This is to certify that the

thesis entitled

Adherence of Candida albicans to human buccal cells in vitro

presented by

Ramon Luis Sandin

has been accepted towards fulfillment
of the requirements for

M.S. degree in Microbiology

Alvin Lee Rogers
Major professor

Date 8/7/81

ADHERENCE OF *CANDIDA ALBICANS* TO HUMAN BUCCAL CELLS *IN VITRO*

By

Ramon Luis Sandin

A Thesis

Submitted to
Michigan State University
in partial fuifillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Microbiology and Public Health

1981

ABSTRACT

ADHERENCE OF CANDIDA ALBICANS TO
HUMAN BUCCAL CELLS IN VITRO

By

Ramon Luis Sandin

Various lectins and sugars were used to study the possible role of saccharide-containing moieties on Candida albicans and human buccal cells in the adherence of this yeast to mucosal surfaces. The lectins used to pretreat C. albicans or buccal cells before the adherence assay possessed affinities towards several sugar moieties. Concanavalin A inhibited adherence of pretreated yeasts to buccal cells and pretreated buccal cells to non-pretreated yeast cells. Preincubating Concanavalin A with a mannose derivative restored adherence but other sugars did not. Lectins that do not recognize mannose did not inhibit adherence. The presence in the medium of α-D-methyl-mannopyranoside, which resembles the units that compose the cell wall α-mannan, inhibited adherence; other sugars did not. These included N-acetyl-D-glucosamine and glucoside which can serve as building blocks for chitin and glucan, the other major cell wall polysaccharides. A procedure utilizing alkali to preferentially extract α-mannan from the wall of C. albicans decreased adherence significantly. Germinated yeasts were more susceptible to adherence inhibition by Concanavalin A than non-germinated yeasts. These experiments show that Con A inhibits adherence of C. albicans by

binding to an alkali-soluble, mannose-containing component. It appears to be concentrated on germinated more than on non-germinated yeasts and could mediate adherence of C. albicans to mannose-related moieties on human buccal cells.

To the four most
important people
in my life:
Peter, Mami, Papi
and Isa.

Table of Contents

Page

List of Tables

List of Figures

INTRODUCTION

One of the research topics in microbiology that currently receives wide attention is the adherence of microorganisms to biological surfaces. The adherence of microorganisms of medical importance, like the opportunistic yeast Candida albicans, to epithelial surfaces is now recognized as an important first step in the colonization and infection of mammalian membranes (29, 79). Several reports that probe into the molecular details of the adherence of C. albicans have been published recently, but the exact structure of the ligands on the yeast cell surface that mediate adherence is still unknown. The major objective of this thesis was to investigate the molecular structure of the entity involved in adherence, both on the yeast surface and on the surface of cells of the oral epithelium.

Understanding the mechanism of adherence of C. albicans at the molecular level has possible clinical applications if we consider that this knowledge could lead to the prevention of adherence and, thus, to the prevention of subsequent infections. It would be an important step in the elucidation of the process of fungal pathogenesis.

Experimental procedures used in most adhesion studies involve the addition to the medium of substances found on the surface of the interacting cells that might act as competitors to the cell ligands that mediate the adherence. This study used sugars and lectins for

this purpose. Information gained in the first part of the research led to the extraction of a possible ligand from the yeast cell wall and to the use of the extracted cells in experiments.

A second objective was to examine the role of the germ tube in the adherence process. The germ tube is important in adherence because germinated yeasts adhered in significantly greater quantities to buccal cells than non-germinated yeast cells. Experiments were designed and performed to pursue this topic.

REVIEW OF THE LITERATURE

Candida and Candidosis

The genus Candida consists of asporogenous yeasts that reproduce by budding and belong to the form-class Deuteromycetes and the form-family Cryptococcaceae (80). There is evidence that some of the species of this genus have sexual stages belonging to the genus Leukosporidium (80). Of the several species of Candida, C. albicans is the only one that can regularly cause fatal disease in humans and animals (62).

Candida albicans is an opportunistic yeast that leads a saprophytic existence in the gastrointestinal (GI) and urogenital tracts of a large percentage of individuals (60). There is no evidence available to disprove the statement that this organism never leaves the host spontaneously during life once it becomes part of the fecal flora (60). The reports on the incidence of C. albicans in normal individuals vary widely. The following figures have been published and are generally recognized as correct. It has been reported that between 30% and 50% of normal humans harbor C. albicans in their gut (60); 39% of normal women have it in their vagina (9); 30% of individuals have it present in their oral cavity (3) and in 46% of normal individuals, it is present in the perianal skin (78).

In addition to leading a saprophytic existence, C. albicans can cause infections known as candidoses that have varied clinical

manifestations. The infections can be acute, sub-acute or chronic and involve the skin, mucous membranes, or internal organs (80). All body systems are susceptible to candidosis, and allergies may be caused by this organism (80). The body counteracts infections by Candida in various ways, from irritation and inflammation, to chronic or acute suppuration, and the formation of granulomas (80). Due to such a wide range of clinical manifestations, Rippon (80) considers it to be perhaps the most protean infectious agent that affects humans with only syphilis being comparable to it.

Thrush in children and vaginitis in women are examples of muco-cutaneous infections by Candida (60). Miles et al. (60) have suggested that the continuous presence of C. albicans in the GI tract of the host may serve as a reservoir from which this yeast can reinfect the vagina in cases of recurrent vaginitis. In that study, women with recurrent vaginitis due to C. albicans always harbored the yeast in the GI tract; conversely, in those women who did not have the organism in the GI tract, the cause of their recurrent vaginal infections was not C. albicans. Holti (37) reports that 30% of vaginitis in pregnant women is caused by C. albicans, whereas it is the cause of vaginitis in only 18% of non-pregnant women with vaginal discharge. In the newborn, the presence of small amounts of organisms in the oral cavity is a prelude to clinical thrush, since the normal resident flora of the GI tract is still unestablished (80). Mothers with vaginitis contribute to thrush in the newborn (80).

Candida albicans differs from the other species of Candida in various ways. Unlike other species, C. albicans is rarely found outside of the natural animal host (19), or as a normal resident on the skin (34). Laboratory methods can be used to carry out the distinction between species. Only C. albicans produces germ tubes when grown in serum at 37°C for one to two hours (89). It is also the only species to produce chlamydospores when grown on chlamydospore agar or corn meal agar to which 'Tween-80 has been added (5). Assimilation and fermentation patterns for carbohydrates can give further proof of identity. Candida albicans is glucose positive, and negative for raffinose, lactose and cellobiose assimilation (5). The colony of C. albicans usually is creamy and smooth, though older colonies can be wrinkled and folded (5). The average size of the yeast form is from 5 - 7 micrometers in length. Candida albicans, and also other species of the genus, produces mycelium and pseudomycelium under certain environmental conditions and media. The pseudomycelium is composed of elongated, undetached spores that have clusters of blastospores at constrictions (5).

Considerable controversy exists as to which phase of the organism - blastospore, spore with germ tube, or filamentous-constitutes the most pathogenic stage and the one that adheres the best. References can be found that support the involvement of each of these stages in pathogenesis and adherence. It is generally accepted, though, that the mere finding of yeasts in body secretions or surfaces is not of

complete diagnostic importance due to the saprophytic existence of the organism in this stage in the body. The presence of mycelial forms connotes that colonization has occurred (80). It follows that only fresh specimens of body fluids or secretions are of diagnostic value for obvious reasons (5). Infected tissue almost always shows the presence of mycelial forms, and this has led investigators to consider it to be the pathogenic or parasitic stage (80). The greater size of the mycelial form should help the organism escape ingestion by the phagocytic cells (52).

Howlet et al. (38) studied epithelia infected with C. albicans by electron microscopy. It was found that epithelial cell membranes were penetrated by hyphae but not by blastospores. No evidence was found for the ability of blastospores to invade. Germination of intracellular hyphae seemed to take place and these were the forms that predominated intracellularly. As will be discussed in other sections, evidence has accumulated which demonstrates that the yeast with germ tube has a greater adherence capability compared to the blastospore stage.

The Cell Wall of Candida Albicans

The cell wall of the blastospore of C. albicans is 200 to 300 nanometers thick (12). Cassone et al. (10) have used procedures that extract the cell wall components by means of alkali and acid, and have determined the distribution and location of the various cell wall

compounds. They identified an outer capsule-like component that consists of spiky, fibrillar protrusions. This component consists chemically, almost in its entirety, of mannan, a homopolymer of mannose. Some mannan is firmly bound to protein (12) which takes place by the esterification of the acid groups of the amino acids to the hydroxyl groups of the sugars (72). The mannan complexes span the entire wall of C. albicans and reach into the inner matrix of the cell wall. This inner component is a rigid, bulky framework composed of glucans and chitin which are closely associated (10, 12). It represents the highly insoluble polysaccharide left after alkaline treatment of the cell wall (10, 65). This inner component was found not to contribute to cell wall layering, but was responsible for providing rigidity and strength to the wall (10). Only when procedures were used which disrupted the inner framework were the yeasts rendered osmotically-fragile (10).

Layering in the cell wall of C. albicans was attributed to the mannose-protein complexes, perhaps with some soluble glucans and unconjugated proteins (10).

Other studies have probed deeper into the molecular structure of the cell wall components. The glucan is a highly branched molecule that contains 72% of its D-glucose units as non-reducing terminal units joined by β 1-6 linkages. The remaining units were linked by β 1-3 linkages (7). A small portion is alkali-soluble and is extracted with the mannan (72). The mannan is also highly branched with short chains

of α 1-2 linked D-mannopyranosyl residues joined together by α 1-6 linkages (7). No furanose rings were found present (7). A small amount of insoluble chitin was found (7). The total lipid content of the wall is approximately 1.1.% and the greater part of it is not chemically bound to the wall (72). Acid phosphatases also exist in association with the cell surface (12).

There is controversy as to the exact delineation of the different layers of the cell wall of C. albicans. From five to eight discrete layers have been identified and variations in reports could be due to the different growth conditions or cytochemical techniques used in different studies (73). McCourtie et al. (58) found that variations occurred in the width of the outer fibrillar-floccular layer of C. albicans when the yeasts were grown in media containing different amounts and types of sugars.

Howlett et al. (38) did scanning and transmission electron microscopy of explants of the oral mucosae of rabbits and rats that had been inoculated with C. albicans. The organ cultures were prepared for microscopy following 12 or 30 hours of incubation with the fungus to study the morphology of the parasite and its relationship to the host. Five distinct wall layers could be identified with their technique. The outermost layer was fibrillar-floccular, in agreement with the descriptions by McCourtie and Cassone (10, 58). This layer could even be clearly identified in intracellular hyphae that were in contact with the host cell cytoplasm. It varied greatly according

to the activity of the fungus. The second inner layer was composed of elements of high electron density, whereas the third one consisted of filaments in an arrangement that was parallel to the cell surface. The fourth layer was composed of dispersed material of only moderate electron density, followed by an innermost electron-dense layer that approximates the plasma membrane.

Studies have been done in which the cell wall structure of blastospores and filamentous forms are compared. It was found that basically the same types of compounds existed in both phases, but that the quantities varied considerably (13). The mycelial forms had three times as much chitin and only one third as much protein as the blastospores, and it was proposed that this could be responsible for the increased resistance of hyphal forms to host defense mechanisms (13). Chitin was also identified as the compound that forms the bulk of the electron-transparent layer that initiates ultrastructural changes leading to germ-tube formation (11).

Adherence as a Physical and Biological Phenomenon

One of the research topics in microbiology that receives the most attention nowadays is the adherence of microorganisms to biological surfaces. The adherence of medically important organisms to epithelial surfaces is now recognized as an important first step in the colonization and infection of mammalian membranes (29, 79).

Various advantages are derived from the ability of an organism to adhere to a surface. Organisms that thrive on skin or mucous

membranes can persist on those surfaces for longer periods of time if they possess a means of attachment and anchorage to the substratum. They face less risk of being sloughed off those surfaces by secretions, fluids and the movements of food and defecation (46). Furthermore, attachment onto solid surfaces enables an organism to benefit from the enriched nutrient status that exists at solid-liquid interfaces (57).

There are organisms that adhere to surfaces by means of non-specific or temporary mechanisms, but it is those that adhere by means of specific mechanisms that have received most attention. Specific adherence is that which takes place between an organism and an interface through bonds formed between complementary molecular structures (56). Perhaps the most widely encountered mechanism of specific adherence is the lectin-carbohydrate interaction. Lectins are globulins or glycoproteins that have a high affinity for specific carbohydrates (17, 88). The two structures interact in a manner that is similar to the lock-and-key model proposed for enzyme-substrate interactions. Indeed, surface enzymes are now considered to be another means of specific adherence (30). Fibroblasts were found to adhere to plastic surfaces that were pre-coated with sialidase or β-galactosidase (75, 76). Conversely, α -mannosidase found on the fibroblast surface can mediate this cell's attachment to surfaces coated with polymers of mannose (77). These reactions occur under physiological conditions in which no hydrolytic cleavage can take place.

Thus, the enzymes were acting as efficiently as, and in a manner similar to, the lectins.

Biological surfaces are usually negatively charged (6). Studies with marine bacteria show that these attach to neutral or positively-charged collectors in greater numbers than to negatively-charged ones (6). Repulsions are bound to occur between the negatively-charged surfaces unless something overcomes or neutralizes these charges. To help bridge the gap more efficiently, some organisms extrude one of the mediators of adherence as an extracellular polymer (6). Other organisms require cations as active participants in the adherence process (84). The primary physical attraction mediated by the cations is usually stabilized by a subsequent biological adhesion step (6), such as a lectin-sugar or enzyme-substrate interaction. In itself, this second biological phenomenon is usually divided into two phases. First, an initial reversible phase that is usually sensitive to sugars and haptens, followed by a second irreversible phase that is insensitive to haptens and in which active, cell-dependent processes are taking place (77).

The Adherence of Microorganisms to Biological Surfaces

The attachment of bacteria to membranes rarely occurs by direct contact between the bacterial cell wall and the epithelial cell membrane (27). Most times, the organisms utilize components of their capsules, mucilage, or fimbriae to effect the link to the host cell

cuticle or carbohydrate-rich cell coat (27). The cell coat, or glycocalyx, is a group of glycoproteins and glycolipids, that surrounds body cells on all sides except those involved in specialized cell-cell junctions (50, 84, 95). Some bacteria, like spirochaetes, are even known to attach to and survive in the mucin layer that overlays the glycocalyx of intestinal villi (49) by using it as a source of food.

