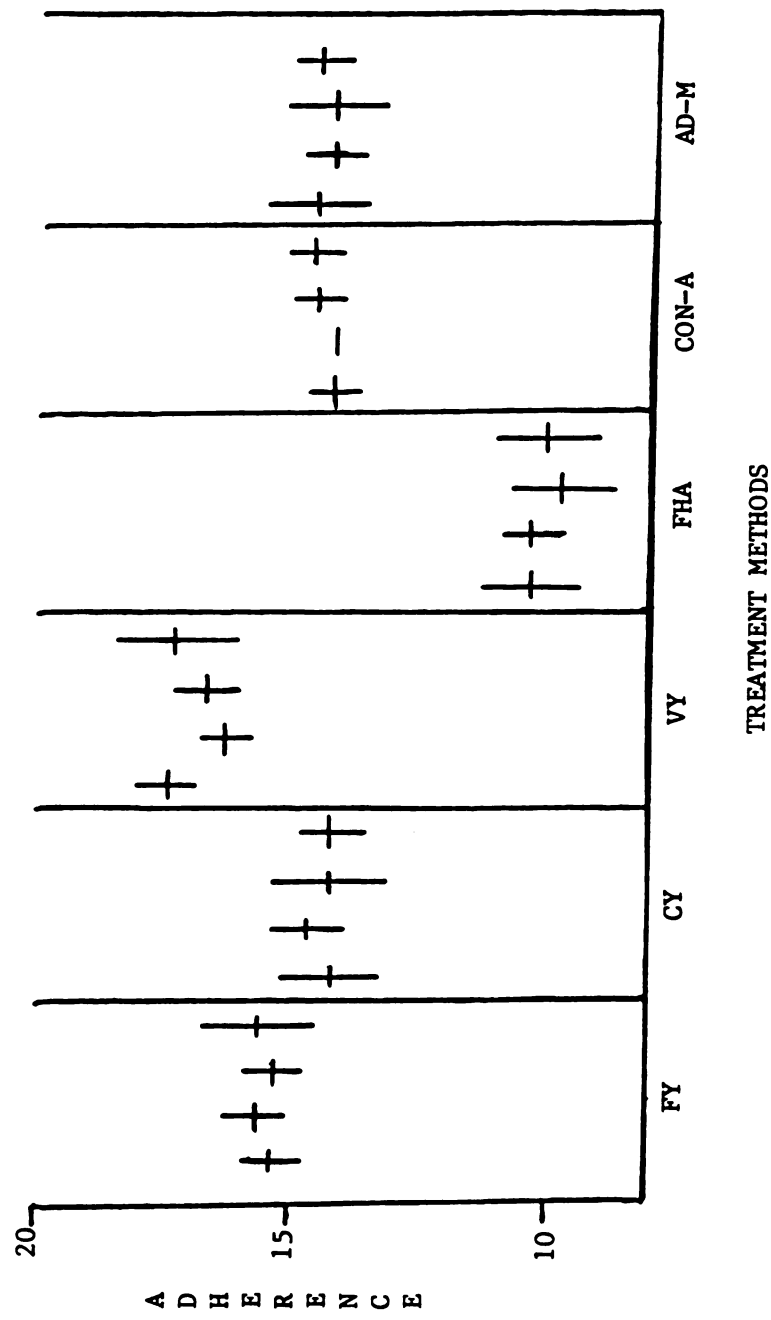

20
15
10
ADHERENCE
FY
CY
VY
FHA
CON-A
AD-M
TREATMENT METHODS

FIGURE 14. Comparison of the effect of different treatment methods on adherence of Candida pseudotropicalis to four different human buccal epithelial cell samples.* Treatment methods include:

FY: formalin treated yeast (kills yeast)
CY: yeast treated with citric acid (decreases cell wall mannan)
VY: viable yeast
FHA: filamentous hemagglutinin treated yeast (binds mannan resulting in inhibition)
Con-A: concanavalin-A treated yeast (binds mannan resulting in inhibition)
A-DM: α-D-methylmannopyranoside treated adherence mixtures (complementary sugar mannan inhibition).

* The vertical error bars indicate the standard deviation within individual buccal cell samples.


20
15
10
ADHERENCE
FY
CY
VY
FHA
CON-A
AD-M
TREATMENT METHODS

treatment method preparation procedures. Candida albicans isolates were germinated prior to treatment and use in the adherence assay, while other organisms used were in the blastospore stage of development. Buccal cells were collected immediately prior to use in the adherence assay, washed, and resuspended. Buccal cell donors included three males (ages 32-66) and three females (ages 22-24).

Adherence for Candida albicans isolate MSU-I (99% germ tubes) viable yeast (63.0-72.3) was similar to and did not significantly differ from adherence for formalized yeast (60.7-70.7) (Figure 3, Appendix B-I, Appendix C). Adherence for CY (17.7-25.7), FHA (10.7-15.7), Con-A (16.7-20.3), and A-DM (17.0-22.7) treatment methods was significantly lower than formalized or viable yeast preparations. These treatment methods did not significantly differ statistically from each other in terms of effect on adherence, although FHA treatment did decrease adherence to the greatest extent. (The numbers in parenthesis for each treatment method represent the range of the average number of adhering yeast to six different epithelial cell donor samples. This applies to all C. albicans and T. glabrata isolates.) Variations between individuals for each treatment method were not significant in most trials, although in some instances, differences did exist which were significant. For instance, the adherence

of the formalized yeast preparation to cells from donor #3 significantly differed from adherence of the formalized yeast preparation to donor #5 cells (Appendix C).

Adherence for C. albicans isolate 10-16-35F (80% germ tubes) viable yeast (64.7-68.3) was similar to and did not significantly differ from adherence for formalized yeast (61.3-67.3) (Figure 4, Appendix B-I, Appendix C). Adherence for CY (21.7-26.7), FHA (17.7-20.0), Con-A (21.3-24.3), and A-DM (21.0-24.3) treatment methods was significantly lower than formalized or viable yeast. These treatment methods did not significantly differ from each other in terms of effect on adherence, although FHA did decrease adherence to the greatest extent. Variations in adherence between individuals for each treatment method were usually not significant, although in some instances, differences were noted. For example, adherence of viable yeast to donor #5 cells significantly differed from adherence of viable yeast to cells from donor #6 (Appendix C).

Adherence for C. albicans isolate ONC-1134 (71% germ tubes) viable yeast (53.7-64.3) was similar to and did not significantly differ from adherence for formalized yeast (52.0-63.3) (Figure 5, Appendix B-I, Appendix C). Adherence for CY (18.7-26.0), FHA (14.0-19.3), Con-A (16.3-25.0), and A-DM (18.0-25) treatment methods was

significantly lower than formalized or viable yeast. These treatment methods did not significantly differ from each other in terms of effect on adherence, although FHA did decrease adherence to the greatest extent. Variations in adherence between individuals for each treatment method were usually not significant, although in some instances, differences were noted. For example, adherence of formalized yeast to buccal cell donor #1 cells significantly differed from adherence of formalized yeast to cells from donor #5. Similar differences in other treatment methods between individuals were also noted (see Appendix C).

Adherence for C. albicans isolate DA-05900 (65% germ tubes) viable yeast (59.0-63.3) was similar to and did not significantly differ from adherence of formalized yeast (48.7-60.3) (Figure 6, Appendix B-I, Appendix C). Adherence for CY (27.3-38.3), FHA (20.0-30.3), Con-A (26.3-37.3), and A-DM (26.7-36.3) treatment methods was significantly lower than formalized or yiable yeast. These treatment methods did not significantly differ from each other in terms of effect on adherence, although FHA did decrease adherence to the greatest extent. Variations in adherence between individuals for each treatment method were usually not significant, although in some instances, differences were noted. For example, adherence of

formalized yeast to donor #2 cells significantly differed from adherence of formalized yeast to cells from donor #5 (Appendix C).

Adherence of C. albicans isolate UCLA (52% germ tubes) viable yeast (44.0-53.7) was similar to and did not significantly differ from adherence for formalized yeast (44.7-48.0) (Figure 7, Appendix B-I, Appendix C). Adherence for CY (19.0-23.0), FHA (15.0-17.0), Con-A (18.7-22.3), and A-DM (20.3-22.7) treatment methods was significantly lower than formalized or viable yeast. These treatment methods did not significantly differ from each other in terms of effect on adherence, although FHA did decrease adherence to the greatest extent. Variations in adherence between individuals for each treatment method were not significant (Appendix C).

Adherence for C. albicans isolate 1840 (5% germ tubes) viable yeast (36.3-45) was similar to and did not significantly differ from adherence for formalized yeast (33.0-41.0) (Figure 8, Appendix B-I, Appendix C). Adherence for CY (6.0-13.3), FHA (5.3-9.3), Con-A (7.7-12.0), and A-DM (8.3-12.3) treatment methods was significantly lower than formalized or viable yeast. These treatment methods did not significantly differ from each other in terms of effect on adherence, although FHA did decrease adherence to the greatest extent. Variations in

adherence between individuals for each treatment method were usually not significant, although in some instances, differences were noted. For example, adherence of viable yeast to donor #5 cells significantly differed from adherence of viable yeast to cells from donor #3 and #4 (Appendix C).

Adherence for T. glabrata #1 viable yeast (30.3-32.0) was similar to and did not significantly differ from adherence for formalized yeast (27.3-29) (Figure 9, Appendix B-II, Appendix C). Adherence for CY (23.7-27.3), FHA (16.7-19.7), Con-A (22.3-27.0), and A-DM (23.0-26.7) treatment methods was significantly lower than formalized or viable yeast. These treatment methods did not significantly differ from each other in termis of effect of adherence, although FHA did decrease adherence to the greatest extent. Variations in adherence between individuals for each treatment method were not statistically significant (Appendix C).