Several groups of organisms have been the subject of in-depth research concerning their adherence mechanisms. One of these groups is the gram negative bacilli that colonize the gastro-intestinal (GI) and urogenital (UG) tracts of humans and animals. Many strains of these bacteria attach to epithelial cells via surface pili (or fimbriae), in particular type I pili. This has been well documented in Escherichia coli (39, 68, 69), Klebsiella pneumoniae (24), Pseudomonas aeruginosa (74, 100), Salmonella typhimurium (6), Proteus sp. (90) and Shigella sp. (20). Piliated strains of these genera adhere in greater numbers to epithelial cells than non-piliated ones. Trypsin treatment of these bacteria which destroys the protein nature of the pili always inhibits adherence. As early as 1959, it was observed that type I pili mediate an adherence that is mannose-sensitive, inhibitable by D-mannose or its derivatives but not by other sugars (6). The type I pili of E. coli have been found to contain a mannose-specific lectin on their tips that recognizes mannose moieties on the surface of epithelial cells (68, 69). The inhibition effected by D-mannose is reversible. Isolated pili can agglutinate yeast cells or the mannan extracted from

them (68). One method that led to the discovery that mannose residues on the epithelial cells also help mediate the adherence was treatment with sodium metaperiodate, which renders sugars inactive and the epithelial cells that contain them non-adherent (69).

Researchers found that various strains of E. coli were insensitive to inhibition by D-mannose. Most of these strains were pathogenic to humans or animals. Further research identified several capsular (K) antigens as mediators of adherence in these strains (84). They resemble pili morphologically, are proteic in nature and plasmid-coded (84). The K88 antigen (6, 40) mediates the adherence of enterotoxigenic E. coli (ETEC) to neonatal swine small intestine epithelium. The epithelial cell receptors to these antigens are believed to be terminal β-D-galactosyl residues of membrane glycoproteins (30, 31).

Other K antigens have also been characterized. The K99 antigen is known to occur on ETEC that affect the small intestine of calves and lambs, and some swine (61). In man, ETEC bind to small bowel epithelium via colonization factors 1 and 2 (CFA 1 and CFA 2) (23).

A second type of adhesin in S. typhimurium has also been found to be mannose-insensitive (6). It is called mannose-resistant hemagglutinin (MRH).

The pili system in Neisseria gonorrhoeae has been well characterized. Colony types 1 and 2, which are piliated, are more virulent than types 3 and 4, which are non-piliated (6, 35). These pili are different from the Type 1 pili of gram negative bacilli and do not

function as mannose-specific lectins. The protein sub-unit of each pilus consisted of 170 amino acids. Cell-membrane receptors to these pili are believed to be galactose units found in gangliosides on the cell surface (35). Other systems of adherence are also believed to be active in N. gonorrhoeae.

Other sugars on epithelial cell surfaces are also found as parts of receptors to various organisms. In the Vibrio cholerae model, the presence of L-fucose or its derivatives in the medium greatly diminishes adherence (41, 84). D-mannose can diminish adherence but to a much lesser degree, while calcium ions enhance binding tremendously. In the helminthic parasite Ascaris suum (66), D-glucosamine and D-galactosamine inhibit adherence to phagocytic cells. Sialic acid residues, on the other hand, are involved in the adherence of chlamydia (21) and Mycoplasma pneumoniae (26) to epithelial cells.

Rhizobium is a genus of gram-negative soil bacilli that fix nitrogen by forming nodules in the roots of specific legumes (18). They first must adhere to the root hairs of the host. Rhizobium trifolii binds to the root hairs of the white clover plant, Trifolium repens, by means of 2-deoxyglucose residues that act as cross-reactive antigens on the surface of both cell types (17). A galacto-protein called trifoliin with lectin-like characteristics, usually found on the root hair surface, mediates the adherence (17). The synthesis of the lectin is inducible and plasmid-coded. It is easily eluted from the intact clover root by 2-deoxyglucose and has a molecular weight of

50,000 daltons (6).

Group A streptococci possess an entirely different method of adherence. Their surface contains lipoteichoic acid (LTA), a huge molecule that possesses a glycerolphosphate terminus and various fatty acids. This molecule is believed to mediate adherence to epithelial cells by the following method. The LTA is released from the cell surface and the hydrophobic chains of the fatty acids are intercalated into the lipid bilayer of the host cell membrane (2, 70). At the same time, the glycerol-phosphate terminus of the molecule is bound by a protein, M protein, which is anchored onto the streptococcal surface (6). This model is consistent with the surface hydrophobicity of S. pyogenes.

Streptococcus mutans, on the other hand, prefers adhering to the surface of teeth rather than epithelial cells (29). It adheres to teeth by means of extracellular glucans and fructans synthesized from sucrose (6) via surface glucosyl and fructosyl transferases (28). Another protein, a glucan-binding protein, keeps the extracellular polymers attached to the cell surface (43). Other accessory methods of attachment have also been proposed (30).

Adherence of Fungi to Biological Surfaces

There have been numerous studies reported in the literature on adherence of various fungi to surfaces and to each other, but most do not probe into the molecular aspect of the process. Those that do

have paved the way for other studies on the adherence mechanisms of fungi which have relevance in various fields: agriculture, industry and medicine. The research on C. albicans benefits from these. Extensive research efforts have been directed at elucidating the mechanisms of adherence of three fungal microorganisms: the yeasts Hansenula wingei and Saccharomyces cerevisiae, and the cellular slime-mold Dictyostelium discoideum.

Perhaps the most widely studied system is that which concerns the molecular basis of mating in the yeast Hansenula wingei. Hansenula is a genus of ascomycetous yeasts that possess, at times, very peculiar-looking spores (1). In 1956, Wickerham observed that the mixing of cells of specific strains of H. wingei in laboratory containers produced a massive agglutination reaction (99). It only occurred when sexually compatible strains, like the haploid strains 5 and 21, came together. The end result of agglutination could be conjugation, a sexual event, and the subsequent production of sexual sacs called asci that give way to haploid meiospores. It is known that specific complementary macromolecules are mediators in the agglutination of spores of strains 5 and 21. These are called 5-factor and 21-factor, and are found on the surface of the cells from strains 5 and 21 (14).

The 5-factor is a multivalent agglutinin of a glycoprotein nature, heterogeneous in size and contains some mannose. Most of the activity resides in the proteic portion since thiol-reducing agents and pronase inactivate it. It is not affected by alkali (56).

The 21-factor is not an agglutinin. It is a small monovalent glycoprotein of 2.8S known to inhibit the action of factor 5. It is found mostly on the cell surface and also contains mannose. This factor is inactivated by alkali, trypsin or heat (14).

Another glycoprotein known as non-specific inhibitor (NSI) is obtained when cells of any strain, and diploids, are heated for 5 minutes at 100°C. This substance can diminish the agglutinability of cells of compatible strains, and once released, can no longer exert that effect. It was observed that diploid yeast did not agglutinate with other diploid cells nor with haploid ones. At first the NSI was held responsible for this phenomenon, but later research demonstrated that this was not the case. The current hypothesis stipulates that a regulator gene in each haploid genome inhibits the formation of the complementary mating factor and when both genomes are together in one cell a mutual repression occurs. In late stationary phase there is a breakdown of the repression mechanism since some diploids can produce 5-factor, though never 21-factor (14).

The mating factors in Hansenula wingei are non-diffusible since cell-cell contact is needed for agglutination (14). This differs widely from the phenomenon that occurs in Saccharomyces cerevisiae, another yeast that has been studied extensively. This yeast is the usual brewers' and bakers' yeast. Cell-cell interactions of the compatible strains, "α' and "a", occur during mating (1). The agglutination of spores could eventually lead to conjugation and,

just as in Hansenula wingei, to the production of haploid ascospores by meiosis. It was observed that changes in the mating yeasts' cell walls which would prepare them for agglutination and, especially conjugation, could occur without cell contact (55). It was thought that diffusible factors might be responsible for such observed phenomena in systems that contain one cell type but are devoid of the complementary mating-type (55).

Diffusible factors were discovered, such as α-factor. Alpha cells produce α-factor that agglutinates "a" cells in the absence of α cells. Washed "a" cells that had been preincubated in the cell-free culture medium of the opposite mating type adhered more strongly to α cells than non-preconditioned "a" cells (25). The changes produced on "a" cells by α-factor include elongation and deformation of the cell, called "shmooing" (55). One could speculate that these changes lead to conjugation by fusion of the cell walls. Four closely related oligopeptides constitute the α-factor.

An "a" factor has not been isolated yet but is believed to exist and possess characteristics similar to those of α-factors. There is a substance known as "Barrier Effect" (BE) that is produced by "a" cells and inactivates the α-factor. It has not been further characterized but could turn out to be similar to "a" factor in function or maybe the "a" factor itself. It is believed that these diffusible factors could also be surface-bound and would function in preparing cells for conjugation when cell-cell contact is impossible (55).

The other system that has undergone extensive molecular characterization in the area of adherence is the aggregation of myxamoebae in the cellular slime-molds. Cellular slime-molds are not true fungi because they do not have cell walls in their vegetative stage and for various other reasons (1). Yet, they have been classified as fungi because the fruiting bodies of some of them resemble those produced by true fungi.

The most widely studied slime-mold is Dictyostelium discoideum. It is a microscopic organism that thrives inconspicuously on forest floors as individual vegetative cells called myxamoebae (1). Certain contrasurvival stimuli in the environment, like starvation, can trigger a phenomenal metamorphosis in the structure of the slime-mold. Numerous individual myxamoebae are called to an aggregation center to become part of a discrete, migratory organism known as a pseudoplasmodium. The dissemination of spores towards environments that could be more conducive to survival becomes possible by the formation of a fruiting body out of the pseudoplasmodium (1).

While the cells are moving towards the aggregation center under the influence of the attractant, cyclic adenosine monophosphate (cAMP), lectins on their surfaces are being synthesized and receptors to those lectins are being modified. These will be functional during the adherence process that accompanies the aggregation (56). The contact lectins are known as slime-mold lectins (SML). In D. discoideum, they are called discoidins, and in Polysphondylium pallidum, another slime-

mold, they are known as pallidins (63). The lectins and the receptors to them are species-specific.

The discoidins have been shown to be agglutinins since they agglutinate formalin-treated sheep red blood cells (sRBC) (63). Their synthesis is induced only in cells that are in the cohesive stage. They are not glycoproteins, but are multimeric proteins. Two discoidins have been discovered: discoidin 1 and discoidin 2. The first is inhibited best by N-acetyl-D-galactosamine. The second, by D-lactose but D-galactose and L-fucose are also known to inhibit its action (63). The pallidins are similar to the discoidins. The receptors for these slime-mold lectins are membrane-bound glycoproteins. Weeks (98) suggested that some of the surface glycoproteins contain mannose since Concanavalin A was found to agglutinate myxamoebae.

Another type of protein has been discovered that, while not being an agglutinin like SML, could play a similar function. It is known as contact sites A and could behave as a second adherence mechanism. Further characterization of this protein is needed. Calcium ions are also known to play a role in aggregation (63).

The Adherence of Candida Albicans to Biological and Non-Biological Surfaces

The literature on the mechanisms of fungal adherence is not as abundant as that which concerns bacterial adherence. Much of what is found, though, relates to C. albicans and this is probably due to the

clinical importance of the organism. Several reports that probe into the more molecular details of the adherence of C. albicans have been published recently. The exact structure of the ligands on the yeast cell surface that mediate the adherence is still unknown but much progress is being made.

It has been shown that C. albicans adheres to buccal cells (44, 45, 46, 92), vaginal cells (46, 92), fibrin-platelet matrixes formed in vitro (53, 54), acrylic surfaces (58), the surface of neutrophils (15) and other cells of the reticuloendothelial system (86, 93, 97).

Among the first studies on the adherence of C. albicans to epithelial cells were those that detail the kinetics of the process (44, 45, 46). Conditions that are conducive to the germination of the yeast, that is, to the production of a protrusion on the yeast body called a germ tube, were found to increase adherence significantly. These conditions included: the performance of the in vitro adherence assays in saliva at 37°C rather than in phosphate-buffered saline (PBS) at 24°C (44, 45); the use of viable rather than non-viable cells (44, 45, 53, 92); and preincubation of the yeasts before the assay in tissue-culture media, like M199, but not in PBS (44, 45, 92). These media are known to favor the production of germ tubes. Once germinated, cells could be killed in formalin with no decrease in adherence but carrying out the treatment before the germination took place decreased adherence significantly (44). If heat treatment follows germination, the cells are rendered non-adherent (92), perhaps by the inactivation

of some surface component. Cysteine, a partial inhibitor of germination, diminished adherence significantly (45). This suggested that changes that occur in the cell wall of C. albicans as it undergoes germination could be responsible for increased adherence (44, 92). There are those who oppose this hypothesis. King et al. (46) have obtained comparable results when they used yeasts grown either in M199 or in PBS for their experiments.

Buccal and vaginal cells are types of stratified squamous non-keratinized epithelium (50) and are both susceptible to infection by Candida. Yet, there is controversy as to which substrate shows greater affinity for C. albicans. Some reports favor buccal cells (92), while others favor vaginal cells (46). It is generally agreed, though, that there exists a great variation in the number of receptor sites for C. albicans on epithelial cells collected from different persons (46, 92). Day to day variations were also detected in swabbings from a single subject (46), and differences were further detected among cells collected in one swabbing, a fact also observed with streptococci (2). One could be led to speculate that this variation could be due to the occurrence of more receptor sites for C. albicans on the surface changes occurring as a result of age or stage in the cell cycle. Yet, the published reports point to the involvement of the indigenous host flora as a protective mechanism that can keep in check the number of C. albicans cells that adhere to and colonize epithelial cell surfaces.

In one report, gnotobiotic mice were inoculated intraorally with C. albicans followed by an inoculation with normal saliva two weeks later. High numbers of yeast cells were retrieved from the feces and from the oral cavity (51). When the procedure was done in reverse and saliva was inoculated into the mouths two weeks before the yeasts, a significant decrease in the adherence of C. albicans occurred. Pure colonies of organisms that are found in normal saliva were grown and used individually in place of the saliva for inoculation. When either Streptococcus salivarius or Streptococcus miteor were used, the adherence of C. albicans was suppressed. Streptococcus mutans, under identical circumstances, could not suppress the adherence of C. albicans even when it was found in great numbers in the oral cavity of the mice. It is interesting that S. mutans preferentially colonizes teeth in humans and is present only in low proportions on oral epithelia. In vitro assays with cells from germ-free mice revealed that higher numbers of C. albicans adhered to those cells than to cells from conventional mice. These researchers (51) hypothesized that the indigenous flora could be suppressing adherence of C. albicans by competing with it for receptor sites on the epithelial cells, modifying these sites to hamper candidal adherence, or enzymatically altering the yeast surface. Competition for nutrients should not be the reason since S. mutans and S. salivarius are generally considered to have similar nutritional requirements but only the latter suppressed the adherence of C. albicans. The secretion of anti-fungal substances

was discarded as a possible reason when no zone of inhibition, indicative of the production of growth-inhibitory materials, was observed upon the cross-streaking of nutrient agar plates with S. salivarious, S. miteor and C. albicans.

The observation that women are more prone to suffer candidal vaginitis during and after menses (59) led to the discovery that lactobacilli are less prevalent in the vagina at that time, when the numbers of C. albicans are elevated (82). This correlates with the finding that C. albicans adheres better to vaginal cells at a pH of 6 than at a pH of 3-4 in in vitro experiments (92), a range that closely corresponds to the normal vaginal pH (42). It is a clinical fact that the pH of the vagina is elevated during and after menstruation (59). Previous coating of vaginal cells with lactobacilli decreased the numbers of adhering C. albicans (92).

Lactobacilli are also known to colonize the keratinized squamous epithelium of the non-secretory portion of the rat and mouse stomachs, whereas Torulopsis pintolopesii, an aciduric yeast, dominates in the mucin of the secretory epithelial surface (85). Only when antibiotics are given that wipe out the lactobacillal population from the non-secretory portion do the yeast colonize that area.

Candida albicans has been found to adhere in greater numbers than C. stellatoidea, C. tropicalis, and others to vaginal cells (46), buccal cells (45, 46) and to fibrin-platelet matrixes formed in vitro (53). It is interesting to mention that there is a direct correlation between

the greater *in vitro* adherence of *C. albicans* to cell surfaces and its ability to colonize and/or infect mucosal surfaces *in vivo*. *Candida albicans* accounts for more than 90% of the yeasts recovered from the normal mouth (101). In *in vitro* assays, *C. albicans* harvested at the stationary phase of growth shows greater adherence values than cells at the logarithmic stage in adherence to epithelial cells (46) and acrylic strips (58). In the experiments on adherence to acrylic strips, several batches of cells were grown in a basic medium supplemented with individual sugars to determine whether cell surface composition varied with the carbon sources used and if that correlated with adherence to acrylic. Additional surface layers that enhanced adherence were revealed only when cells were grown to stationary stage; mid-exponential stage cells adhered poorly to acrylic (58).

It is reasonable and perhaps, logical, that buccal and vaginal cells would constitute the substrate for the first *in vitro* experiments dealing with the adherence mechanism of *C. albicans*. Thrush and vaginitis are, after all, rather common diseases in the population. Yet, novel *in vitro* assays have been recently evolved to study the role of candidal adherence in diseases that are found less commonly in the population and/or that only now are beginning to capture the attention of researchers. Infective endocarditis is a disease of the heart valves (53). Although *C. albicans* is responsible for only a small percentage of the occurrence of this disease, candidal endocarditis can be fatal (81) since the clinical signs and symptoms are not

always present (87). In a rabbit model, it was found that vegetations of C. albicans occur on areas of endothelial trauma on fibrin-platelet matrixes (clots) (83). Maisch and Calderone (53, 54) developed an in vitro assay in which platelet-rich plasma was mixed with thrombin to produce clots that could be inoculated with radiolabelled C. albicans. Incubation for 30 minutes could be followed by a radiometric measurement of adherence. Results showed that C. albicans adhered to clots and that anti-Candida antiserum used to pretreat the yeasts would decrease adherence significantly, but normal rabbit serum did not. Antibodies could play a role in vivo in protection against candidosis.

Candida albicans is also known to infect the mucosa under the upper denture of denture wearers. This disease is known as denture stomatitis ("chronic atrophic candidosis") (58) and is regarded now as the most common form of oral candidosis (67). Acrylic is used to manufacture dentures and it has been implicated as a possible reservoir for C. albicans from which it can spread to the mucosa (58). Since sucrose rinses can promote denture stomatitis in infection-free denture wearers (71), McCourtie et al. (58) decided to experimentally test the relationship between the presence of sugars in the yeast's environment and the occurrence of disease. It is well known that dental plaque can be caused by Streptococcus mutans and it is known to be increased by the presence of high amounts of sucrose in the diet (36). These researchers developed an in vitro assay in which transparent acrylic strips were placed in the wells of serology plates, inoculated with

yeasts and incubated at room temperature for one hour. Adherence could then be quantitated visually with the use of a microscope. Growth of C. albicans in media that contained high amounts of a single sugar as the sole carbon source gave increased adherence values. The increase was proportional to the amount of sugar added. Saliva used to pretreat the strips significantly decreased the adherence of yeasts. Electron microscopy of the yeast surface showed the presence of additional cell wall layers that could be considered responsible for the increased adherence. The finding that the presence of glucose and other sugars, besides sucrose, could increase the adherence of C. albicans to acrylic is important because there are increased levels of salivary glucose (48) in diabetic patients and those undergoing antibiotic or steroid therapy. All three cases are predisposed to candidosis (67).

The most recently published studies on adherence of C. albicans probe into the molecular aspects of the phenomenon. Pretreatment of C. albicans with trypsin, chymotrypsin or pronase reduced adherence significantly (46, 53, 92). This suggests that the adhesin on the surface of C. albicans might be proteic in nature. Preincubation of the yeasts in L- or D-fucose, but not in D-mannose or D-galactose, reduced adherence significantly whereas preincubation in D-glucose increased adherence (92). Various recent articles tend to contradict these latter findings by pointing consistently at the yeast mannan as the entity that mediates the adherence in C. albicans. The blastospores of C. albicans are agglutinated by Concanavalin A (Con A) (10),

a mannose and glucose-specific lectin (88). When a procedure is followed that uses alkali and acid to preferentially extract the mannan from the cell wall of C. albicans, the blastospores are rendered unagglutinable by Con A in direct proportion to the amount of mannan extracted (10). Thiol-reducing agents which did not completely extract the small amounts of mannan found in the inner portions of the wall, did not completely abolish agglutination. A conclusion arrived at by Cassone et al., was that the mannan is the entity involved in the agglutination of blastospores by Con A and constitutes the bulk of the outer fibrillar-floccular layer of the cell wall of C. albicans (10).

Treatment of C. albicans with papain renders it nonadherent to vaginal cells (47). Purification of the products of the digestion with papain yielded two mannoproteins, A and B. The latter was shown to bind to vaginal cells. When these cells were pretreated with the B mannoprotein, there was partial blockage of the adherence of blastospores. Non-adhering yeasts were shown to have small amounts of the B substance.

An alkali-soluble cell wall extract of C. albicans was conjugated to sheep red blood cells (sRBC) via periodate oxidation or Con A. These cells adhered to clots formed in vitro whereas non-conjugated sRBC's did not (54). Pretreatment of the alkali-soluble fraction with α-mannosidase, or degradation by acetolysis, followed by conjugation to sRBC, rendered the cells non-adherent. Antisera to C. albicans blastospores that could abrogate their adherence to clots was unable

to do so when preabsorbed with the extract. The extract consisted mostly of polysaccharides, with mannose being the predominant sugar. The conclusion of these researchers (54) was that surface mannan could be involved in the process of adherence of C. albicans to clots.

The attachment of C. albicans to cells of the reticulo-endothelial system has received much attention lately. Diamond et al., found that neutrophils can cause damage to pseudohyphae of C. albicans in the absence of serum in vitro but that this can be inhibited by 2-deoxy-glucose (15), or Candida mannans (16). Dextran, chitin, Con A or mannose had no effect (16). The sugars interfered with the spreading of neutrophils over the surface of Candida. The neutrophils could attach to other filamentous fungi but not to the blastospore stage of C. albicans nor to other fungi that lack a hyphal phase.

Alveolar macrophages were found to bind glycoproteins that have mannose, N-acetylglucosamine or glucose in the exposed nonreducing position (93). An excess of yeast mannan completely abolishes the binding whereas glycoproteins with terminal galactose residues do not (93). The lung macrophages have also been shown to bind and ingest whole yeast blastospores in the absence of serum factors in vitro but 30mM concentrations of D-mannose or D-glucosamine inhibited the binding (97). D-mannitol, D-glucose, D-galactose or L-fucose had no effect.

Schwocho et al., found recently that D-mannose can impair the trapping of C. albicans in the livers of normal mice, whereas D-mannitol or D-glucose had no effect (86).

Literature Cited

LITERATURE CITED

1. Alexopoulos, C. J. 1962. In: J. Wiley and Sons (ed.), Introductory Mycology. New York.

2. Bartelt, M., and J. L. Duncan. 1978. Adherence of group A streptococci to human epithelial cells. Infect. Immun. 20: 200-208.

3. Baum, G. L. 1960. The significance of Candida albicans in human sputum. N. Engl. J. Med. 263: 70.

4. Beachey, E. H. 1981. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. J. Infect. Dis. 143: 325-345.

5. Beneke, E. S., and A. L. Rogers. 1980. Yeast of medical importance. Candidosis. pp. 124-131. In: Medical mycology manual. Burgess Publishing Company. Minneapolis.

6. Berkeley, Lynch, Melling, and Rutter. 1981. In: Microbial adhesion to surfaces. John Wiley and Sons. New York.

7. Bishop, C. T., F. Blank, and P. E. Gardner. 1960. The cell wall polysaccharides of C. albicans: glucan, mannan and chitin. Can. J. Chem. 38: 868-881.

8. Bitton, G., and K. C. Marshall. 1980. Microbial adhesion in perspective. pp. 1-5. In: G. Bitton and K. C. Marshall (eds.). Adsorption of microorganisms to surfaces. J. Wiley and Sons. New York.

9. Carter, B., C. P. Jones, and R. Creadnik. 1959. The vaginal fungi. Ann. N.Y. Acad. Sci. 83: 265.

10. Cassone, A., E. Mattia, and L. Boldrini. 1978. Agglutination of blastospores of Candida albicans by Concanavalin A and its relationship with the distribution of mannan polymers and the ultrastructure of the cell wall. J. Gen. Microbiol. 105: 263-273.

11. Cassone, A., N. Simonetti, and V. Strippoli. 1973. Ultrastructural changes in the wall during germ-tube formation in blastospores of Candida albicans. J. Gen. Microbiol. 77: 417-426.

12. Chattaway, F. W., S. Shenolikar, and J. O'Reilly. 1976. Changes in the cell surface of the dimorphic forms of Candida albicans by treatment with hydrolytic enzymes. J. Gen. Microbiol. 95: 335-347.

13. Chattaway, F. W., M. R. Holmes, and A. J. E. Barlow. 1968. Cell wall composition of the mycelial and blastospore forms of Candida albicans. J. Gen. Microbiol. 51: 367-376.

14. Crandall, M. A., and T. D. Brock. 1968. Molecular basis of mating in the yeast Hansenula wingei. Bacteriological Reviews. 32: 139-163.

15. Diamond, R. D., R. Krzesicki, and W. Jao. 1978. Damage to pseudohyphal forms of Candida albicans by neutrophils in the absence of serum in vitro. J. Clinic Invest. 61: 349-359.

16. Diamond, R. D., R. Krzesicki, and W. Jao. 1978. Mechanism of attachment of neutrophils to Candida albicans pseudohyphae in the absence of serum, and of subsequent damage to pseudohyphae by microbicidal processes of neutrophils in vitro. J. Clinic. Invest. 61: 360-369.

17. Dazzo, F. 1980. Adsorption of microorganisms to roots and other plant surfaces. pp. 253-316. In: G. Bitton and K. C. Marshall (eds.). Adsorption of microorganisms to surfaces. J. Wiley and Sons. New York.

18. Dazzo, F. B., C. A. Napoli, and D. H. Hubbell. 1976. Adsorption of bacteria to roots as related to host specificity in the Rhizobium-Clover symbiosis. Appl. Environ. Microb. 32: 166-171.

19. Drake, T. E., H. I. Maiback. 1973. Candida and candidiasis. Postgrad. Med. 53: 83-88, 120-126.

20. Duguid, I. P., M. R. Darekar, and D. W. F. Wheather. 1976. Fimbriae and infectivity in Salmenella typhimurium. J. Med. Microbiol. 9: 459-473.

21. Ellwood, D. C., J. Melling, and P. Rutter. 1979. Adhesion of Vibrio cholerae. pp. 174-176. In: Ellwood, Melling, and Rutter (eds.). Adhesion of microorganisms to surfaces. Academic Press. London.

22. Etzler, M. E., and M. L. Branstrator. 1974. Differential localization of cell surface and secretory components in rat intestinal epithelium by use of lectins. J. Cell. Bio. 62: 329-343.

23. Evans, D. G., and D. J. Evans. 1978. New surface-associated heat-labile colonization factor antigen (CFA/II) produced enterotoxigeni Escherichia coli of serogroups 06 and 08. Infect. Immun. 21: 638-647.

24. Fader, R. C., A. E. Avots-Avotins, and C. P. Davis. 1979. Evidence for pili mediated adherence of Klebsiella pneumoniae to rat bladder epithelial cells in vitro. Infect. Immun. 25: 729-737.

25. Fehrenbacher, G., K. Perry, and J. Thorner. 1978. Cell-cell recognition in Saccharomyces cerevisiae: regulation of mating-specific adhesion. J. Bacteriol. 134: 893-901.

26. Feldner, J., W. Bredt, and S. Razin. 1979. Adherence of Mycoplasma pneumoniae to glass surfaces. Infect. Immun. 26: 70-75.

27. Fletcher, M., and G. D. Floodgate. 1973. An electron-microscopic demonstration of an acidic polysaccharide involved in the adhesion of a marine bacterium to solid surfaces. J. Gen. Microbiol. 74: 325-334.

28. Germaine, G. R., and C. F. Schachtele. 1976. Streptococcus mutans dextransucrase: mode of interaction with high-molecular-weight dextran and role in cellular aggregation. Infect. Immun. 13: 365-372.

29. Gibbons, R. P., and J. Van Houte. 1975. Bacterial adherence in oral microbial ecology. Annu. Rev. Microbiol. 29: 19-44.

30. Gibbons, R. J. 1977. Adherence of bacteria to host tissue. pp. 395-406. In: D. Schlessinger (ed.). Microbiology 1977. American Society for Microbiology. Washington.

31. Gibbons, R. A., G. W. Jones, and R. Sellwood. 1975. An attempt to identify the intestinal receptor for the K88 adhesin by means of a hemagglutination inhibition test using glycoproteins and fractions from sow colostrum. J. Gen. Microbiol. 86: 228-240.

32. Goldstein, I. J., C. E. Hollermon, and J. M. Merrick. 1965. Protein-carbohydrate interactions. I. The interaction of polysaccharides with Concanavalin A. Biochim. Biophys. Acta 97: 68-76.

33. Goldstein, I. J., and L. L. So. 1965. Protein-carbohydrate interactions III. Agar-gel diffusion studies on the interaction of Concanavalin A, a lectin, isolated from Jack bean, with polysaccharides. Arch. Biochem. Biophys. 8: 407-414.

34. Greshem, G. A., and C. H. Whittle. 1961. Studies on the invasive, mycelial form of Candida albicans. Sabouraudia. 1: 30-33.

35. Gubish, E. R., M. L. Mace, S. M. Steiner, and R. P. Williams. 1979. Assessment of attachment of Neisseria gonorrhoeae to HeLa cells by double radiolabeling. Infect. Immun. 25: 1043-1050.

36. Hamada and Slade. 1980. Biology, immunology and cariogenicity of Streptococcus mutans. Microbiol. Reviews 44: 331-384.

37. Holti, G. 1966. p. 73. In: E. and S. Livingstone (eds.). Symposium on Candida infections. Edinburgh.

38. Howlett, J. A., and C. A. Squier. 1980. Candida albicans ultrastructure: colonization and invasion of oral epithelium. Infect. Immun. 29: 252-260.