Adherence for T. glabrata isolate #3 viable yeast (34.3-36.8) was similar to and did not significantly differ from adherence for formalized yeast (30.0-32.0) (Figure 10, Appendix B-II, Appendix C). Adherence for CY (26.0-28.3), FHA (17.7-20.0), Con-A (25.7-27.7), and A-DM (25.0-27.0) treatment methods was significantly lower than formalized or viable yeast. These treatment methods did not signifi-

cantly differ from each other in terms of effect on adherence, although FHA did decrease adherence to the greatest extent. Variations in adherence between individuals for each treatment method were not statistically significant (Appendix C).

Adherence for C. stellatoidea viable yeast (25.3-26.3) was similar to and did not significantly differ from adherence for formalized yeast (23.7-25.3), CY (19.7-22.0), Con-A (20.3-21.7), and A-DM (19.7-22.3) (Figure 11, Appendix B-III, Appendix C). Adherence of FHA treated yeast (13.7-14.7) was lower than and significantly differed from all other treatment methods (Appendix C). (The numbers in parenthesis after each treatment method represent the range of the average number of yeast adhering to 100 buccal cells in four different donor samples. This applies to non-C. albicans Candida spp. isolates.) Variation in adherence between individuals for each treatment method were usually not significant, although in some instances, differences were noted. For example, adherence of A-DM yeast to donor #1 cells significantly differed from adherence of A-DM yeast to cells from donor #3 (Appendix C).

Adherence for C. parapsilosis viable yeast (21.0-22.7) was similar to and did not significantly differ from adherence for formalized yeast (20.3-20.7), CY (17.7-19.0),

Con-A (18.0-19.0), and A-DM (14.3-14.7) (Figure 12, Appendix B-III, Appendix C). FHA treated yeast (13.0-14.0) adhered the least, and significantly differed from all other treatment methods. Variations in adherence between individuals for each treatment method were not statistically significant (Appendix C).

Adherence for C. tropicalis viable yeast (16.3-17.3) was similar to and did not significantly differ from adherence for formalized yeast (15.3-15.7), CY (14.3-14.7), Con-A (14.3-14.7), and A-DM (14.3-14.7) (Figure 13, Appendix B-III, Appendix C). FHA treated yeast (9.7-10.3) adhered the least, and significantly differed from all other treatment methods. Variations in adherence between individuals for each treatment method were not statistically significant (Appendix C).

Adherence for C. pseudotropicalis viable yeast (18.7-20.3) was similar to and did not significantly differ from adherence for formalized yeast (17.7), CY (15.7-16.7), Con-A (15.7-16.7), and A-DM (15.0-16.0) (Figure 14, Appendix B-III, Appendix C). FHA treated yeast (13.0-14.3) adhered the least, and significantly differed from all other treatment methods. Variations in adherence between individuals for each treatment method were not statistically significant (Appendix C).

Overall, there was little variation in adherence of a particular organism preparation to an individual buccal cell sample (tests were done in triplicate) (Figures 3-14, Appendix B and C). Similarly, there was little variation in adherence of a particular organism preparation to all samples of buccal epithelial cells, although variations did exist in some cases, especially for epithelial cell donor #5 in most organism preparations where a difference exists. Formalized and viable yeast preparations did not statistically differ in adherence to buccal cells for each organism (see Appendix C for ANOVA results). Other treatment methods decreased adherence, with FHA treated cells adhering the least for all organisms, in comparison to other treatment methods. In some instances, even though FHA did decrease adherence to the greatest extent, this amount did not statistically differ from the decrease in adherence seen with other treatment methods used. CY, Con-A, and A-DM effects on adherence were not different statistically within treatment groups for a particular organism tested (see Appendix C for ANOVA results). For statistical analysis of data for each organism, refer to Appendix B (Candida albicans isolates, B I; Torulopsis glabrata isolates, B II; other Candida spp., B III).

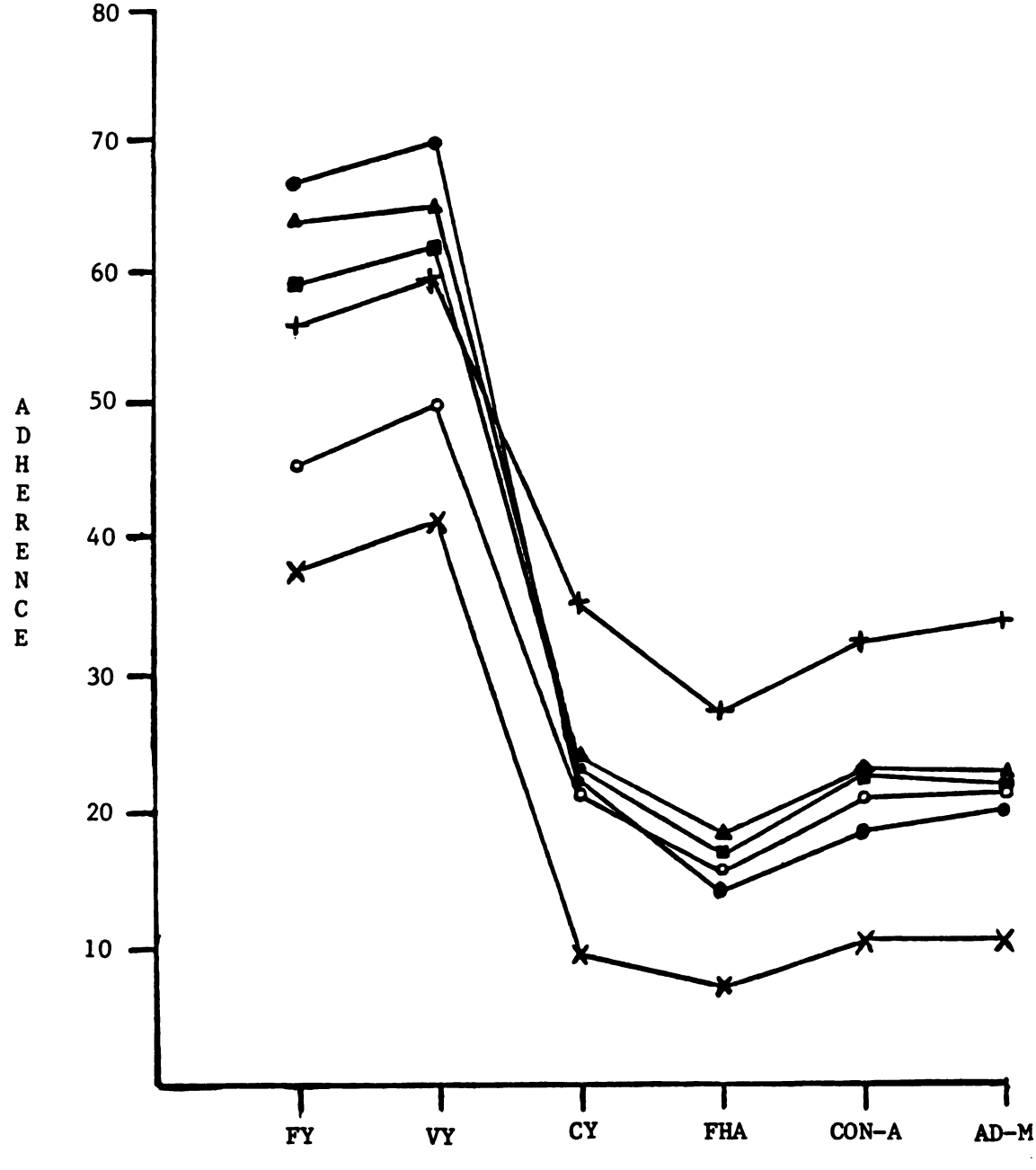
Figure 15 represents a comparison of adherence after treatment methods, explained previously, of germinated

FIGURE 15. Comparison of effects of treatment methods on adherence of *Candida albicans* strains to human buccal epithelial cells. Organisms include:

- • MSU-I
- ▲ 10-16-35-F
- ▪ ONC-1134
- \+ DA-05900
- o UCLA
- x 1840

a Treatment methods include:

FY: formalin treated yeast kills yeast)
CY: yeast treated with citric acid (decreases cell wall mannan)
VY: viable yeast
FHA: filamentous hemagglutinin treated yeast (mannan inhibition)
Con-A: concanavalin-A treated yeast (mannan inhibition)
A-DM: α-D-methylmanno pyraonside treated adherence mixtures (complementary sugar mannan inhibition)


80
70
60
50
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30
20
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ADHERENCE
FY
VY
CY
FHA
CON-A
AD-M
TREATMENT METHODS

Candida albicans isolates to pooled human buccal epithelial cells (six donors). (The numbers in parenthesis following each treatment method represent the average number of yeast the standard deviation adhering to 100 pooled buccal epithelial cells.) FHA treatment of cells decreased adherence to the greatest extent as compared to Con-A, A-DM, or CY samples, which decreased adherence approximately equally within trials for each organism. For example, adherence for Candida albicans isolate MSU-I viable yeast was (69.6±3.9), formalized yeast was (66.7±3.9), CY was (22.3±3.1), FHA was (14.2±2.3), Con-A was (18.6±1.9), and A-DM was (20.1±2.3). Adherence seen with formalized and viable yeast for MSU-I and other Candida albicans isolates for each organism was similar (see Table 1 for statistical data). Decreases in adherence seen with CY, Con-A, A-DM treatment methods were similar within treatment groups for MSU-I and other organisms tested (Figure 15, Table I), and FHA treatment decreased adherence to the greatest extent for all isolates tested (Figure 15, Table I).

Figure 16 represents a comparison of the effect of the treatment methods explained previously on adherence of Torulopsis glabrata strains, C. stellatoidea, C. parapsilosis, C. tropicalis, and C. pseudotropicalis to pooled human buccal epithelial cells. For Torulopsis

Table 1. Comparison of the effect of treatment methods including formalization of the yeast, cell wall mannan removal, mannan inhibition, and incubation of a complementary sugar of mannan in the adherence assay, on the adherence of isolates of *Candida albicans*, other *Candida* spp., and *Torulopsis glabrata* to human buccal epithelial cells.

Subject	Treatment Methods (x ± SD) FY[a]	VY[b]	CY[c]	FHA[d]	Con-A[e]	A-DM[f]
Candida albicans						
MSU-I	66.7±3.9	69.6±3.9	22.3±3.1	14.2±2.3	18.6±1.9	20.1±2.3
Range:	(59-72)	(62-74)	(15-28)	(9-17)	(15-22)	(16-24)
10-16-35F	63.9±2.4	64.8±7.1	24.1±2.3	18.4±1.3	22.9±1.7	22.8±1.8
	(60-69)	(64-69)	(21-28)	(16-22)	(20-26)	(20-26)
ONC-1134	58.9±4.2	61.9±4.1	23.1±3.4	17.2±2.2	22.8±3.4	22.2±2.9
	(51-66)	(52-66)	(17-29)	(13-21)	(14-27)	(17-26)
DA-05900	55.8±4.0	59.4±3.8	35.2±3.9	27.5±3.9	32.6±3.8	33.9±3.8
	(47-61)	(51-65)	(27-40)	(19-32)	(24-39)	(25-37)
UCLA	45.5±2.5	49.8±3.5	21.4±1.6	15.8±1.1	21.0±1.9	21.7±1.4
	(40-49)	(41-56)	(18-24)	(14-17)	(18-24)	(20-24)
1840	37.7±2.9	41.2±3.1	9.6±2.5	7.3±1.8	10.5±1.9	10.4±1.9
	(32-42)	(35-46)	(5-14)	(4-10)	(7-14)	(8-14)
Torulopsis glabrata						
TG-1	28.3±1.7	31.1±0.9	25.8±1.7	18.1±1.8	24.9±1.7	24.7±1.6
	(27-31)	(30-33)	(22-28)	(15-20)	(21-28)	(22-28)
TG-3	29.6±6.9	35.2±0.9	27.4±1.4	18.8±1.3	26.4±1.1	24.8±4.1
	(29-33)	(34-37)	(26-30)	(16-21)	(24-28)	(24-29)
Other *Candida* sp.						
C. stellatoidea	24.5±1.0	25.8±0.8	21.0±1.2	14.3±0.9	20.9±1.0	21.1±1.4
	(22-26)	(25-27)	(19-23)	(13-16)	(19-22)	(19-23)
C. parapsilosis	20.5±0.5	22.0±0.9	18.3±0.9	13.7±0.9	18.5±0.7	18.3±1.4
	(20-21)	(20-23)	(17-20)	(12-15)	(18-20)	(17-21)
C. tropicalis	15.5±0.5	16.9±0.7	14.4±0.5	10.1±0.7	13.7±2.8	14.5±0.8
	(15-16)	(16-18)	(14-15)	(9-11)	(14-15)	(13-16)
C. pseudo-tropicalis	17.7±0.7	19.7±1.0	16.2±0.8	13.3±1.0	16.2±0.6	15.5±0.8
	(17-19)	(18-21)	(15-18)	(12-15)	(15-17)	(14-17)

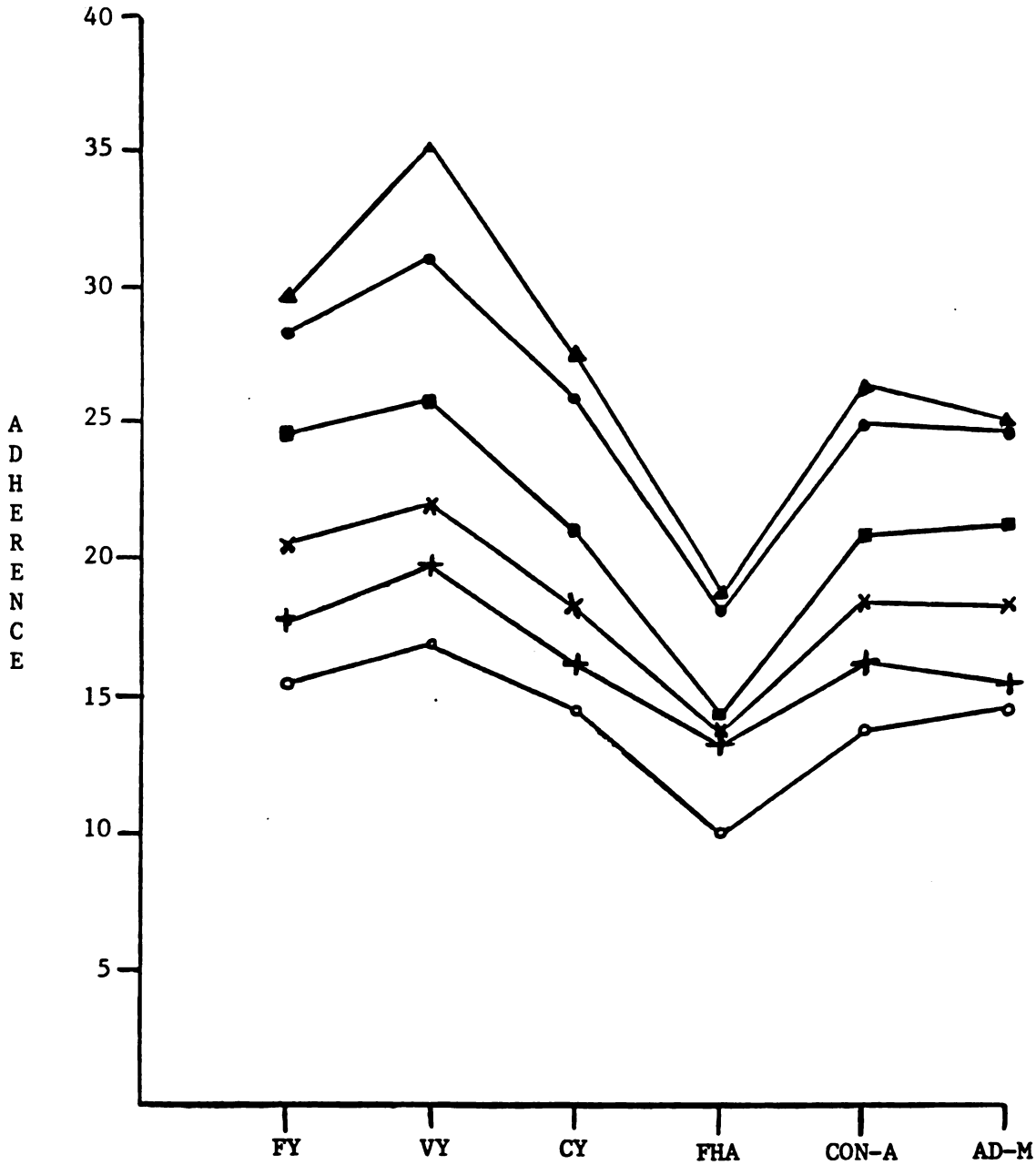
[a]Formal in treated yeast: Treatment methods and effect kills organism.
[b]Viable yeast.
[c]Citric acid treated yeast: Mannan removal.
[d]Filamentous Hemagglutinin treated yeast (Mannan inhibition).
[e]Concanavalin-A treated yeast (Mannan inhibition).
[f]α-D methylmannopyranoside adherence treatment (complementary sugar).

FIGURE 16: Comparison of effects of treatment methods on adherence of isolates of Torulopsis glabrata and Candida spp. (non-C. albicans) to human buccal epithelial cells. Organisms include:

- • Torulopsis glabrata #1
- ▲ Torulopsis glabrata #3
- • Candida stellatoidea
- x Candida parapsilosis
- o Candida tropicalis
- \+ Candida pseudotropicalis

a Treatment methods include:

FY: formalin treated yeast (kills yeast)
CY: yeast treated with citric acid (decreases cell wall mannan)
VY: viable yeast
FHA: filamentous hemagglutinin treated yeast (mannan inhibition)
Con-A: concanavalin-A treated yeast (mannan inhibition)
A-DM: α-D-methylmannopyranoside treated adherence mixtures (complementary sugar mannan inhibition)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AD-M
TREATMENT METHODS

glabrata isolate #1, FHA treatment of yeast cells (18.1±1.8) decreased adherence to the greatest extent as compared to Con-A (24.9±1.7), A-DM (24.7±1.6), or CY (25.8±1.7) samples, which decreased adherence approximately equally within trials, while adherence of viable yeast (31.0±0.9) differed little from formalized yeast (28.3±1.7) (see Table 1 for statistical data). Similar results were obtained for other organisms tested (Table 1).

Table 1 shows the statistical data for Figures 15-16, and indicates the average adherence for the treatment methods of each organism, standard deviations, and ranges for each particular treatment test. The ranges for specific tests in some instances are broad, although standard deviation values are not, indicating that even though a significant difference exists numerically between highest and lowest samples, variation between individual readings was not significant.