39. Johanson, W. G., D. E. Woods, and T. Chaudhuri. 1979. Association of respiratory tract colonization with adherence of gram-negative bacilli to epithelial cells. J. Infect. Dis. 139: 667-673.

40. Jones, G. W., and J. M. Rutter. 1972. Role of the K88 antigen in the pathogenesis of neonatal diarrhea caused by Escherichia coli in piglets. Infect. Immun. 6: 918-927.

41. Jones, G. W., and J. M. Rutter. 1974. The association of K88 antigens with hemaglutinating activity in porcine strains of Escherichia coli. J. Gen. Microbiol. 84: 135.

42. Karnaky, K. J. 1959. Comparison of battery pH readings of the vagina in Trichomonas vaginalis infestation. Am. J. Obstet. Gynecol. 77: 149-154.

43. Kelstrup, J., and T. D. Funder-Nielsen. 1974. Adhesion of dextran to Streptococcus mutans. J. Gen. Microbiol. 81: 485-489.

44. Kimura, L. H., and N. H. Pearsall. 1978. Adherence of Candida albicans to human buccal epithelial cells. Infect. Immun. 21: 64-68.

45. Kimura, L. H., and N. H. Pearsall. 1980. Relationship between germination of Candida albicans and increased adherence to human buccal epithelial cells. Infect. Immun. 28: 464-468.

46. King, R. D., J. C. Lee, and A. L. Morris. 1980. Adherence of Candida albicans and other Candida species to mucosal epithelial cells. Infect. Immun. 27: 667-674.

47. King, R. D., and J. C. Lee. 1981. Characterization of surface components which mediate Candida albicans adherence to mucosal cells. p. 313. Abst. Annu. Meet. Amer. Soc. Microbiol. 1981.

48. Knight, L., and J. Fletcher. 1971. Growth of Candida albicans in saliva: stimulation by glucose associated with antibiotics, corticosteroids and diabetes mellitus. J. Infect. Dis. 123: 371-377.

49. Lee, A. 1980. Normal flora of animal intestinal surfaces. Pp. 145-174. In: G. Bitton and K. C. Marshall (eds.). Adsorption of microorganisms to surfaces. J. Wiley and Sons. New York.

50. Leeson, C. R., and T. S. Leeson. 1976. The cell. p. 26. In: Histology. W. B. Saunders Company. Philadelphia.

51. Liljemark, W. F., and R. J. Gibbons. 1973. Suppression of Candida albicans by human oral streptococci in gnotobiotic mice. Infect. Immun. 8: 846-849.

52. Louria, D. B., and R. G. Brayton. 1964. The behavior of Candida cells within leucocytes. Proc. Soc. Exp. Biol. Med. 115: 93-98.

53. Maisch, P., and R. A. Calderone. 1980. Adherence of Candida albicans to a fibrin-platelet matrix formed in vitro. Infect. Immun. 27: 650-656.

54. Maisch, P. A., and R. A. Calderone. 1981. Role of surface mannan in the adherence of Candida albicans to fibrin-platelet clots formed in vitro. Infect. Immun. 32: 92-97.

55. Manney, T. R., and J. H. Meade. 1977. Cell-cell interactions during mating in Saccharomyces cerevisiae. pp. 283-304. In: J. L. Reissig (ed.). Microbial interactions. Receptors and recognition, Series B, Vol. 3. Chapman and Hall. London.

56. Marshall, K. C. 1976. In: Interfaces in microbial ecology. Harvard Univ. Press. Cambridge.

57. Marshall, K. C. 1976. pp. 5, 44. In: Interfaces in microbial ecology. Harvard Univ. Press. Cambridge.

58. McCourtie, J., and L. J. Douglas. 1981. Relationship between cell surface composition of Candida albicans and adherence to acrylic after growth on different carbon sources. Infect. Immun. 32: 1234-1241.

59. Mead, P. B. 1974. Candida albicans. pp. 242-267. In: G. R. G. Monif (ed.). Infectious diseases in obstetrics and gynecology. Harper and Row. New York.

60. Miles, M. R., L. Olsen, and A. L. Rogers. 1977. Recurrent vaginal candidiasis. J. Amer. Med. Assoc. 238: 1836-1837.

61. Moon, H. W., B. Nagy, R. E. Isaacson, and I. Orskov. 1977. Occurrence of K99 antigen on Escherichia coli isolated from pigs and colonization of pig ileum by $K99^+$ enterotoxigenic E. coli from calves and pigs. Infect. Immun. 15: 614-620.

62. Mourad, S., and L. Friedman. 1961. Pathogenicity of Candida. J. Bacteriol. 81: 550-556.

63. Newell, P. C. 1977. Aggregation and cell surface receptors in cellular slime molds. pp. 3-49. In: J. L. Reissig (ed.). Microbial interactions. Receptors and recognition, Series B, Vol. 3. Chapman and Hall. London.

64. Newman, H. N. 1980. Retention of bacteria on oral surfaces. pp. 207-252. In: G. Bitton and K. C. Marshall (eds.). Adsorption of microorganisms to surfaces. J. Wiley and Sons. New York.

65. Northcote, D. H. 1963. The structure and organization of the polysaccharides of yeast. P. Appl. Chem. 7: 669-675.

66. Nuessen, M. E. and E. L. Jeska. 1981. The involvement of sugars and Mg^{+2} in the attachment of phagocytic cells to Ascaris suum juveniles. J. Infect. Dis. 143: 71-75.

67. Odds, F. C. 1979. Candida and candidosis. University Park Press. Baltimore.

68. Ofek, I., and E. H. Beachey. 1978. Mannose binding and epithelial cell adherence of Escherichia coli. Infect. Immun. 22: 247-254.

69. Ofek, I., D. Mirelman, and N. Sharon. 1977. Adherence of Escherichia coli to human mucosal cells mediated by mannose receptors. Nature. 265: 623-625.

70. Ofek, I., E. H. Beachey, W. Jefferson, and G. I. Campbell. 1975. Cell membrane binding properties of group A streptococcal lipoteichoic acid. J. Exp. Med. 141: 990-1003.

71. Olsen, I., and J. M. Birkeland. 1976. Initiation and aggravation of denture stomatitis by sucrose rinses. Scand. J. Dent. Res. 84: 94-97.

72. Phaff, H. J. 1963. Cell wall of yeasts. Annu. Rev. Microbiol. 17: 15-30.

73. Poulain, D., G. Tronchin, J. F. Dubremetz, and J. Biguet. 1978. Ultrastructure of the cell wall of Candida albicans blastospores: study of its constitutive layers by the use of a cytochemical technique revealing polysaccharides. Ann. Microbiol. (Inst. Pasteur). 129: 141-153.

74. Ramphal, R., P. M. Small, J. W. Shands, W. Fischtschweiger, and P. A. Small. 1980. Adherence of Pseudomonas aeruginosa to tracheal cells injured by influenza infection or by endotracheal intubation. Infect. Immun. 27: 614-619.

75. Rauvala, H., W. G. Carter, and S. Hakomori. 1981. Studies on cell adhesion and recognition I. Extent and specificity of cell adhesion triggered by carbohydrate-reactive proteins (glycosidases and lectins) and by fibronectin. J. Cell. Biol. 88: 127-137.

76. Rauvala, H., W. G. Carter, and S. Hakomori. 1981. Studies on cell adhesion and recognition II. The kinetics of cell adhesion and cell spreading on surfaces coated with carbohydrate-reactive proteins (glycosidases and lectins) and fibronectin. J. Cell. Biol. 88: 138-148.

77. Rauvala, H., W. G. Carter, and S. Hakomori. 1981. Studies on cell adhesion and recognition III. The occurrence of α-mannosidase at the fibroblast cell surface, and its possible role in cell recognition. J. Cell. Biol. 88: 149-159.

78. Recio, P. M., and A. DeLeon. 1957. Candidiasis of the rectoanal tract. J. Phil. Med. Assoc. 33: 293.

79. Reed, W. P., and R. C. Williams. 1978. Bacterial adherence: first step in pathogenesis of certain infections. J. Chron. Dis. 31: 67-72.

80. Rippon, J. W. 1974. Candidosis. pp. 175-204. In: W. B. Saunders (ed.). Medical mycology. The pathogenic fungi and the pathogenic actinomycetes. Saunders. Philadelphia.

81. Rubinstein, E., E. R. Noriega, M. S. Simberkoff, R. Holzman, and J. J. Rahal. 1975. Fungal endocarditis, analysis of 24 cases and reviews of the literature. Medicine. 54: 331-344.

82. Saigh, J. H., C. C. Sanders, and W. E. Sanders. 1978. Inhibition of Neisseria gonorrhoeae by aerobic and facultatively anaerobic components of the endocervical flora: evidence for a protective effect against infection. Infect. Immun. 19: 704-710.

83. Sande, M. A., C. R. Bowman, and R. A. Calderone. 1977. Experimental Candida albicans endocarditis: characterization of the disease and response to therapy. Infect. Immun. 17: 140-147.

84. Savage, D. C. 1980. Colonization by and survival of pathogenic bacteria on intestinal mucosal surfaces. pp. 175-206. In: G. Bitton and K. C. Marshall (eds.). Adsorption of microorganisms to surfaces. J. Wiley and Sons. New York.

85. Savage, D. C., and R. J. Dubos. 1967. Localization of indigenous yeast in the murine stomach. J. Bacteriol. 94: 1811-1816.

86. Schwocho, L. R. 1981. Clearance and killing of Candida albicans in the perfused mouse liver. M.S. Thesis. Michigan State University. East Lansing, Michigan.

87. Seeling, M. S., C. P. Speth, P. J. Kozinn, E. F. Tonl, and C. L. Taschdjian. 1973. Candida endocarditis after cardiac surgery. Clues to early diagnosis. J. Thor. Cardiovasc. Surg. 65: 583-601.

88. Sharon, N., and H. Lis. 1972. Lectins: cell-agglutinating and sugar-specific proteins. Science. 177: 949-958.

89. Silva-Hutner, M., and B. H. Cooper. 1974. Chapter 56. Medically important yeasts. pp. 491-507. In: E. H. Lennette, E. H. Spaulding and J. P. Truant (eds.). Manual of clinical microbiology. American Society for Microbiology. Washington.

90. Silverblatt, F. J. 1974. Host-parasite interaction in the rat renal pelvis. J. Exp. Med. 140: 1696-1711.

91. So, L. L., and I. J. Goldstein. 1968. Protein-carbohydrate interaction. XIII. The interaction of Concanavalin A with α-mannans from a variety of microorganisms. J. Biol. Chem. 243: 2003-2007.

92. Sobel, J. D., P. G. Myers, D. Kaye, and M. E. Levison. 1981. Adherence of Candida albicans to human vaginal and buccal epithelial cells. J. Infec. Dis. 143: 76-82.

93. Stahl, P. D., J. S. Rodman, M. J. Miller, and P. H. Schlesinger. 1978. Evidence for receptor-mediated binding of glycoproteins glycoconjugates and lysosomal glycosidases by alveolar macrophages. Proc. Nat'l Acad. Sci. U.S.A. 75: 1399-1403.

94. Stoddart, R. W., and B. M. Herbertson. 1977. The use of lectins in the detection and identification of human fungal pathogens. pp. 233-235. Biochem. Soc. Transactions. 566th meeting. 1977.

95. Swift, J. G., and T. M. Mukherjee. 1976. Demonstration of the fuzzy surface coat of rat intestinal microvilli by freeze-etching. J. Cell Biol. 69: 491-494.

96. Tkacz, J. S., E. B. Cybulska, and J. O. Lampen. 1971. Specific staining of wall mannan in yeast cells with fluorescein-conjugated Concanavalin A. J. Bacteriol. 105: 1-5.

97. Warr, G. A. 1980. A macrophage receptor for (mannose/glucosamine)-glycoproteins of potential importance in phagocytic activity. Biochem. Biophys. Res. Comms. 93: 737-745.

98. Weeks, G. 1973. Agglutination of growing and differentiating cells of Dictyostelium discoideum by Concanavalin A. Exp. Cell Res. 76: 467-470.

99. Wickerham, L. J. 1958. Sexual agglutination of heterothallic yeasts in diverse taxonomic areas. Science. 128: 1504-1505.

100. Woods, D. E., D. C. Strauss, W. G. Johanson, V. K. Berry, and J. A. Bass. 1980. Role of pili in adherence of Pseudomonas aeruginosa to mammalian buccal epithelial cells. Infect. Immun. 29: 1146-1151.

101. Young, G., H. G. Resca, and M. T. Sullivan. 1951. The yeasts of the normal mouth and their relation to salivary acidity. J. Dent. Res. 30: 426-430.

Inhibition of Adherence of *Candida albicans* to Human Epithelial Cells.

Ramon L. Sandin and Alvin L. Rogers

Submitted to Mycopathologia for Publication.
Accepted for publication May 4, 1981.

Inhibition of adherence of Candida albicans to human epithelial cells.

R. L. Sandin[1] A. L. Rogers[1,2]

Department of Microbiology and Public Health (1), and the Department of Botany and Plant Pathology (2), Michigan State University, East Lansing, Michigan 48824

Abstract

A mannose-specific lectin, Concanavalin A, was used to pretreat Candida albicans before using the yeasts in an in vitro adherence assay. Adherence to buccal cells was inhibited but could be restored by preincubation of the lectin with a specific haptenic sugar, α-D-methylmannopyranoside, prior in the assay but not by using D-galactose, D-ribose and D-raffinose, sugars which the lectin does not recognize.

Introduction

Candida albicans is an opportunistic yeast that is known to live in a saprophytic existence in the gastrointestinal and urinary tracts of a large percentage of individuals (10). In addition to a saprophytic existence C. albicans can cause mucocutaneous infections (16) like thrush in children, vaginitis in women and the recurrent attacks of these illnesses could be due to (10) the continuous presence of the organism in the gut of the patient. This yeast can also lead to systemic infection and even death in compromised individuals (16).

If a microorganism is going to survive on the mucous membranes of the host and either remain in a symbiotic existence or proceed to cause an infection, a means of adherence is necessary to bind it to host cells. Otherwise, secretions and food moving down the GI tract and menstrual flows or urine moving down the urogenital tract would lead to dislodging and eradication of the organism from the body. Understanding the mechanism of adherence might permit us to prevent the process and subsequent infections and shed some light on fungal pathogenesis.

The adherence of many microorganisms to cells has been studied in depth. Most gram negative bacteria studied attach to epithelial cells by means of pili on their surfaces (i.e.Escherichia coli (11, 12), Klebsiella pneumoniae (4), Pseudomonas aeruginosa (19) and others). In the E. coli model (11, 12) mannose moieties on the surface of epithelial cells serve as antigens that are recognized by a mannose-specific lectin on the surface of the pili. For group A streptococci (1) a lipid-like substance, lipoteichoic acid, found on the bacterial surface influences adherence. The hydrophobic chains of the fatty acids in this compound may be responsible for the adherence of this microorganism to the epithelium by their intercalation into the lipid bilayers of the host cell membranes. Species of the gram negative bacterium Rhizobium, a genus of nitrogen-fixing organisms, adhere selectively to the surface of root hairs of clover plants (3). Rhizobium trifolii binds to the root hairs of white clover plants by

means of 2-deoxyglucose residues, cross-reactive antigens, on the surface of both cell types. A galactoprotein called trifoliin with lectin-like characteristics and usually found on the root hair surface mediates adhesion to the epithelium for this species. A sugar-lectin interaction appears to be the most common microbial mechanism of adherence but novel methods of adherence have been postulated recently (i.e. enzyme-substrate interactions, fibronectin, etc. (13, 14, 15)). The glycocalyx or cell coat has been mentioned as the possible receptor site involved in adherence (2).

Recently, results of several studies on the various aspects of the adherence mechanism of C. albicans and other Candida species have been published but little is known about the molecular structure of the receptors on the yeast cell surface that mediate the adherence. Candida albicans is known to adhere to buccal cells (6, 7, 8) and to vaginal cells (8) and a protrusion called the germ tube that arises as the organism grows into the filamentous form could be responsible for this adherence in vivo, since germination of the yeasts in vitro increases adherence significantly (6, 7). In vitro experiments show that the adherence process is temperature dependent (6, 7, 8) and can be suppressed by the presence of bacteria on the surface of epithelial cells (9). Candida albicans adheres in greater quantities than other species of Candida to vaginal cells (8). A direct relationship exists between species of Candida that adhere the most to epithelial cells in in vitro assays and those found more commonly in the body as saprophytes

or as causative agents of infection (8).

There is a controversy as to which dimorphic form, blastospore stage or filamentous stage, is more important in the process of pathogenesis. Evidence for the greater involvement of either has been collected by different researchers (5, 18) but we decided to use the germ tube, an intermediate stage between the yeast and filamentous form, as mycelial elements are usually found in infected tissue.

Lectins have been used in our studies to probe the surface of epithelial and yeast cells in attempts to identify the molecular entities involved in the yeast-buccal cell interaction. The following data includes work done with one of the lectins, Concanavalin A (Con A), a mannose and glucose-specific lectin (17).

Materials and Methods

Buccal Cells

These cells were collected by gently rubbing the inside cheek area with sterile swabs and swirling the swabs in PBS (phosphate-buffered saline, pH 7.2). The cells were washed three times in PBS and standardized to 2 X 10^5 cells per ml.

Yeasts

Candida albicans clinical isolate MSU-1 was grown on Sabouraud's dextrose agar slants for 48h, at 37C. A loop of cells was transferred to 100ml of tryptic soy broth (BBL) plus 4% glucose and incubated at 37C on a rotary shaker (180 RPM) for approximately 15h to develop the

stationary phase stage. An aliquot washed three times in PBS and resuspended in tissue culture medium M199 (Gibco) was incubated for one hour at 37C for the development of germ tubes and resuspended in 0.5% formaldehyde in PBS for 30 minutes at 4C to kill the cells. After removal of the formaldehyde by washing with PBS the yeasts were standardized to 1 X 10^6 cells per ml in PBS.

Adherence Test

Adherence of C. albicans was studied by use of a modification of a previously (6) used adherence test. Briefly, 0.2ml aliquots of buccal and yeast cells were pipetted into tubes (12 X 75mm) and incubated on a shaker at 180 RPM for one hour at 37C. Three tubes were used for the control and for each experimental test followed by repetition of each experiment. Polycarbonate filters 12 microns in pore-size (Nucleopore Corp.) were used for collection of the adherence mixtures from each tube and washed with 100ml of PBS under continual agitation. The filters were stained with Gram crystal violet and the number of yeasts adhering to 200 buccal cells was determined by light microscopy at 430X. Double-blind conditions were used in all studies.

Pre-incubation of the yeasts with Con A

Con A (ICN Pharmaceuticals) was used at a concentration of 10 micrograms per ml of PBS containing added cations (0.002M salts of magnesium, calcium and manganese). Two ml aliquots of the yeasts were centrifuged and resuspended in 5ml of the Con A solution and incubated

for 45 minutes at room temperature on a shaker. The cells were washed, resuspended in PBS to 2ml and mixed with buccal cells for the assay as described before.

Pretreatment of the Con A with sugars

Six percent solutions of α-D-methylmannopyranoside (Sigma), D-galactose, D-raffinose and D-ribose (Nutritional Biochemicals Corp.) were prepared by adding the appropriate concentration of sugar to 5ml of PBS containing added cations that had the lectin already dissolved in it at 10 micrograms per ml. The solutions were incubated at 24C on a shaker for 2h, after which 2ml aliquots of the standardized yeast suspension were resuspended in each of the sugar solutions and incubated at 24C on a shaker for 45 minutes. After washing the yeast cells and resuspending in 2ml of PBS, these were mixed with buccal cells for the adherence assay.

Results and Discussion

Preincubation of the yeast cells with the Con A prior to mixing with the buccal cells reduced the yeast adherence significantly (see figure 1). Since high molecular weight compounds like Con A might inhibit adherence by acting as mechanical coats on the surfaces of the organisms, we proceeded to test the specificity of the observed inhibitory effect. Lectins are known to have immunoglobulin-like qualities (17) so a hapten inhibition test was carried out on Con A.

The lectin was pretreated with a haptenic sugar, α-D-methylmannopyranoside (α-D-mM), for which it is known to have a high affinity (17) before using it to pretreat the yeasts (see figure 1). Increasing concentrations of the α-D-mM diminished significantly the inhibitory effect of the Con A. To determine if we could obtain the same results as above with other sugars, the lectin was pretreated with D-ribose, D-galactose and D-raffinose (see figure 2). These sugars did not abolish the inhibitory effect of the lectin; thus, the effect shown by Con A is a specific one. Lectins with other specificities (manuscript in preparation) have been unable to inhibit adherence. Research in progress has also identified mannose residues on the buccal cell surface (manuscript in preparation) as possible cross-reactive antigens involved in the adherence of this yeast to buccal cells.

Conclusion

Our results with the lectin Concanavalin A and with the sugars used in its treatment suggest that a mannose-containing moiety on the yeast cell surface is probably involved in the mechanism of adherence of Candida albicans to human buccal cells. Further work should identify these moieties as either mannose-containing receptors or as part of an indigenous lectin, or both.

Acknowledgments

We wish to thank Drs. Ron J. Patterson and E. S. Beneke for their generous advice in the experimental procedures.

Bibliography

1. Bartelt, M. and J. L. Duncan. 1978. Adherence of group A Streptococci to human epithelial cells. Infect. Immun. 20: 200-208.

2. Costerton, W. Personal communication.

3. Dazzo, F. B., C. A. Napoli and D. H. Hubbell. 1976. Adsorption of bacteria to roots as related to host specificity in the Rhizobium-Clover symbiosis. Appl. Environ. Microb. 32: 166-171.

4. Fader, R. C., A. E. Avots-Avotins and C. P. Davis. 1979. Evidence for pili mediated adherence of Klebsiella pneumoniae to rat bladder epithelial cells in vitro. Infect. Immun. 25: 729-737.

5. Hill, D. W. and L. P. Gebhardt. 1956. Morphological transformation of Candida albicans in tissues of mice. Proc. Soc. Exp. Biol. Med. 92: 640-644.

6. Kimura, L. H. and N. H. Pearsall. 1978. Adherence of Candida albicans to human buccal epithelial cells. Infect. Immun. 21: 64-68.

7. Kimura, L. H. and N. H. Pearsall. 1980. Relationship between germination of Candida albicans and increased adherence to human buccal epithelial cells. Infect. Immun. 28: 464-468.

8. King, R. D., J. C. Lee and A. L. Morris. 1980. Adherence of Candida albicans and other Candida species to mucosal epithelial cells. Infect. Immun. 27: 667-674.

9. Liljemark, W. F. and R. J. Gibbons. 1973. Suppression of Candida

albicans by human oral streptococci in gnotobiotic mice. Infect. Immun. 8: 846-849.

10. Miles, M. R., L. Oslen and A. L. Rogers. 1977. Recurrent vaginal candidiasis. J. Amer. Med. Assoc. 238: 1836-1837.

11. Ofek, I. and E. H. Beachey. 1978. Mannose binding and epithelial cell adherence of Escherichia coli. Infect. Immun. 22: 247-254.

12. Ofek, I. and E. H. Beachey. 1977. Adherence of Escherichia coli to human mucosal cells mediated by mannose receptors. Nature 265: 623-625.

13. Rauvala, H., W. G. Carter, and S. Hakomori. 1981. Studies on cell adhesion and recognition. I. Extent and specificity of cell adhesion triggered by carbohydrate-reactive proteins (glycosidases and lectins) and by fibronectin. J. Cell Biol. 88: 127-137.

14. Rauvala, H., W. G. Carter and S. Hakomori. 1981. Studies on cell adhesion and recognition. II. The kinetics of cell adhesion and cell spreading on surfaces coated with carbohydrate-reactive proteins (glycosidases and lectins) and fibronectin. J. Cell Biol. 88: 138-148.

15. Rauvala, H., W. G. Carter and S. Hakomori. 1981. Studies on cell adhesion and recognition. III. The occurrence of α-mannosidase at the fibroblast cell surface, and its possible role in cell recognition. J. Cell Biol. 88: 149-159.

16. Rippon, J. W. 1974. Candidosis. Medical Mycology. The Pathogenic Fungi and The Pathogenic Actinomycetes. Saunders, Philadelphia.

175-204.

17. Sharon, N. and H. Lis. 1972. Lectins: cell-agglutinating and sugar-specific proteins. Science 177: 949-958.

18. Simonetti, N. and V. Strippoli. 1973. Pathogenicity of the Y form as compared to M form in experimentally induced Candida albicans infections. Mycopathol. Mycol. Appl. 51: 19-28.

19. Woods, D. E., D. C. Strauss, W. G. Johanson, V. K. Berry and J. A. Bass. 1980. Role of pili in adherence of Pseudomonas aeruginosa to mammalian buccal epithelial cells. Infect. Immun. 29: 1146-1151.

Figure 1. Inhibition of adherence of Con A-pretreated Candida albicans to buccal cells and the effect of the sugar α-D-methylmannopyranoside on the inhibition by this lectin. Control = cells pretreated only with PBS and taken as 100% adherence. Con A used at 10 micrograms per ml of PBS with added cations.

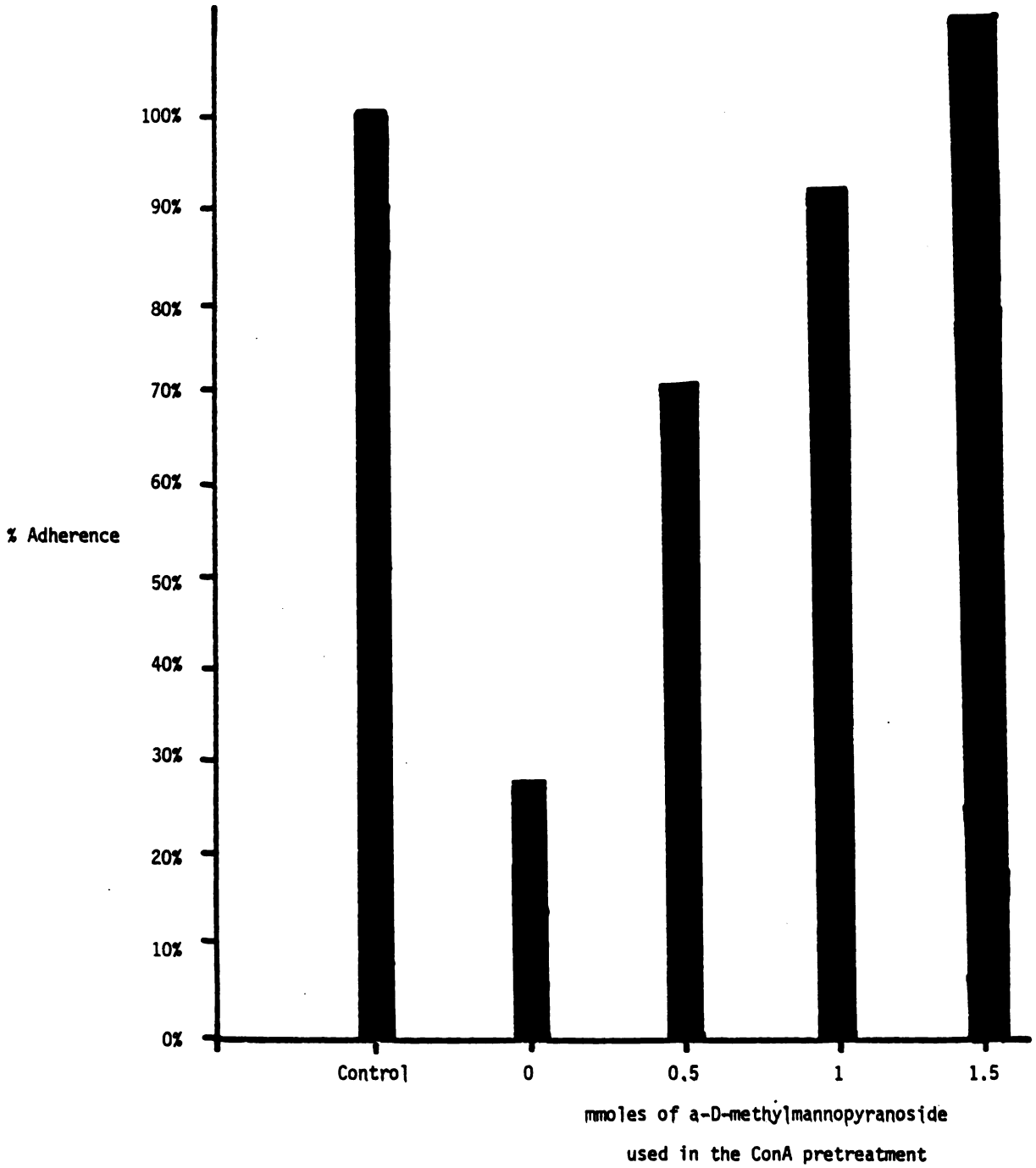
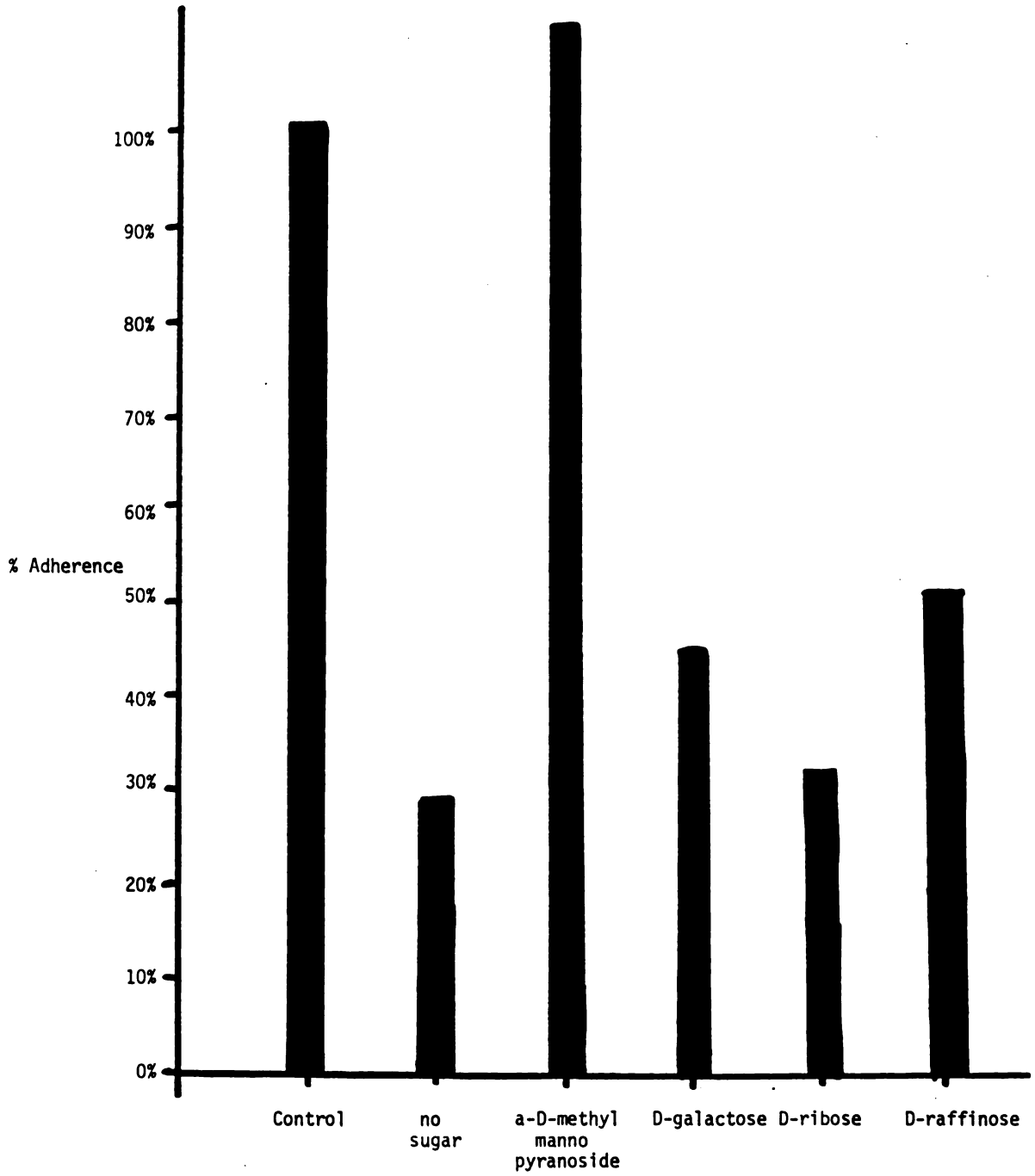