The comparison of the remaining relative percentage of adhering yeast to human buccal epithelial cells after various treatment methods is shown in Table 2. The treatment of yeast with a citric acid extraction procedure decreased adherence of Candida albicans isolates (range: 23.3-59.3%) much greater than Torulopsis glabrata strains (77.8-82.9%) or other Candida spp. tested (81.6-85.2%). FHA (17.7-46.3%) and Con-A (26.7-54.9%) pretreatment of

Table 2. Comparison of the remaining relative percentage of adhering yeast to human buccal epithelial cells after various treatment methods (treatment/viable yeast x 100)

	Treatment Methods (x ± SD)			
Subject	CY	FHA	Con-A	A-DM
Candida albicans				
MSU-I	32.0	20.4	26.7	28.9
10-16-35F	37.2	28.4	35.3	35.2
ONC-1134	37.3	27.8	36.8	35.9
DA-05900	59.3	46.3	54.9	57.1
UCLA	43.0	31.7	42.2	43.6
1840	23.3	17.7	25.5	25.2
Torulopsis glabrata				
TG-1	82.9	58.2	80.1	79.4
TG-3	77.8	53.4	75.0	70.5
Other *Candida* sp.				
C. stellatoidea	81.6	55.4	81.0	81.8
C. parapsilosis	83.2	62.3	84.1	83.2
C. tropicalis	85.2	59.8	81.1	85.8
C. pseudo-tropicalis	82.2	67.5	82.2	78.7

yeast isolates, and A-DM (25.2-57.1%) treatment of adherence mixtures also decreased adherence of Candida albicans isolates greater than adherence of the other organisms tested, with ranges of adherence for each treatment being 77.8-85.2% for CY, 53.4-67.5% for FHA, 75.0-84.1% for Con-A, and 70.5-85.5% for A-DM (see Table 2 for results for each organism). Comparison of percent germ tube formation (Figure 1) and remaining adherence after treatment methods (Table 2) for C. albicans isolates indicates that there is no correlation between ability to produce germ tubes and degree of decreased adherence seen using treatment methods which inhibited mannan, as would be expected if ability to adhere is related to ability to produce germ tubes. For example, MSU-I (99% germ tube formation) CY remaining adherence (32%) was greater than CY remaining adherence (23.3%) for isolate 1840 (Table 2). Similar results were found with FHA, Con-A, and A-DM treatment methods (Table 2).

DISCUSSION

Adherence of microorganisms to biological surfaces is considered to be an initial and essential stage in colonization and infection of the host (Beachey et al., 1981). Candida albicans, other Candida sp., and Torulopsis (Candida) glabrata are the causative agents of candidiasis, a primary or secondary opportunistic infection of host epithelial cell surfaces caused by a member of the genus Candida (Rippon, 1980). Candida albicans is the most frequently isolated member of the genus in human patient populations (Rippon, 1980), although in specific disease states, other Candida sp. are more commonly seen such as in infective endocarditis of fungal origin where C. parapsilosis is most frequently found (Rippon, 1980). Disease states caused by different Candida sp. are clinically indistinguishable and affected areas must be cultured to determine the causative agent of the disease process (Braunwald, et al., 1987).

Characterization of environmental, developmental, and growth factors which optimize adherence of C. albicans to biological surfaces has been studied extensively. Organisms in the stationary phase of growth have been found to adhere in greater numbers than organisms in the logarhythmic phase of development (King et al., 1980). Many in vitro studies have shown that the germinated form of C. albicans, an intermediate form between the blastospore and filamentous

stage of development, adheres in greater numbers than non-germinated forms (Kimura and Pearsall, 1978,1980; King et al., 1980; Sandin and Rogers, 1982; Rotresen et al., 1986). Although both blastoconidial and mycelial forms of C. albicans can adhere, invade, and proliferate in an infected host (Shepherd, 1985), another study showed that the capacity of C. albicans to produce hyphae appeared to be an important but nonessential virulence factor in the pathogenesis of candidal vaginitis in vivo (Sobel et al., 1984).

Preincubation of C. albicans isolates in tissue culture medium M-199 prior to use in the adherence assay favors germ tube formation (Kimura and Pearsall, 1978, 1980; Sandin and Rogers, 1982). Researchers have postulated that changes in the cell wall of C. albicans as it germinates could be responsible for the increased adherence seen (Kimura and Pearsall, 1978; Sobel et al., 1981). With increasing germ tube formation capability for C. albicans isolates, increased adherence is seen (Figure 1). All C. albicans isolates used in this study were of clinical origin (Appendix A-I) and were in the stationary phase of development prior to being subjected to conditions permitting germination to occur. This study indicates that for C. albicans isolates, increased germ tube production

tubes results in increased ability to adhere to human buccal epithelial cells.

Adherence of Candida sp. to biological surfaces is important in establishment of colonization and infection of the host (Beachey et al., 1981), for without an effective means of adhesion to host epithelial surfaces, bodily secretions, peristalsis, or mechanical trauma would result in dislodgement of the organisms from affected tissues (King et al., 1980). C. albicans has been found to adhere in greater numbers than C. stellatoidea, C. tropicalis, and other Candida sp. to vaginal cells (King et al., 1980), buccal cells (Kimura and Pearsall, 1980; King et al., 1980; Rotresen et al., 1986) and to fibrin platelet matrixes formed in vitro (Rotresen et al., 1985). Candida albicans also adheres more strongly to epithelial cells than fungal cells of other Candida sp. (Macura, 1985). Results indicate that C. albicans isolate MSU-I adheres to a greater extent than T. glabrata strains and other Candida sp. tested (Figure 2). T. glabrata isolates adhered greater than (in decreasing order of adherence) C. tropicalis, C. pseudotropicalis, C. parapsilosis, and C. stellatoidea (Figure 2). These results are in agreement with published data and parallel the heirarchy of clinical isolates of Candida obtained obtained from patient specimens (Rippon, 1980). The C. albicans isolate with the least germ tube

formation capability (1840) adhered greater than T. glabrata strains or other non-C. albicans sp. tested (comparison of Figures 1 and 2). This data supports the concept that C. albicans isolates adhere to a greater extent to human buccal epithelial cells than other Candida spp. and T. glabrata, even when germ tube production is low, and is also in agreement with published data supporting the concept that the germ tube is important in adherence of C. albicans isolates to human buccal epithelial cells.

It is generally agreed that there is great variation in the number of receptor sites for C. albicans on epithelial cells collected from different persons (King et al., 1980; Sandin et al., 1987), although in a study by Cox, 1983, adherence of C. albicans to human buccal epithelial cells was found to be the same in normal adults and children, suggesting that a stable cell receptor system in present which is not age dependent. Researchers have hypothesized that indigenous flora could suppress adherence of C. albicans by competing with it for receptor sites on epithelial cells, modifying these sites to hamper candidal adherence, or enzymatically altering the yeast surface (Liljemark and Gibbons, 1973). Specific host defense factors include cell mediated and humoral immunity as well as non-specific immune mechanisms which may influence adherence (Rogers and Balish, 1980). Non-specific host

factors include local defense mechanisms and environmental conditions (Rippon, 1980). Other factors which might affect adherence of Candida spp. to epithelial cells include stage of development and other characteristics of epithelial cells. Scanning electron microscopy studies have shown non-uniform distribution of adhering microorganisms with diminished adherence in areas of active mitosis and proliferation, and increased adherence to mature flat cells often in the process of desquamation in studies of adherence of C. albicans to vaginal epithelial cells (Sobel et al., 1982). For C. albicans isolates, T. glabrata isolates, and other Candida sp. tested, variation existed between individuals in terms of adherence of viable or treated organisms to human buccal epithelial cells (Figures 3-14), but that variation was not statistically significant in most cases (see Appendix C for ANOVA analysis data, and Appendix B for individual trial results). One particular individual (male, age 31; position 5 in Figures 3-10) in many cases differed significantly from the other five individuals tested, and this can possibly be attributed to the fact that he had worked with C. albicans and other Candida spp. for a considerable period of time prior to use of his buccal cell samples in adherence assays. Specific host defense factors such as immune mechanisms could also account for these results. Possible explanations for the non-variation

between individuals in adherence of particular yeasts, treated or untreated, to buccal epithelial cells is that the cells were collected at approximately the same time on days of experiments, buccal cell donors were candidal free as determined by oropharyngeal culture, donors were not receiving antibiotics or antifungal agents at the time. Other experimental conditions which may have affected adherence include that buccal cell specimens were processed immediately upon collection and placed on ice until use (less than two hours), buccal cell specimens were used for only one set of experiments and not held over for future use in adherence experiments, and buccal cells for each individual were resuspended after processing according to viable cell count as determined by Methylene Blue staining and hemocytometer count. The stable cell receptor system for adherence of C. albicans suggested by Cox, 1983, could also account for these observations. Significant variation did exist in terms of number of yeast adhering to individual buccal cells in each particular test which supports published data (Sandin et al., 1987), although specific numbers of yeast adhering to individual buccal cells in each trial were not recorded. Stages of development of individual buccal cells collected from donors were not determined, which may have had an effect on adherence seen.

The study of adherence of C. albicans to biological surfaces has included both viable and formalized cells in different growth stages. Kimura and Pearsall, 1978, found that once germinated, C. albicans cells could be killed in formalin with no significant decrease in adherence, while treatment before germination decreased adherence. In a study of attachment to endothelial cells, viable or killed Candida organisms were enveloped by membrane processes from endothelial cell surfaces and were incorporated into the endothelial cells in phagosomes (Rotresen et al., 1985). For C. albicans isolates, no significant difference was found between adherence of viable or formalized germinated yeast specimens (Figures 3-8, Appendix C). No significant difference between formalized and viable yeast (blastospore form) is present for T. glabrata isolates tested or for other non-C. albicans Candida spp. tested (Figures 9-14, Appendix C). Similar results were seen using pooled buccal epithelial cells for all organisms (Figures 15 and 16, Table 1). Non-C. albicans organisms were used in the blastospore stage of development, and C. albicans isolates were in the germinated form. Studies were not performed to determine if germination of these organisms increased adherence ability, or if formalization before germination of non-C. albicans organisms decreased adherence.