100%
90%
80%
70%
60%
% Adherence
50%
40%
30%
20%
10%
0%
Control
0
0.5
1
1.5
mmoles of a-D-methylmannopyranoside
used in the ConA pretreatment

Figure 2. The effect of various sugars on the inhibition by Con A when used to pretreat the lectin. The control cells were pretreated with PBS and all other cells had Con A at 10 micrograms per ml of PBS with added cations, that had been pretreated with no sugar or with the indicated sugar. All sugars used at 6% solutions as described in the text.


% Adherence
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Control
no sugar
a-D-methyl manno pyranoside
D-galactose
D-ribose
D-raffinose

Evidence for Mannose-mediated Adherence of *Candida albicans* to Human Buccal Cells *In Vitro*

Ramon L. Sandin[1], Alvin L. Rogers[1,2*]

Submitted to Infection and Immunity
for publication.

[1]Department of Microbiology and Public Health
[2]Department of Botany and Plant Pathology
Michigan State University
East Lansing, Michigan 48824
(517) 355-5076

ABSTRACT

Various lectins and sugars were used to study the possible role of saccharide-containing moieties on the surface of Candida albicans and human buccal cells in the adherence of this yeast to mucosal surfaces. The lectins used possessed affinities towards several different sugar moieties and were used to pretreat C. albicans or buccal cells before mixing and incubating in the adherence assay. It was found that Concanavalin A (Con A), a lectin that recognizes mannose and glucose, inhibited adherence of the pretreated yeasts to buccal cells and also adherence of the pretreated yeasts to buccal cells. Adherence was restored by preincubating the Con A with a mannose derivative but preincubation of Con A with other sugars did not produce this effect. Lectins that do not recognize mannose did not inhibit adherence. The presence of α-D-methyl mannopyranoside in the incubation medium during the assay inhibited adherence while other sugars did not. Germinated yeasts were more susceptible to adherence inhibition by Con A than nongerminated yeasts. Thus, mannose containing moieties on the surface of Candida albicans and buccal cells could mediate the adherence of this yeast to human epithelium.

INTRODUCTION

The adherence of microorganisms to epithelial cell surfaces is now recognized as an important first step in the colonization and infection of mammalian membranes (6). Escherichia coli (13, 14) Klebsiella pneumoniae (5), Pseudomonas aeruginosa (22) and other gram-negative bacteria attach to epithelial cells by means of pili on their surfaces. A mannose-specific lectin on the pili of E. coli (13, 14) and mannose residues on buccal cell surfaces help mediate the adherence of this organism. The lipoteichoic acid on the surface of group A streptococci (1, 15) is believed to be involved in the adherence of these bacteria to epithelial cells presumably by the intercalation of this substance into the lipid bilayer of the host cell membrane.

Candida albicans adheres to buccal cells (7, 8, 9), vaginal cells (9) and fibrin-platelet matrixes (11) in vitro. The germ tube, an intermediate stage between the blastospore and the filamentous form of the fungus, has been implicated (7, 8, 20) in the adherence process since germinated yeasts adhere in vitro in greater numbers than non-germinated yeasts. Candida albicans adheres in greater numbers than other species of Candida to vaginal cells (9) and to fibrin-platelet matrixes formed in vitro (11).

Little is known about the molecular structure of the surface receptors that mediate the adherence of C. albicans. Recently, Lee and King reported the extraction of a soluble, biologically active compound of a mannoprotein nature that was involved in the adherence

of C. albicans to epithelial cells (J. C. Lee and R. D. King, Abst. Annu. Meet. Amer. Soc. Microbiol. 1981, F1, p. 313). This study identifies the general nature of the surface receptors on C. albicans and on human buccal cells involved in in vitro adherence.

MATERIALS AND METHODS

Buccal cells. These cells were collected by gently rubbing the inside cheek area of one of the authors (R. S.) with sterile swabs and swirling the swabs in PBS (phosphate-buffered saline, pH 7.2). The cells were washed three times in PBS and resuspended to concentrations of 2×10^5 cells per ml of PBS.

Yeasts. Candida albicans MSU-1, a clinical isolate, was grown on Sabouraud's dextrose agar slants for 48 h at 37°C. A loop of cells was tranferred to 100 ml of tryptic soy broth (BBL, Cockeysville, Maryland) plus 4% glucose and incubated at 37°C on a rotary shaker (180 RPM) for approximately 15 h to develop the stationary phase stage. An aliquot was removed, washed three times in PBS then resuspended in tissue culture medium M199 (Gibco, Santa Clara, California) adjusted to a pH of 7.2. The suspension was incubated for 1 h at 37°C for the development of germ tubes and resuspended in 0.5% formaldehyde in PBS for 30 min at 4°C to kill the cells. The majority of the yeast cells possessed germ tubes after the treatment. The yeast cells were killed to prevent growth of longer germ tubes during experimentation involving sugars and lectins. After removal of the formaldehyde by washing with PBS the yeasts were resuspended at 1×10^6 cells per ml of PBS.

Adherence test. Adherence of C. albicans was studied by use of a modification of a previously described adherence test (7). Briefly, 0.2 ml aliquots of buccal and yeast cells were pipetted into tubes

(12 X 75 mm) and incubated on a shaker at 180 RPM for 1 h at 37°C. Three tubes were used for each control and for each experimental test followed by repetitions of each experiment. Polycarbonate filters, 12 microns in pore-size (Nucleopore Corp., Pleasanton, California), were used for collection of the adherence mixtures from each tube and washed with 100 ml of PBS under continual agitation. This pore size allowed the nonadhering yeasts to pass through while retaining those adhering to buccal cells. The filters were stained with Gram crystal violet and the number of yeasts adhering to 200 buccal cells was determined by light microscopy at 430X. Double-blind conditions were used in all studies.

Lectin pretreatment of the yeasts. All lectins were used at a concentration of 10 micrograms per ml of PBS containing added cations (0.002 M concentration of magnesium, calcium and manganese salts). Concanavalin A was obtained from ICN Pharmaceuticals, Cleveland, Ohio; Phytohaemagglutinin (PHA), from Wellcome Reagents, Beckenham, England while the other lectins (soybean agglutinin, wheat germ agglutinin, Dolichos biflorus agglutinin, Ulex europaeus agglutinin 1, peanut agglutinin and Ricinus communis agglutinin 1) were from a lectin screening kit from Vector Labs., Burlingame, California. The lectins were supplied in crystallized or salt-free and lyophilized form. Two ml aliquots of the yeasts were pelleted, resuspended in 5 ml of the lectin solution and incubated for 45 min at room temperature on a shaker. The

cells were washed, resuspended in PBS to 2 ml and mixed with buccal cells for the adherence assay.

Saccharide pretreatment of the Con A. Solutions of 2, 4, and 6% of α-D-methyl mannopyranoside (α-D-mM) (Sigma, St. Louis, Missouri) and 6% solutions of D-galactose, D-ribose or D-raffinose (Nutrition Biochemicals, Cleveland, Ohio) were prepared by adding the appropriate concentrations of carbohydrate to 5 ml of PBS containing added cations and the lectin at 10 micrograms per ml. The solutions were incubated at 24°C on a shaker at 180 RPM for 2 h, after which 2 ml aliquots of the standardized yeast suspension were resuspended in each of the sugar solutions and incubated at 24°C on a shaker for 45 min. After washing the yeast cells and resuspending in 2 ml of PBS, these were mixed with buccal cells for the adherence assay.

Saccharide inhibition of adherence. 200 mg of α-D-mM, D-ribose, D-galactose or D-xylose were dissolved in 0.5 ml of PBS and added to tubes that already contained a total of 0.4 ml of the standardized yeast and buccal cell suspensions. The contents of each tube were mixed by vortexing and were immediately set on a shaker at 180 RPM to proceed with the adherence test as described earlier.

RESULTS

Adherence of C. albicans to buccal cells. Every experiment performed included a control(s) that showed the number of C. albicans adhering to buccal cells in nontreated systems. Each table or graph which follows includes these controls. For instance, 326 ± 18.2 yeast cells adhered to 200 buccal cells in the control shown in Table 1.

Inhibition of adherence of Con A-pretreated C. albicans to buccal cells. Preincubation of the yeast cells with various concentrations of Con A prior to mixing with buccal cells reduced adherence significantly. Table 1 shows two such concentrations. Since there was no significant difference between the results obtained using these two concentrations of Con A, we decided to use 10 micrograms per ml of Con A in PBS for all our assays. All other lectins were used at this same concentration after finding no inhibition of adherence at either high or low concentrations.

Suppression of Con A's inhibitory effect on pretreated C. albicans' adherence to buccal cells. The specificity of the observed inhibitory effect by Con A on pretreated yeasts was examined. A hapten-inhibition test was performed in which increasing concentrations of a sugar hapten with affinity for Con A, i.e. α-D-methylmannopyranoside (19), were used to pretreat the lectin prior to pretreatment of the yeast. Figure 1 shows that increasing concentrations of α-D-mM diminish significantly the inhibitory effect of the Con A, prompting the number of adhering

yeasts to increase within the range of the control.

Further proof of the specificity of the inhibitory effect of Con A on the yeast cells was acquired by pretreating the lectin with various other sugars for which it is not known to have affinity. Figure 2 compares the effect of D-ribose, D-galactose and D-raffinose on the lectin with that of α-D-mM. The only sugar that significantly abolished the inhibitory effect of the Con A was α-D-mM.

Inhibition of adherence of C. albicans to Con A-pretreated buccal cells. Preincubation of the buccal cells with Con A prior to mixing with C. albicans reduced the number of yeasts adhering to their surfaces significantly (Table 2). When the lectin was incubated with α-D-mM before being used to pretreat the buccal cells, its inhibitory effect on adherence was suppressed and adherence of yeasts increased significantly. Other sugars such as D-ribose, D-raffinose and D-galactose used to pretreat the Con A did not suppress the inhibitory effect of the lectin.

Effect of various other lectins on the adherence of treated-C. albicans to buccal cells. Seven other lectins were used to pretreat C. albicans before mixing with buccal cells for the 1 h adherence assay. Included in this group of lectins were some specific for L-fucose, N-acetyl galactosaminides, N-acetyl glucosamine and others, none of which were specific for mannose or glucose. None of these lectins produced significant inhibition of adherence when compared to the nonlectin

treated controls (Table 3).

Effect of Con A on the adherence of germinated vs. nongerminated C. albicans to buccal cells. Candida albicans cells submitted to the germination process of 1 h incubation in M-199 adhered to buccal cells in greater quantities than those pretreated with PBS (Table 4). Con A pretreatment of the germinated yeasts before the adherence assay inhibited adherence significantly, as expected, as did adding the lectin to the incubation medium immediately before the 1 h assay. Neither Con A pretreatment of the nongerminated yeasts prior to the adherence assay nor incorporation of Con A into the medium produced any significant diminution in already markedly low adherence values.

Effect on adherence of saccharide pretreatment of C. albicans and buccal cells and of incorporation of saccharides into the medium prior to the adherence assay. When both the yeast cells and the buccal cells were pretreated with 1 mM concentrations of α-D-mM prior to the adherence assay there was significant inhibition of adherence (Table 5). Including this sugar into the medium immediately before the assay gave significant inhibition (Fig. 3). Inclusion of other sugars (D-ribose, D-galactose and D-xylose) in the incubation medium at the same concentrations produced nonsignificant or smaller decreases in adherence than did α-D-mM.

DISCUSSION

Candida albicans is an opportunistic yeast known to reside in the gastro intestinal (GI) and urogenital tracts of many individuals (12). It can remain as a saprophyte or cause mucocutaneous infections (18) like thrush in children or vaginitis in women. In debilitated individuals or in compromised hosts, such as recipients of organ transplants or patients undergoing intensive antibiotic therapy, this organism may spread systemically and even lead to death (18). Miles and Rogers (12) have suggested that the continuous presence of the organism in the GI tract of the host may serve as a reservoir from which this yeast can reinfect the vagina anew in cases of recurrent vaginitis. In that study, women with recurrent vaginitis due to C. albicans in the vagina also had the yeast in the GI tract; conversely, those who did not harbor the organism in the GI tract did not have it in the vagina. To persist as part of the gastrointestinal microflora it is likely that this yeast possesses a means of attachment and anchorage to the substrate epithelium. If not, secretions, movement of food products and defecation might be expected to dislodge the organism from its substratum.

Studies defining the parameters of adherence of C. albicans to epithelial cells have been published in recent years. There appears to be a direct relationship between those species of Candida that adhere to epithelial cells or fibrin platelet matrixes in vitro (9, 11) and those that are known to colonize host tissues or cause disease.

Candida albicans ranks first in both phenomena, thus this species was used for our studies. The form of this dimorphic organism used here, the yeast with germ tube, is an intermediate stage between the blastospore and the filamentous forms. Numerous reasons prompted this decision. Our experiments with the germinated stage demonstrated significantly greater adherence to buccal cells than those conducted with the blastospore stage, a fact previously reported in the literature (7, 8). Aside from this, germ tubes can be produced in vitro from blastospores in relatively short periods of time, which validates our utilization of this form even when a report in the literature suggested that the blastospore is an important stage in the colonization of the host (21). Last of all, the fact that mycelial elements are usually found in infected tissue further supports our use of this form for experimentation. Buccal cells are ideal due to their ease of collection and because they are a natural mucocutaneous substrate, as in thrush. Histologically, they are similar to vaginal cells that serve as substrate for vaginitis infections.

The experiments with Con A reported here indicate that pretreatment of C. albicans with this lectin inhibits adherence of the yeast to human buccal cells by binding to mannose-containing moieties on the yeast surface. This process is specific and could occur because these moieties are saturated by the Con A and thus are made less available for binding. Previous studies on the structure of the cell wall of C. albicans further support this conclusion. Cassone et al. (2) found

that the outer wall portion of this yeast consists of a capsule-like component composed of spiky protrusions that consist essentially of mannan. Extraction of the yeast mannan by acid or alkali treatment resulted in loss of yeast agglutinability by Con A. They concluded that the mannan and mannan-protein complexes play a major role in the layering of the cell wall of C. albicans. These studies on the wall of Candida also minimize the possibility that Con A inhibits yeast adherence by binding to the glucan or to glucan-protein complexes on the cell wall since the glucan was only found in the inner layers of the cell wall forming part of a rigid and alkali-insoluble glucan-chitin matrix. None of the other lectins used in our study to pretreat yeast cells had predilection for mannose residues, and none resulted in significant inhibition of adherence, including those with an affinity for N-acetyl D-glucosamine, the monomeric sugar that forms the polymer chitin that is part of the inner cell wall portion of C. albicans.

The Con A inhibition of adherence of pretreated yeasts to nonpretreated buccal cells may occur as a result of binding to and blocking mannose-containing receptors on the yeast surface and/or mannose moieties of an indigenous lectin associated with the yeast cell surface.

Additionally, mannose-containing moieties on the buccal cell surface could be acting as receptors for the C. albicans. Pretreatment of the buccal cells with Con A inhibited the adherence of nontreated C. albicans, an effect that was specific and a possible consequence of

the blockage of receptors. Previous studies with this lectin have shown that mannose-containing compounds are widely distributed on the membranes of mammalian cells (19). Similar models have been proposed to explain the adherence of other microorganisms. A lectin on the pili of E. coli has affinity for the mannose residues on the surface of buccal cells (13, 14). Identical residues of 2-deoxyglucose on the surface of root hair cells of the white clover plant and on the surface of Rhizobium trifolii function as cross-reactive antigens to a lectin that recognizes and binds each of them, joining together both cell types (3).

We have inhibited adherence significantly by saturating the surface of both C. albicans and buccal cells before the adherence assay with α-D-mM, a mannose derivative. This effect could also be obtained by adding the sugar to the adherence medium before the incubation to act as a competitor for the binding sites on the cell surfaces. This inhibition could not be reproduced using other sugars like D-galactose, D-xylose and D-ribose. It is interesting to mention that the recent work by Lee and King also points to the involvement of a mannose-containing compound, a mannoprotein, on the cell wall of C. albicans in the adherence of this organism to epithelial cells. Our results are in partial disagreement with those of Sobel et al. (20) who found that pretreating C. albicans with L- or D-fucose, but not with mannose, mannoside or galactose inhibited adherence. The discrete number of receptor sites for C. albicans on epithelial cells has also been

studied by saturating those cell surfaces with lactobacilli (20) and streptococci (10) and finding that adherence of C. albicans is inhibited.

We have observed that Con A inhibits adherence of germinated C. albicans to buccal cells to a greater extent that it does non-germinated yeasts. The adherence values for the control of the non-germinated yeasts were low compared to the control of the germinated yeasts, but still the Con A experiments point to a specific chemical entity as the responsible factor for adherence. This entity may be concentrated on the germ tube surface since it is the lack of a germ tube that distinguishes a nongerminated yeast from a germinated one. Previous stuides have identified antigens present on the germ tube surface that are not found on the yeast forms (4). We found that live germinated C. albicans adhere equally well to buccal cells compared to germinated yeasts that are killed in 0.5% formaldehyde in PBS for 1 h or less. Prolonged standing in formalin for a period of weeks, though, did cause shrinkage of the yeasts and diminished adherence significantly. Others have found that heat or overnight treatment in formalin does inhibit adherence of germinated yeasts (20). Concerning yeast-yeast interactions, we found that Con A agglutinated equally well germinated and nongerminated yeasts and live for formalin killed cells (data not shown). Our studies, nevertheless, are concerned with yeast-epithelial cell interactions and agglutination of yeasts was never observed in our systems since all pretreatments and the 1 h adherence tests were carried out under conditions of continual agitation on a shaker.

Washing the cells that had been collected on the filter was also done under continual agitation.

Observations concerning the receptor sites for C. albicans on the epithelial cell surfaces show that there is a great variation in the number of these receptors from person to person (20, 9) and from day to day in the same person (9). We have also confirmed this last observation in our statistical studies of the buccal cells. In addition, we found great variations in the number of yeasts adhering per buccal cell from swabbings of the mouth done for each single experiment, a fact also observed with streptococci (1). We found that a majority of the cells had little or no C. albicans attached to their surfaces (data not shown), while a minority of them had greater numbers of attached yeasts which was responsible for elevation in adherence. Consequently, controls were included in each experiment.

Novel mechanisms of adherence of cells to surfaces have been proposed (16, 17). We are presently studying the kinetics of adherence of C. albicans to human buccal cells and preliminary data show that adherence can be inhibited by adding sugar or lectins up to a certain point, after which time treatments with these substances produce no inhibition of adherence.

The knowledge that we have gained by the use of Con A and other lectins and sugars is a contribution to the field of fungal adherence. Understanding the mechanism of adherence of C. albicans to human mucosal surfaces might permit us to prevent the process and prevent subsequent infections, thus shedding light on fungal pathogenesis.

LITERATURE CITED

1. Bartelt, M., and J. L. Duncan. 1978. Adherence of group A steptococci to human epithelial cells. Infect. Immun. 20: 200-208.
2. Cassone, A., E. Mattia, and L. Boldrini. 1978. Agglutination of blastospores of Candida albicans by Concanavalin A and its relationship with the distribution of mannan polymers and the ultrastructure of the cell wall. J. Gen. Microbiol. 105: 263-273.
3. Dazzo, F. B., C. A. Napoli, and D. H. Hubbell. 1976. Adsorption of bacteria to roots as related to host specificity in the Rhizobium-Clover symbiosis. Appl. Environ. Microb. 32: 166-171.
4. Evans, E. G. V., M. D. Richardson, F. C. Odds, and K. T. Holland. 1973. Relevance of antigenicity of Candida albicans growth phases to diagnosis of systemic candidasis. Br. Med. J. 4: 86-87.
5. Fader, R. C., A. E. Avots-Avotins, and C. P. Davis. 1979. Evidence for pili mediated adherence of Klebsiella pneumoniae to rat bladder epithelial cells in vitro. Infect. Immun. 25: 729-737.
6. Gibbons, R. P., and J. Van Houte. 1975. Bacterial adherence in oral microbial ecology. Annu. Rev. Microbiol. 29: 19-44.
7. Kimura, L. H., and N. H. Pearsall. 1978. Adherence of Candida albicans to human buccal epithelial cells. Infect. Immun. 21: 64-68.
8. Kimura, L. H., and N. H. Pearsall. 1980. Relationship between germination of Candida albicans and increased adherence to human buccal epithelial cells. Infect. Immun. 28: 464-468.

9. King, R. D., J. C. Lee, and A. L. Morris. 1980. Adherence of Candida albicans and other Candida species to mucosal epithelial cells. Infect. Immun. 27: 667-674.

10. Liljemark, W. F., and R. J. Gibbons. 1973. Suppression of Candida albicans by human oral streptococci in gnotobiotic mice. Infect. Immun. 8: 846-849.

11. Maisch, P., and R. A. Calderone. 1980. Adherence of Candida albicans to a fibrin-platelet matrix formed in vitro. Infect. Immun. 27: 650-656.

12. Miles, M. R., L. Olsen, and A. L. Rogers. 1977. Recurrent vaginal candidasis. J. Amer. Med. Assoc. 238: 1836-1837.

13. Ofek, I., and E. H. Beachey. 1978. Mannose binding and epithelial cell adherence of Escherichia coli. Infect. Immun. 22: 247-254.

14. Ofek, I., D. Mirelman, and N. Sharon. 1977. Adherence of Escherichia coli to human mucosal cells mediated by mannose receptors. Nature. 265: 623-625.

15. Ofek, I., E. H. Beachey, W. Jefferson, and G. I. Campbell. 1975. Cell membrane binding properties of group A streptococcal lipoteichoic acid. J. Exp. Med. 141: 990-1003.

16. Rauvala, H., W. G. Carter, and S. Hakomori. 1981. Studies on cell adhesion and recognition 1. Extent and specificity of cell adhesion triggered by carbohydrate-reactive proteins (glycosidases and lectins) and by fibronectin. J. Cell Biol. 88: 127-137.

17. Rauvala, H., W. G. Carter, and S. Hakomori. 1981. Studies on cell adhesion and recognition III. The occurrence of α-mannosidase at the fibroblast cell surface, and its possible role in cell recognition. J. Cell Biol. 88: 149-159.

18. Rippon, J. W. 1974. Candidosis, p. 175-204. In W. B. Saunders (ed.) Medical Mycology. The pathogenic fungi and the pathogenic antinomycetes. Saunders, Philadelphia.

19. Sharon, N., and H. Lis. 1972. Lectins: cell-agglutinating and sugar-specific proteins. Science. 177: 949-958.

20. Sobel, J. D., P. G. Myers, D. Kaye, and M. E. Levison. 1981. Adherence of Candida albicans to human vaginal and buccal epithelial cells. J. Infect. Dis. 143: 76-82.

21. Taschdjian, B. S., and P. J. Kozinn. 1957. Laboratory and clinical studies on candidiasis in the newborn infant. J. Pediatr. 50: 426-433.

22. Woods, D. E., D. C. Strauss, W. G. Johanson, V. K. Berry, and J. A. Bass. 1980. Role of pili in adherence of Pseudomonas aeruginosa to mammalian buccal epithelial cells. Infect. Immun. 29: 1146-1151.

Table 1: Inhibition of adherence of Con A-pretreated C. albicans to buccal cells.

Treatment	Adherence[a]	Percent Adherence[b]	Probability
None[c]	326 ± 18.2	100	
Con A (10μg/ml)	60 ± 15.3	18.4	< 0.001[d]
Con A (1mg/ml)	47.7 ± 5.83	14.6	< 0.001[e]

[a]Number of yeast cells adhering per 200 buccal cells; means of triplicate determination ± standard error of the mean.

[b]$\frac{\text{treated}}{\text{control}}$ x 100. Control is taken to be 100%

[c]PBS-treated cells

[d]Probability of occurrence between control system and yeasts pretreated with Con A at 10 μg/ml, according to the Student's t test. Probabilities reported in subsequent tables, if not otherwise specified, were based on this same test.

[e]Probability of occurrence between control system and yeasts pretreated with Con A 1 mg/ml.

Table 2: Inhibition of adherence of C. albicans to Con A-pretreated buccal cells and the effect of sugars on the lectin's inhibitory effect.

Treatment	Number of adhering yeast per 200 buccal cells ± SEM (% adherence)	Probability[a]
None (control)[b]	440 ± 19.36 (100)	
Con A[c]	77 ± 9.88 (17.5)	< 0.001
Con A pretreated with:		
α-D-mM[d]	293 ± 28.84 (66.6)	< 0.01
D-galactose	80.7 ± 22.31 (18.3)	> 0.8
D-ribose	49 ± 8.03 (11.1)	> 0.05
D-raffinose	78.7 ± 21.45 (17.9)	> 0.9

[a]The first probability is that between the control and the Con A-pretreated cells; all others are between the Con A-treated cells and those pretreated with the lectin that had undergone pretreatment with the individual sugars.

[b]PBS-treated buccal cells.

[c]Con A 100 μg/ml with added cations.

Table 2 continued.

[d]20% solutions of the sugars were prepared by adding the appropriate amounts of sugar to 5 ml of PBS containing added cations that had the lectin already dissolved to 100 μg/ml PBS. All other procedures are identical to those used in the saccharides pretreatment of the Con A used to pretreat yeasts, as described in text.

Table 3: Effect of pretreatment of C. albicans with various lectins on adherence to buccal cells.

Treatment	% Adherence	Probability[a]
Control[b]	100	
Soybean Agglutinin[c]	92.6	
Ulex europaeus Agglutinin 1	103.4	> 0.05
Dolichos biflorus Agglutinin	112.6	
Wheat germ Agglutinin	98.9	
Peanut Agglutinin	84.8	
Ricinus communis Agglutinin 1	74.5	
PHA (Phyto haemagglutinin)	92.8	

[a]All probabilities came out higher than 5% according to the Student's t test, as compared to the control.

[b]Control = yeasts pretreated with PBS. A typical control in all of these experiments represented approximately 150 adhering yeasts per 200 buccal cells.

[c]All lectins were used at 10 µg/ml following the procedure used for lectin pretreatment of yeast cells as described in the text.

Table 4: Effect of Con A on the adherence of germinated vs. non-germinated C. albicans to buccal cells.

Treatment	Number of adhering yeasts per 200 buccal cells ± SEM (% adherence)[a]	Probability[b]
Germinated yeasts:[c]		
Control[d]	206.5 ± 1.22 (100)	
Pretreated with Con A (10 µg/ml)	21.7 ± 5.26 (10.5)	< 0.001
Con A included during[e] the adherence test	20.7 ± 3.93 (10)	< 0.001
Non-germinated yeasts:		
Control[d]	4 ± 0.58 (1.93)[f]	
Pretreated with Con A (10 µg/ml)	3.7 ± 1.21 (1.83)	> 0.8
Con A included during the adherence test	1.7 ± 1.21 (0.82)	> 0.1

[a] % Adherence = $\frac{\text{treated yeasts}}{\text{control germinated yeasts}}$ x 100; the control germinated yeasts is taken as 100%.

[b] The first two are compared to the control of the germinated C. albicans; the last two are compared to the control of the non-germinated yeasts.

[c] Yeasts were germinated in M199 for one hour following the procedure in text; non-germinated yeasts remained in PBS during this time.