Characterization of the adhesin which mediates adherence of C. albicans to biological surfaces has received much attention. The cell wall of C. albicans is composed of chitin, mannan, glucan, and other chemical constituents including protein (SanBlas, 1982). Five to eight layers of the cell wall of C. albicans have been identified depending on growth conditions or cytochemical techniques utilized (Poulain et al., 1978), with chitin identified as the compound composing the bulk of the most electron transparent layer in transmission electron microscopy studies of germ tube formation (Cassone et al., 1973). The localization of mannan in the cell wall of C. albicans has been confirmed by Djaczenko and Cassone, 1971; Poulain et al., 1978; and Evron and Drewe, 1984. Studies by Kimura and Pearsall, 1978, showed that enhanced adherence of C. albicans after incubation in saliva was related to changes in the fungus itself, and Sobel et al., 1981, found that adherence of C. albicans was enhanced by a surface component of the germinated yeast, postulated to be a glycoprotein. Cassone et al., 1978, found that mannan is the major constituent of the fibrillar-floccular layer of the cell wall of C. albicans using concanavalin-A. Pope and Cole, 1981, found that yeast were associated with a mucus layer on epithelial surfaces throughout the gastrointestinal tract in scanning electron microscopy studies, and a study by Tronchin et al.,

1984, indicated that attachment of C. albicans to buccal epithelial cells appeared to involve spatial rearrangement of their cell wall surface through development of a fibrogranular surface layer which was detected using specific carbohydrate staining techniques and concanavalin-A binding, indicating that attachment of yeast to epithelial cells was mediated by a mannose receptor. Similar studies have not been completed for non-C. albicans Candida sp.

Inhibition of adherence of C. albicans to different epithelial cell surfaces has been accomplished through the use of many different and varied compounds. Pretreatment of C. albicans with trypsin, chymotrypsin, or proteinase decreased adherence significantly (King et al., 1980; Maisch and Calderone, 1980). Loss of adherence after treatment of yeast cells with α-mannosidase or papain suggests that the cell wall mannoprotein is an essential component of the C. albicans adhesin (Lee and King, 1983). Concanavalin-A (con-A) pretreatment of C. albicans inhibited adherence (Sandin and Rogers, 1982), and addition of α-D-meh tylmannopyranoside in the adherence assay also inhibited adherence (Sandin et al., 1982). Studies of controlled degradation of the cell surface of C. albicans indicated that mannan and mannoproteins appeared to be important constituents of the adhesin mediating adherence (Rotresen et al., 1986). The effect of various treatment methods on adherence of C.

albicans isolates to pooled (Figure 15) and individual (Figures 3-8) human buccal epithelial cell samples using a citric acid (CY) extraction technique which decreased cell wall mannan (Tronchin et al., 1984), cell wall mannan ligand inhibition with the use of con-A and filamentous hemagglutinin (FHA), a purified protein adhesin of Bordetella pertussis which binds mannan and mediates adherence of the organism to ciliated respiratory epithelial cells (Sato et al., 1984), and α-D-methylmannopyranoside treatment of adherence mixtures, which is a complementary sugar of mannan that inhibits adherence of C. albicans isolates to human buccal epithelial cells (Sandin et al., 1982) showed that all treatment methods decreased adherence of C. albicans isolates to human buccal epithelial cells. Filamentous hemagglutinin treatment decreased adherence to the greatest extent. Little variation between adherence of organisms (viable or treated) to individual (Figures 3-8) or pooled (Figure 15) buccal cells was seen. Wide variation in remaining adherence for each treatment method between C. albicans isolates was noted (Table 2). Comparison of remaining adherence after treatment methods (Table 2) to percent germination of these isolates (Figure 1) reveals no correlation of these factors. The degree of inhibition of adherence seen with FHA and Con-A, decreasing cell wall mannan (CY), or complementary sugar inhibition (A-DM) is not

dependent on germ tube formation capability, indicating that differences in cell wall mannan concentration and structure could be present in different isolates of germinated C. albicans. These results indirectly support the concept that changes in the cell wall of C. albicans as it germinates are responsible for the increased adherence seen (Kimura and Pearsall, 1978, 1980; Sobel et al., 1981), although the lack of correlation between germ tube formation capability and inhibition of adherence suggests that other factors such as growth conditions or cell wall differences for different strains of C. albicans may be playing a role in adherence capacity of individual organisms.

The effect of treatment methods on the adherence of T. glabrata isolates and Candida spp. other than C. albicans to individual (Figures 9-14) and pooled (Figure 16) human buccal epithelial cells indicates that all treatment methods inhibit adherence. Filamentous hemagglutinin treatment had the most dramatic effect, decreasing adherence to the greatest extent. Changes in adherence to individual or pooled buccal cell preparations using the treatment methods indicated were significant from viable yeast preparations (see ANOVA analysis results for Figures 9-14 in Appendix C), although the changes in adherence seen between CY, con-A, and A-DM treated yeast for each organism were minimal and and less than that of FHA. No differences in adherence

inhibition were noted when comparing adherence of organisms to individual or pooled buccal epithelial cells. Little variation in remaining adherence between non-C. albicans Candida spp. or T. glabrata strains within trials for a particular treatment method were noted (Table 2). This suggests that differences in mannan mediated adherence for these organisms are small, and that other mechanisms of attachment using other adhesin systems could be operational for these organisms.

The ability of FHA to inhibit adherence greater than other mannan inhibitors or other treatment methods for C. albicans isolates (Figures 3-8 and 15), and for non-C. albicans organisms (Figures 9-14 and 16) was demonstrated. Filamentous hemagglutinin is a protein secretion of Bordetella pertussis which mediates adherence of the organism to human ciliated respiratory epithelial cells in vitro (Tuomanen and Hendley, 1983; Urisu et al., 1986). FHA was originally thought to be a component of the fimbriae of B. pertussis (Sato et al., 1984) which was identical to hemagglutinating proteins present in the fimbriae of other piliated bacteria which mediated adherence or these organisms to biological surfaces (Korhonen et al., 1982), but it is now believed to be an extracellular protein adhesin secretion of the organism (Zhang et al., 1985). FHA is a large protein with a molecular weight approaching 10^6

(Urisi et al., 1986). It has been shown to enhance the adherence of Hemophilus influenza Type B and inhibit the adherence of Streptococcus pneumoniae to human respiratory epithelial cells in vitro, suggesting that piracy of adhesins may contribute to superinfection in mucosal diseases, although it was impossible to determine if the interaction between heterologous bacteria and FHA was receptor mediated or nonspecific (Tuomanen, 1986). The potential pathophysiological importance of this interaction is suggested by the idea that piracy of adhesins could allow or enhance adherence of organisms to surfaces, or inhibit the adherence of other organisms to specific biological surfaces. The interaction seen between FHA and organisms (Seen in Figures 3-8 and 15 for C. albicans, and Figures 9-14 and 16 for non-C. albicans isolates) indicates that the binding of this protein to yeast (germinated C. albicans isolates) decreases adherence ability. The FHA used in this study was secreted by B. pertussis, but similar compounds are secreted by other piliated strains of bacteria (Korhonen et al., 1982). In addition, it has been shown that the presence of certain piliated strains of bacteria such as Klebsiella pneumoniae (Centeno et al., 1983) and Escherichia coli (Makrides and MacFarlane, 1983) can increase Candida adherence to epithelial cells. It is recognized also that indigenous microflora of specific site

areas in humans can suppress the adherence of C. albicans, such as in the gastrointestinal tract (Rippon, 1980). Treatment of humans with antibiotics, antimetabolites, and corticosteroids disturbs the normal flora distribution in humans and predisposes them to opportunistic invasion by Candida sp. (Braunswald et al., 1987). Other disease states, metabolic abnormalities, and immunosuppression, are factors which have also been found to predispose patients to candidiasis (Rippon 1980). The piracy of adhesins secreted by remaining flora and other microorganisms in such situations could possibly help explain and partially account for the increased incidence of candidiasis seen clinically. The results presented in Figures 3-16 regarding FHA support the concepts of adhesin piracy in vitro, and indirectly demonstrate the ability of microorganisms to affect the adherence of other microbials to biological surfaces. Because the effect of FHA on adherence was greater than that of the other treatment methods which decreased adherence through mannan inhibition or decreased cell wall mannan for all organisms, the results suggest that adherence may be mediated by other factors in addition to mannan receptor function in Candida spp. and T. glabrata. Comparative studies were not performed which would have quantified the cell wall mannan removed with the citric acid extraction technique for each organism, nor were studies done to

determine if all mannan receptor sites were inhibited or blocked with FHA, con-A, or α-D-methylmannopyranoside. Titration assays were performed to determine the maximum inhibition of adherence possible using the methods indicated for a representative organism (C. albicans isolate MSU-I).

The relative percent remaining adherence after treatment methods for all organisms tested showed that variation for all treatment methods exists for C. albicans isolates, but the change in adherence for other organisms used is relatively constant within a particular treatment method (Table 2). CY, con-A, and A-DM decreased adherence approximately equally within tests for one organism, which is different than the effect of these treatment methods on adherence of C. albicans isolates seen also in Table 2. Percent adherence of C. albicans isolates after subjection to treatment methods was less than remaining adherence for all other organisms tested. Possible reasons for these observations include differences in cell wall structure and mannan concentration between different Candida sp. and within the C. albicans species which could affect the characteristics of the adhesins responsible for adherence of particular organisms. Since only C. albicans isolates were in the germinated form in the adherence assays, the differences in adherence seen with the treatment methods used for C. albicans, and the differences noted between non-C.

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The relative percent remaining adherence after treatment methods for all organisms tested showed that variation for all treatment methods exists for C. albicans isolates, but the change in adherence for other organisms used is relatively constant within a particular treatment method (Table 2). CY, con-A, and A-DM decreased adherence approximately equally within tests for one organism, which is different than the effect of these treatment methods on adherence of C. albicans isolates seen also in Table 2. Percent adherence of C. albicans isolates after subjection to treatment methods was less than remaining adherence for all other organisms tested. Possible reasons for these observations include differences in cell wall structure and mannan concentration between different Candida sp. and within the C. albicans species which could affect the characteristics of the adhesins responsible for adherence of particular organisms. Since only C. albicans isolates were in the germinated form in the adherence assays, the differences in adherence seen with the treatment methods used for C. albicans, and the differences noted between non-C.

albicans isolates and T. glabrata strains as a group compared to C. albicans could be related to the germination process in addition to cell wall structural differences, and possible differences in adhesin characteristics.

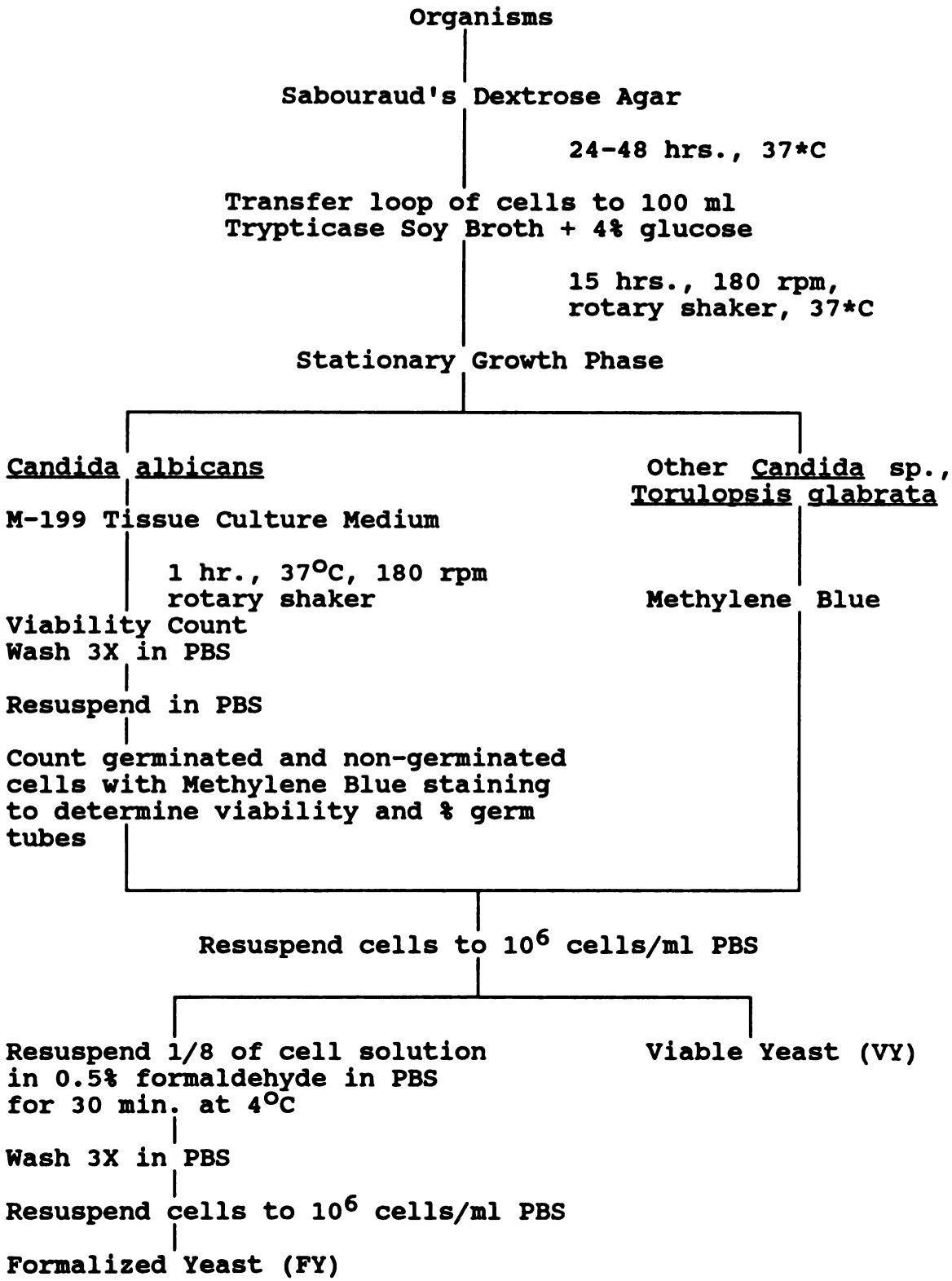
APPENDIX A

APPENDIX A-I

Source of organisms used in studies:

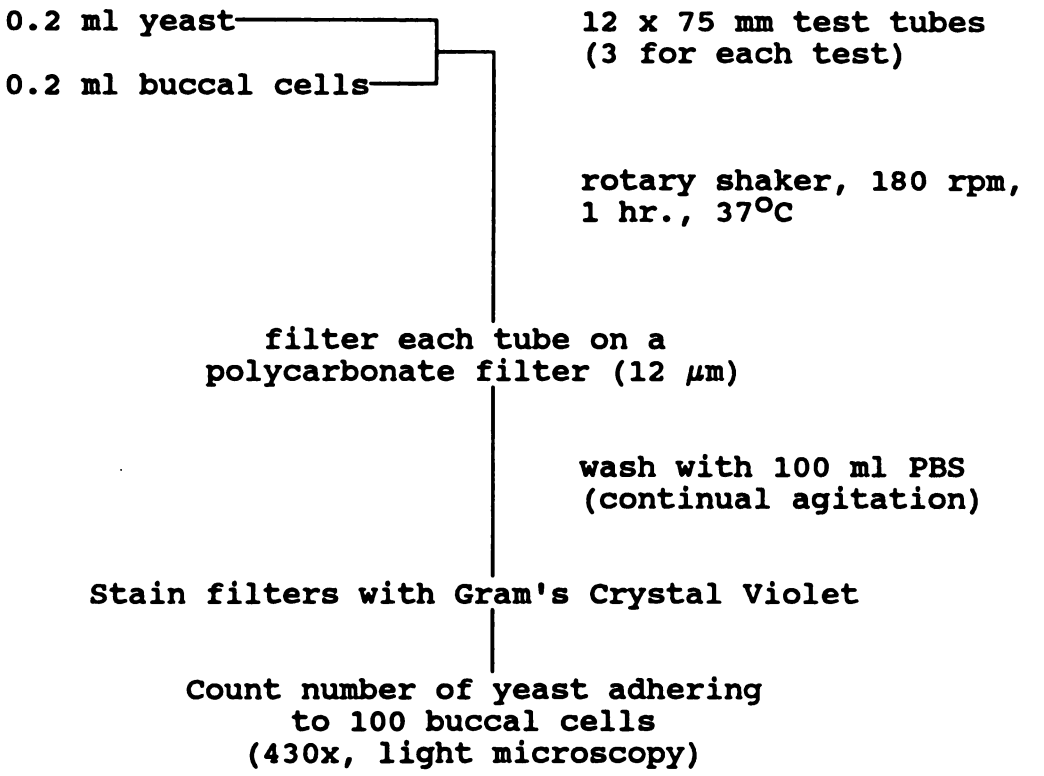
ORGANISM	SOURCE
Candida albicans	
MSU-I	Fecal specimen isolate collected by Linda Olsen, MSU Med. Myc. Lab.
10-16-35F	Vaginal isolate from Sparrow Hosp., Lansing, MI.
ONC-1134	Vaginal isolate from Olin Health Center, MSU.
DA-05900	Vaginitis case isolate from Dr. Cooper's lab., Dallas, TX.
UCLA	Vaginal isolate from Dr. Howard's lab., UCLA.
1840	Recurrent vaginitis case isolate from Dr. Magee's lab., MSU.
Torulopsis glabrata	
1	Vaginal isolate from Lansing General Hosp., Lansing, MI.
3	Vaginal isolate from Sparrow Hosp., Lansing, MI.
Other *Candida* spp.	
C. stellatoidea	Vaginal isolate from CDC Proficiency Test.
C. parapsilosis	Vaginal isolate from CAP Proficiency Test.
C. tropicalis	Vaginal isolate from Sparrow Hosp., Lansing, MI.
C. pseudotropicalis	Vaginal isolate from Med. Myc. Lab., Duke Univ.

APPENDIX A-II



APPENDIX A-III

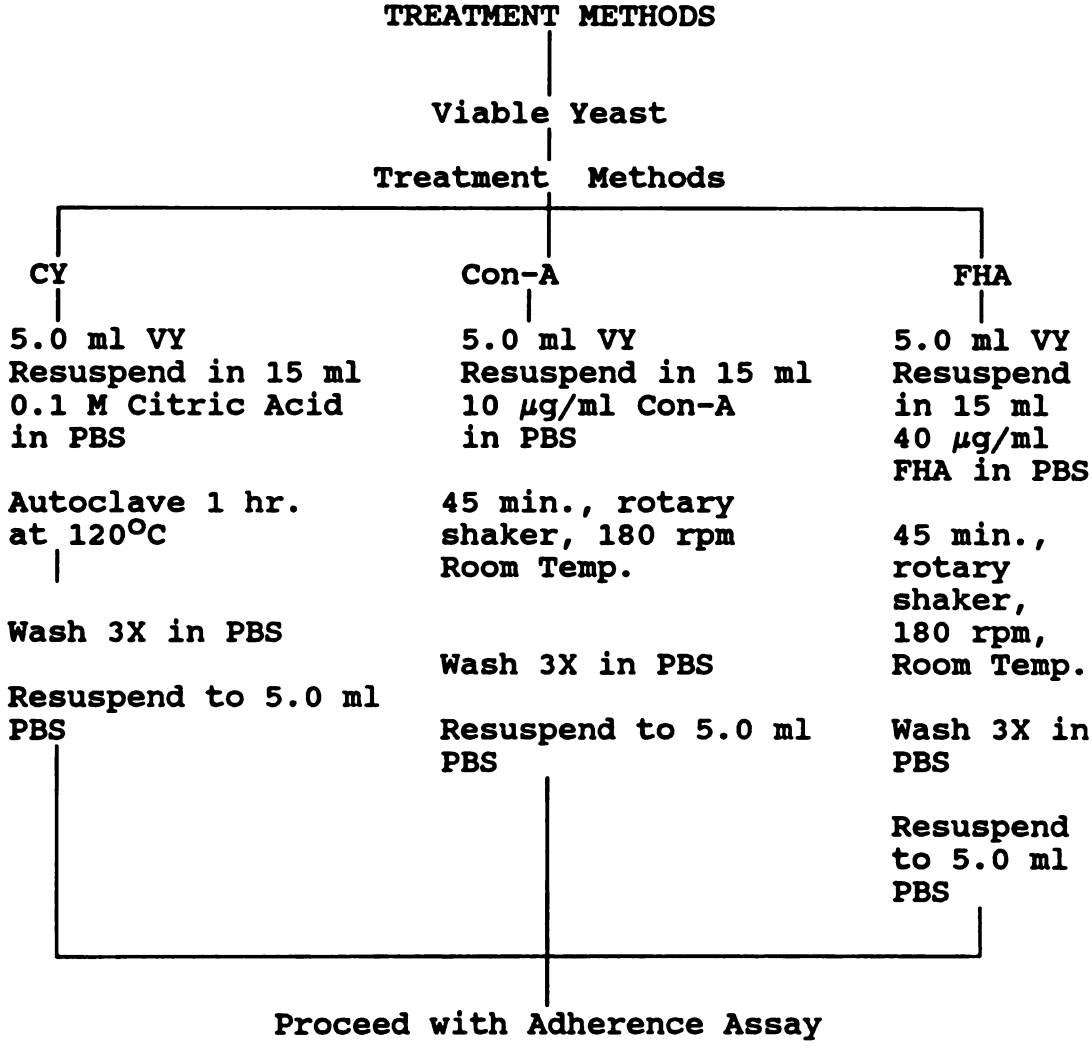
ADHERENCE ASSAY



* Double blind conditions were used in all studies

* α-D-methylmannopyranoside added after buccal cells and yeast were pipetted into test tubes

APPENDIX A-IV



* α-D-methylmannopyranoside was added to three adherence test tubes containing 0.2 ml viable yeast and 0.2 ml buccal cells as an adherence treatment method.

* Formalized yeast were also used in adherence assays.

APPENDIX B

APPENDIX B

1. Comparison of the effect of different treatment methods on adherence of six clinical isolates of Candida albicans with differing germ-tube formation ability to six different human buccal epithelial cell samples.

Organism: MSU-I -- 99% Germ Tubes

Subject	Treatment Methods ($\bar{x}$ ± SD)					
	FY	VY	CY	FHA	Con-A	A-DM
1	69.0 ± 1.0	71.7 ± 1.5	22.0 ± 1.0	15.3 ± 1.5	18.3 ± 2.1	21.7 ± 2.1
2	69.0 ± 1.0	71.7 ± 0.6	23.7 ± 1.5	15.7 ± 1.5	20.1 ± 1.0	19.7 ± 1.5
3	70.7 ± 1.5	72.3 ± 1.5	24.0 ± 1.0	10.7 ± 1.5	18.3 ± 2.1	21.0 ± 2.0
4	68.0 ± 1.0	72.0 ± 2.6	25.7 ± 2.5	15.7 ± 1.5	20.3 ± 1.5	22.7 ± 1.5
5	60.7 ± 1.5	63.0 ± 1.0	17.7 ± 2.3	13.7 ± 1.5	17.7 ± 0.6	18.7 ± 0.6
6	63.3 ± 1.5	67.3 ± 1.0	21.0 ± 2.0	14.0 ± 2.0	16.7 ± 1.5	17.0 ± 1.0

Organism: 10-16-35F -- 80% Germ Tubes

Subject	Treatment Methods ($\bar{x}$ ± SD)					
	FY	VY	CY	FHA	Con-A	A-DM
1	63.3 ± 1.5	67.3 ± 0.6	24.3 ± 0.6	20.0 ± 1.7	23.0 ± 1.0	23.3 ± 1.2
2	64.7 ± 2.1	67.0 ± 1.7	25.3 ± 3.8	18.7 ± 0.6	23.0 ± 2.6	23.3 ± 2.1
3	64.3 ± 0.6	66.7 ± 0.6	24.0 ± 1.0	17.7 ± 0.6	24.3 ± 1.5	22.7 ± 2.1
4	62.7 ± 2.1	64.7 ± 0.6	22.3 ± 1.5	19.0 ± 1.0	21.3 ± 1.5	22.0 ± 1.0
5	61.3 ± 1.5	64.7 ± 1.6	21.7 ± 0.6	17.7 ± 0.6	22.0 ± 1.0	21.0 ± 1.7
6	67.3 ± 1.5	68.3 ± 0.6	26.7 ± 1.6	17.7 ± 1.5	23.7 ± 1.5	24.3 ± 1.5

Organism: ONC-1134 -- 71% Germ Tubes

Subject	Treatment Methods ($\bar{x}$ ± SD)					
	FY	VY	CY	FHA	Con-A	A-DM
1	62.3 ± 2.5	63.3 ± 2.5	22.7 ± 2.1	16.3 ± 1.5	23.3 ± 1.2	20.7 ± 2.1
2	63.3 ± 2.5	64.3 ± 2.1	22.7 ± 1.5	19.3 ± 0.6	24.7 ± 2.5	23.0 ± 1.0
3	60.7 ± 1.5	63.7 ± 1.5	26.0 ± 4.4	17.7 ± 1.5	25.0 ± 2.0	24.7 ± 1.5
4	58.3 ± 2.1	63.7 ± 1.5	25.7 ± 3.2	18.7 ± 2.1	24.3 ± 2.1	25.0 ± 1.0
5	52.0 ± 1.0	53.7 ± 1.5	18.7 ± 1.5	14.0 ± 1.0	16.3 ± 2.1	18.0 ± 1.0
6	57.0 ± 2.0	63.0 ± 1.0	23.0 ± 2.6	16.7 ± 1.5	23.0 ± 1.0	22.7 ± 1.5

Organism: DA-05900 -- 65% Germ Tubes

Subject	Treatment Methods ($\bar{x}$ ± SD)					
	FY	VY	CY	FHA	Con-A	A-DM
1	58.7 ± 0.6	61.7 ± 2.1	35.7 ± 0.6	26.7 ± 1.5	32.3 ± 1.5	33.7 ± 3.2
2	60.3 ± 0.6	63.3 ± 1.5	38.3 ± 1.5	30.0 ± 1.0	37.3 ± 2.1	36.3 ± 0.6
3	54.7 ± 1.5	59.0 ± 1.0	37.0 ± 1.0	29.7 ± 1.5	34.0 ± 2.0	36.3 ± 1.5
4	56.3 ± 2.5	61.3 ± 1.5	36.7 ± 2.1	30.3 ± 1.5	33.7 ± 1.5	36.0 ± 1.0
5	48.7 ± 1.5	52.3 ± 1.5	27.3 ± 0.6	20.0 ± 1.0	26.3 ± 2.5	26.7 ± 1.5
6	56.3 ± 1.5	59.0 ± 1.0	36.3 ± 2.1	28.3 ± 1.2	31.7 ± 1.5	34.7 ± 0.6

I. Comparison of the effect of different treatment methods on adherence of six clinical isolates of *Candida albicans* with differing germ-tube formation ability to six different human buccal epithelial cell samples, cont'd.

Organism: UCLA -- 52% Germ tubes

Subject	Treatment Methods ($\bar{x}$ ± SD) FY	VY	CY	FHA	Con-A	A-DM
1	46.7 ± 1.5	50.0 ± 1.0	20.7 ± 0.6	15.7 ± 1.5	21.0 ± 2.0	21.7 ± 0.6
2	46.7 ± 2.1	50.3 ± 0.6	21.7 ± 0.6	15.0 ± 1.0	19.7 ± 1.5	20.3 ± 0.6
3	48.0 ± 1.0	52.3 ± 1.5	23.0 ± 1.0	15.7 ± 1.5	22.3 ± 1.5	22.0 ± 2.0
4	45.7 ± 0.6	53.7 ± 2.5	22.0 ± 1.0	17.0 ± 0.0	22.3 ± 1.5	23.0 ± 1.0
5	41.0 ± 1.0	44.0 ± 2.6	19.0 ± 1.0	15.7 ± 0.6	18.7 ± 0.6	20.3 ± 1.2
6	44.7 ± 0.6	48.7 ± 2.1	22.3 ± 1.5	16.0 ± 1.0	22.0 ± 1.0	22.7 ± 0.6

Organism: 1840 -- 5% Germ tubes

Subject	Treatment Methods ($\bar{x}$ ± SD) FY	VY	CY	FHA	Con-A	A-DM
1	37.7 ± 1.5	40.0 ± 1.0	6.0 ± 1.0	5.3 ± 0.6	7.7 ± 0.6	8.3 ± 0.6
2	39.3 ± 2.1	43.3 ± 1.5	10.7 ± 0.6	8.3 ± 0.6	12.0 ± 1.0	12.3 ± 1.5
3	38.0 ± 2.6	42.3 ± 1.5	8.7 ± 0.6	5.3 ± 1.5	9.3 ± 0.6	9.7 ± 2.1
4	37.3 ± 2.1	40.3 ± 0.6	9.7 ± 2.5	9.3 ± 0.6	12.0 ± 1.7	11.0 ± 1.0
5	33.0 ± 1.0	36.3 ± 2.3	9.3 ± 0.6	6.7 ± 0.6	10.0 ± 1.0	9.7 ± 1.5
6	41.0 ± 1.0	45.0 ± 1.0	13.3 ± 0.6	8.7 ± 1.5	12.0 ± 1.0	11.7 ± 2.1

II. Comparison of the effect of different treatment methods on adherence of two clinical isolates of *Torulopsis glabrata* to six different human buccal epithelial cell samples.

Organism: Torulopsis glabrata 1

Subject	Treatment Methods ($\bar{x}$ ± SD) FY	VY	CY	FHA	Con-A	A-DM
1	28.0 ± 1.0	30.3 ± 0.6	23.7 ± 1.5	19.7 ± 0.6	22.3 ± 1.5	23.7 ± 0.6
2	28.7 ± 1.5	31.3 ± 1.5	26.3 ± 1.5	18.0 ± 1.0	25.7 ± 0.6	24.3 ± 0.6
3	28.0 ± 2.0	30.7 ± 0.6	26.7 ± 0.6	19.0 ± 1.0	27.0 ± 1.0	25.0 ± 1.0
4	27.3 ± 2.3	32.0 ± 1.2	27.3 ± 1.2	18.3 ± 2.1	25.0 ± 1.0	26.7 ± 2.3
5	29.0 ± 1.0	31.3 ± 1.5	25.3 ± 1.5	16.7 ± 2.1	24.7 ± 1.2	25.3 ± 1.5
6	29.0 ± 2.6	31.0 ± 1.5	25.7 ± 1.5	16.7 ± 2.1	25.0 ± 1.0	23.0 ± 1.0

Organism: Torulopsis glabrata 3

Subject	Treatment Methods ($\bar{x}$ ± SD) FY	VY	CY	FHA	Con-A	A-DM
1	32.0 ± 1.0	34.3 ± 0.6	28.0 ± 1.0	20.0 ± 1.0	27.7 ± 0.6	27.0 ± 1.0
2	31.7 ± 1.5	34.7 ± 0.6	28.3 ± 0.6	19.0 ± 1.0	25.7 ± 1.5	26.0 ± 2.6
3	30.0 ± 1.0	35.0 ± 1.0	27.0 ± 1.0	17.7 ± 1.5	26.3 ± 1.2	25.0 ± 1.0
4	31.0 ± 1.0	35.7 ± 0.6	28.0 ± 2.0	19.0 ± 1.0	26.7 ± 0.6	25.7 ± 2.1
5	31.0 ± 1.0	35.3 ± 0.6	26.0 ± 2.0	18.0 ± 1.7	26.0 ± 1.0	25.7 ± 0.6
6	32.0 ± 1.0	36.8 ± 1.2	27.3 ± 0.6	19.3 ± 0.6	26.0 ± 1.0	26.0 ± 1.0

III. Comparison of the effect of different treatment methods on adherence of four clinical isolates of Candida sp. other than Candida albicans to four different human buccal epithelial cell samples.

Organism: Candida stellatoidea

	Treatment Methods ($\bar{x}$ ± SD)					
Subject	FY[a]	VY[b]	CY[c]	FHA[d]	Con-A[e]	A-DM[f]
1	24.7 ± 0.6	26.3 ± 0.6	21.3 ± 0.6	13.7 ± 0.6	21.0 ± 1.0	22.3 ± 0.6
2	24.3 ± 0.6	25.3 ± 0.6	21.0 ± 1.0	14.7 ± 1.2	20.3 ± 1.5	20.3 ± 1.2
3	23.7 ± 1.5	25.3 ± 0.6	19.7 ± 1.2	14.3 ± 0.6	20.7 ± 0.6	19.7 ± 0.6
4(6)	25.3 ± 0.6	26.0 ± 1.0	22.0 ± 1.0	14.3 ± 1.2	21.7 ± 0.6	22.0 ± 1.0

Organism: Candida parapsilosis

	Treatment Methods ($\bar{x}$ ± SD)					
Subject	FY[a]	VY[b]	CY[c]	FHA[d]	Con-A[e]	A-DM[f]
1	20.7 ± 0.6	22.7 ± 0.6	19.0 ± 1.0	13.7 ± 0.6	18.7 ± 0.6	20.0 ± 1.0
2	20.3 ± 0.6	21.0 ± 1.0	17.7 ± 0.6	14.0 ± 1.0	18.3 ± 0.6	17.3 ± 0.6
3	20.7 ± 0.6	22.3 ± 0.6	18.0 ± 1.0	13.0 ± 1.0	18.0 ± 0.0	17.7 ± 0.6
4(6)	20.7 ± 0.6	22.0 ± 1.0	18.7 ± 0.6	14.0 ± 1.0	19.0 ± 1.0	18.3 ± 1.5

Organism: Candida tropicalis

	Treatment Methods ($\bar{x}$ ± SD)					
Subject	FY[a]	VY[b]	CY[c]	FHA[d]	Con-A[e]	A-DM[f]
1	15.3 ± 0.6	17.3 ± 0.6	14.3 ± 0.6	10.3 ± 0.6	14.3 ± 0.6	14.7 ± 0.6
2	15.7 ± 0.6	16.3 ± 0.6	14.7 ± 0.6	10.3 ± 0.6	14.3 ± 0.6	14.3 ± 0.6
3	15.3 ± 0.6	16.7 ± 0.6	14.3 ± 0.6	9.7 ± 0.6	14.7 ± 0.6	14.3 ± 0.6
4(6)	15.7 ± 0.6	17.3 ± 0.6	14.3 ± 0.6	10.0 ± 1.0	14.7 ± 0.6	14.7 ± 1.5

Organism: Candida pseudotropicalis

	Treatment Methods ($\bar{x}$ ± SD)					
Subject	FY[a]	VY[b]	CY[c]	FHA[d]	Con-A[e]	A-DM[f]
1	17.7 ± 0.6	19.7 ± 0.6	16.0 ± 1.0	13.0 ± 1.0	15.7 ± 0.6	15.0 ± 1.0
2	17.7 ± 0.6	19.7 ± 0.6	16.3 ± 0.6	14.3 ± 0.6	16.0 ± 0.0	15.7 ± 0.6
3	17.7 ± 0.6	20.3 ± 0.6	16.7 ± 1.2	13.0 ± 1.0	16.3 ± 0.6	16.0 ± 1.0
4(6)	17.7 ± 1.2	18.7 ± 1.2	15.7 ± 0.6	13.0 ± 1.0	16.7 ± 0.6	15.7 ± 0.6

[a]Formal in treated yeast: Treatment methods and effect kills organism.
[b]Viable yeast.
[c]Citric acid treated yeast: Mannan removal.
[d]Filamentous Hemagglutinin treated yeast (Mannan inhibition).
[e]Concanavalin-A treated yeast (Mannan inhibition).
[f]α-D methylmannopyranoside adherence treatment (complementary sugar).

APPENDIX C

APPENDIX C

ANOVA results of statistical analysis of data:

I. Graph position	Person
1	LRH
2	ALR
3	AO
4	EB
5	MK
6	LS

II. Organism: Graph # = Figure #

1.....3
2.....4
3.....5
4.....6
5.....7
6.....8
7.....9
8.....10
9.....11
10.....12
11.....13
12.....14

GENERALIZATIONS

FACTOR A:

Tests 1-6: FY and VY significantly differed from the rest except each other.

Tests 7-12: FHA significantly differed from the rest in many cases.

FACTOR B:

MK is significantly different in all cases where a significant difference exists in FACTOR B.

RESULTS OF GRAPH #1

FACTOR A

FY	significantly differs from	rest except VY
VY	significantly differs from	rest except FY
CY ALR	significantly differs from	FHA ALR
AD	significantly differs from	AO
EB	significantly differs from	EB
FHA AO	significantly differs from	α-DM AO

FACTOR B

FY AO	significantly differs from	FY MK

WITHIN SUBGROUPS

FY AO	significantly differs from	All except VY

RESULTS OF GRAPH #2

FACTOR A

FY	significantly differs from	all but VY
VY	significantly differs from	all but FY

FACTOR B

VY MK	significantly differs from	VY LS

RESULTS OF GRAPH #3

FACTOR A

FY	significantly differs from	all but VY
VY	significantly differs from	all but FY
CY LRH	significantly differs from	FHA LRH

FACTOR B

FY LRH	significantly differs from	FY MK
CY ALR	significantly differs from	CY MK
VY MK	signficantly differs from	All VY except LS and AO
FMA MK	significantly differs from	FMA ALR AO EB
ConA ALR	significantly differs from	ConA MK
α-DM MK	significantly differs from	α-DM ALR AO EB

RESULTS OF GRAPH #4

FACTOR A

FY	significantly differs from	All except VY
VY	significantly differs from	All except FY

FACTOR B

FY ALR	significantly differs from	FY MK

RESULTS OF GRAPH #5

FACTOR A

FY	significantly differs from	All except VY
VY	significantly differs from	All except FY
CY LAS	significantly differs from	FHA LAS

RESULTS OF GRAPH #6

FACTOR A

FY	significantly differs from	All except VY
VY	significantly differs from	All except FY
FY ESB	significantly differs from	VY ESB

FACTOR B

VY MAL	significantly differs from	VY AO ESB

RESULTS OF GRAPH #7

FACTOR A

FY	significantly differs from	FHA
VY	significantly differs from	FHA
VY LRH	significantly differs from	ConA LRH
FHA ALR	significantly differs from	FY ALR, CY ALR
AO	significantly differs from	FY AO, ConA AO
ESB	significantly differs from	All ESB except ConA
MAK	significantly differs from	All MAK
LAS	significantly differs from	All LAS
VY LAS	significantly differs from	α-DM LAS

RESULTS OF GRAPH #8

FACTOR A

FHA	significantly differs from	All
VY	significantly differs from	All except FY

RESULTS OF GRAPH #9

FACTOR A

FHA	significantly differs from	All
VY ALR	significantly differs from	ConA ALR, α-DM ALR CY AO, α-DM AO
AO	significantly differs from	α-DM AO

RESULTS OF GRAPH #10

FACTOR A

FY	significantly differs from	FHA
VY	significantly differs from	FHA
FHA LRH	significantly differs from	CY LRH
	significantly differs from	ConA LRH
	significantly differs from	α-DM LRH
FHA AO	significantly differs from	CY AO
	significantly differs from	ConA AO
	significantly differs from	α-DM AO
FHA LS	significantly differs from	CY LS
	significantly differs from	ConA LS

RESULTS OF GRAPH #11

FACTOR A

FHA	significantly differs from	All

RESULTS OF GRAPH #12

FACTOR A

VY	significantly differs from	FHA
FY LRH	significantly differs from	FHA LRH
VY AO	significantly differs from	ConA AO, α-DM AO
FY AO	significantly differs from	FHA AO
LS	significantly differs from	LS
VY LRH	significantly differs from	α-DM LRH

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