Table 4 continued.

[d]PBS-treated yeasts.

[e]Con A added to tubes before incubation for the 1-hour adherence test at 10 μg/ml.

[f]$\frac{\text{Control non-germinated yeasts}}{\text{Control germinated yeasts}} \times 100$

Table 5: Effect on adherence when both C. albicans and buccal cells are pretreated with α-D-mM.

Treatment	# of adhering yeasts per 200 buccal cells + SEM (% adherence)	Probability
Control[a]	177.7 ± 19.36 (100)	
Pretreatment with α-D-mM[b]	108 ± 11.56 (60.8)	< 0.05

[a]PBS-treated cells

[b]Aliquots of standardized yeast and buccal cells were pretreated separately in 1 mM concentration solutions of α-D-mM in PBS for 1 hour at room temperature on a shaker. The cells were then washed several times in PBS and resuspended in PBS before being mixed for the 1-hour adherence assay.

Figure 1. Effect of incubation of Con A with various concentrations of α-D-mM prior to pretreatment of C. albicans. All groups were treated with either Con A (10μg/ml) or Con A pretreated with the indicated concentration of α-D-mM. Sugar pretreatment of the lectin is described in text. % adherence = $\frac{\text{treated}}{\text{control}}$ x 100 and the control is taken to be 100% adherence. Typical control tubes in these experiments had approximately 150 yeasts adhering per 200 buccal cells.

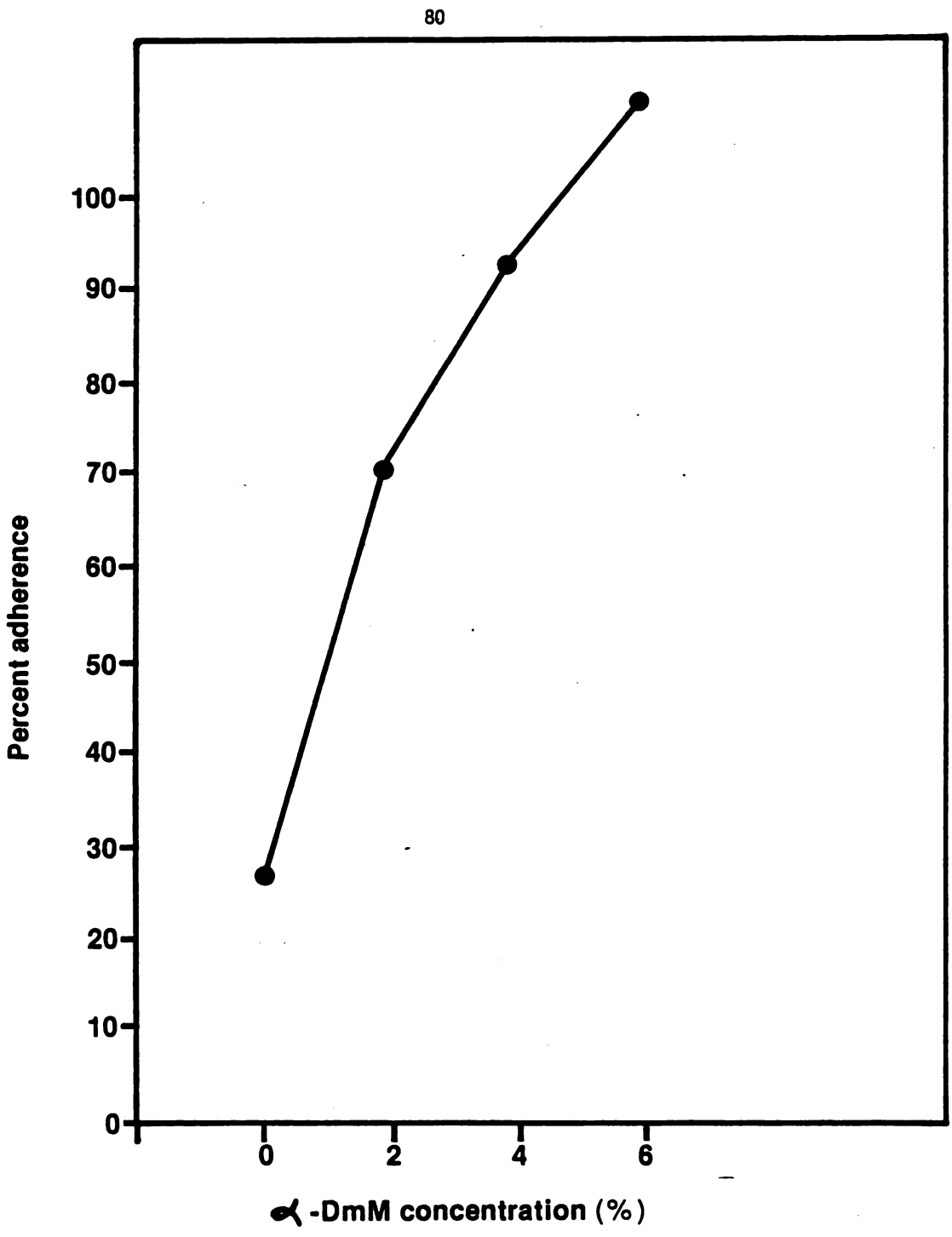

Percent adherence
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
α-DmM concentration (%)

Figure 2. The effect of various sugars on the ability of Con A to inhibit adherence of C. albicans to buccal cells. Control = PBS-treated yeasts. All other groups were treated with either Con A (10μg/ml) or Con A pretreated with 6% solutions of the indicated sugar. The results are the mean of triplicate determinations.

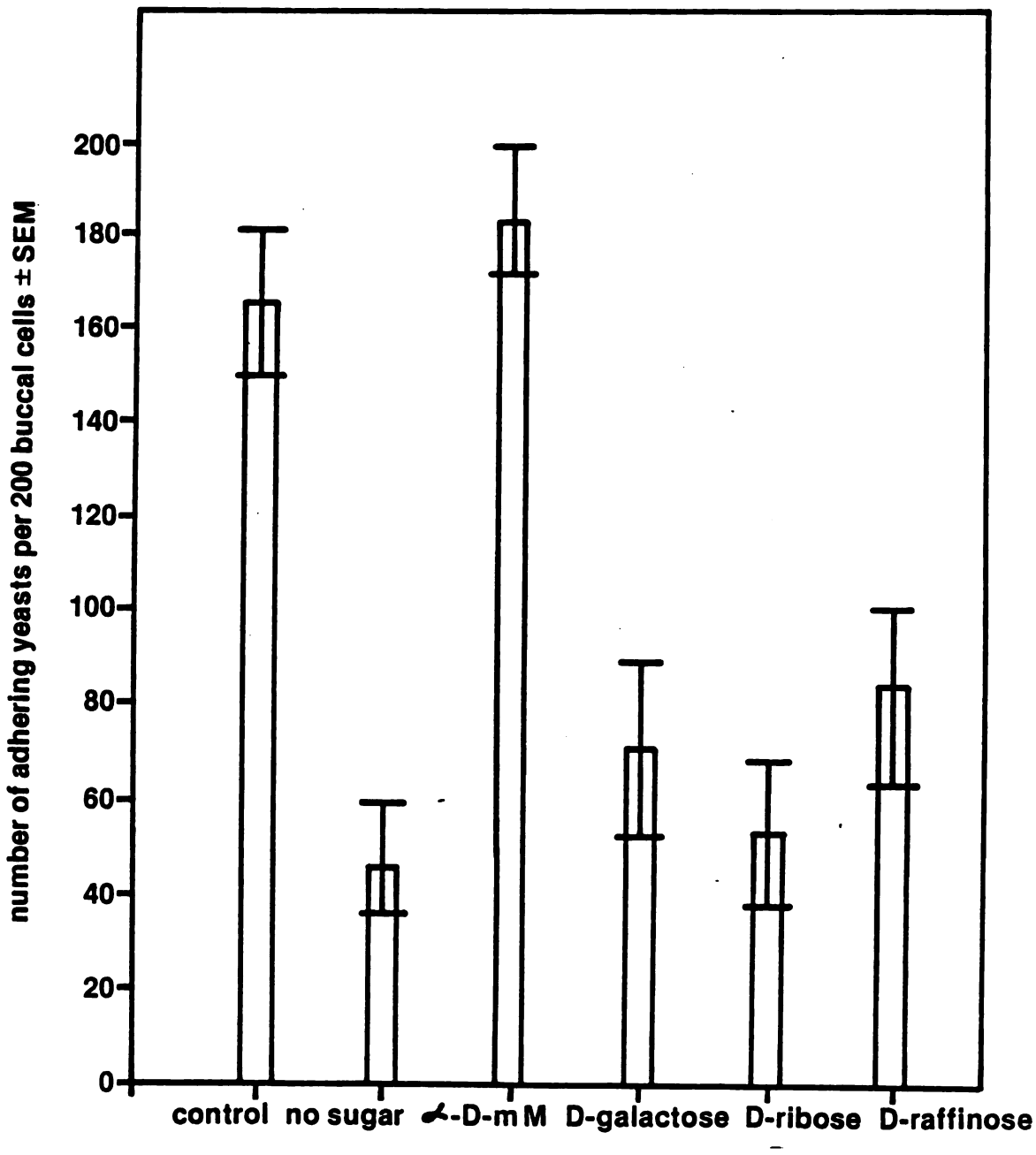
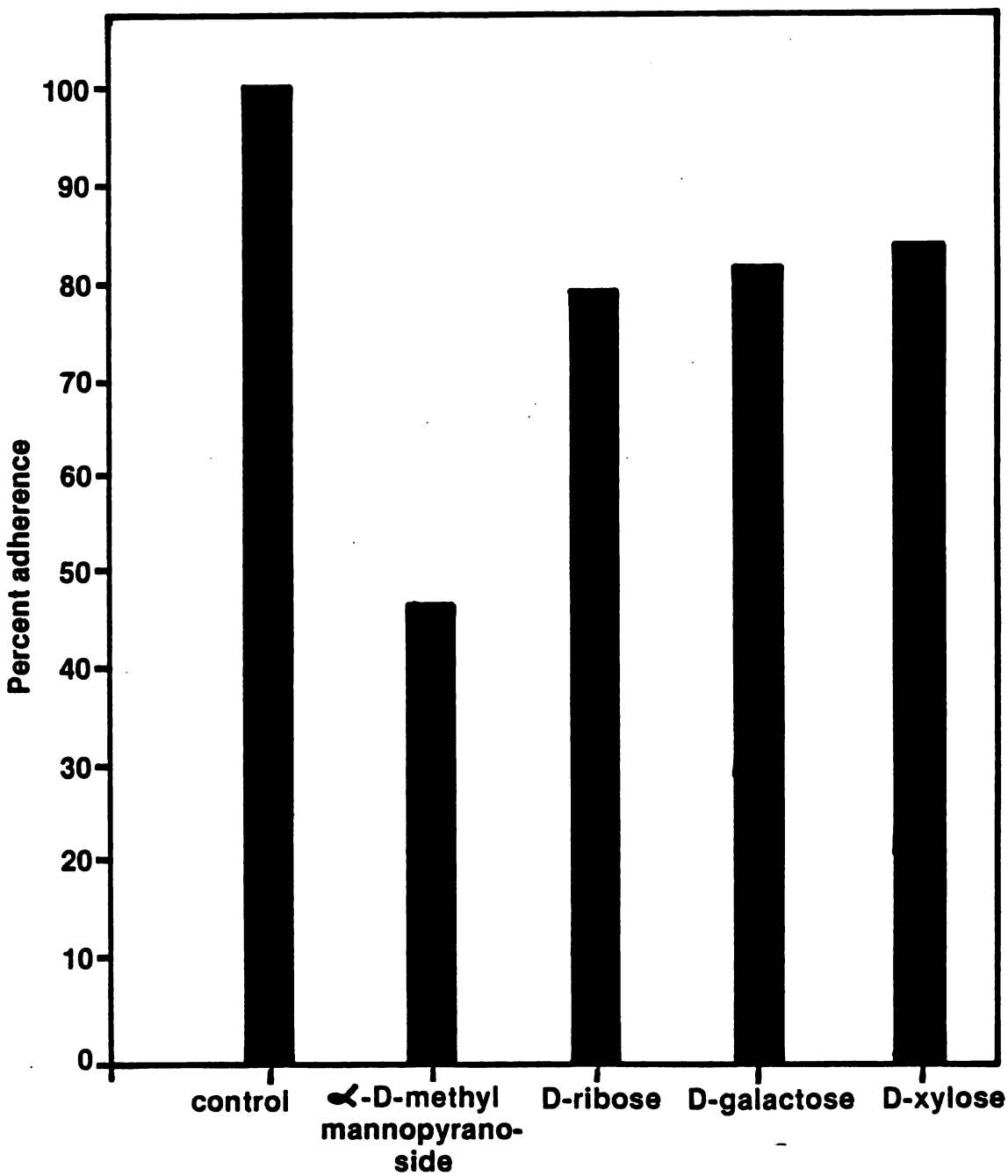

number of adhering yeasts per 200 buccal cells ± SEM
200
180
160
140
120
100
80
60
40
20
0
control
no sugar
α-D-m M
D-galactose
D-ribose
D-raffinose

Figure 3. Effect on adherence when different sugars are included in the incubation medium prior to the adherence assay. Control 100% adherence. Typical controls in these experiments showed an average of 200 yeast cells adhering per 200 buccal cells.


Percent adherence
100
90
80
70
60
50
40
30
20
10
0
control
α-D-methyl mannopyrano-side
D-ribose
D-galactose
D-xylose

EXPERIMENTS PERFORMED SUBSEQUENTLY TO THE SUBMISSION OF ARTICLES 1 and 2

The experiments that follow have been completed since articles 1 and 2 were submitted for publication. These experiments add more information to our knowledge of the adherence of C. albicans to buccal cells.

MATERIALS AND METHODS

Saccharide Inhibition of Adherence: Additional Sugars

These experiments followed the same procedure as described in article 2, under "Saccharide Inhibition of Adherence". The sugars N-acetyl-D-glucosamine, and α-D-methylglucopyranoside were obtained from Sigma Chemicals.

Adherence of Germinated and Non-germinated Yeasts

Non-germinated yeasts were standardized to the following concentrations: 1 x 10^6ml, 1 x 10^7ml, 1 x 10^8ml, 1 x 10^9ml in PBS (7.2). Germinated yeasts (control) were resuspended in PBS to 1 x 10^6ml. Aliquots of the yeast suspensions were mixed with buccal cells before carrying out the standard adherence test, as described in the Materials and Methods section of Article 2.

Extraction of Polysaccharides from the Cell Wall of Candida albicans

A procedure reported previously by Cassone et al. (10) was followed. It preferentially extracts α-mannan from the cell wall of C. albicans.

Briefly, yeasts were germinated in M199 as usual and divided into two aliquots. The first was to serve as control. These cells were treated with 0.5% formaldehyde in PBS for 30 minutes, washed twice in PBS and resuspended to a concentration of 1 x 10^6/ml. These were kept in the cold. The second aliquot was submitted to the experimental extraction procedure. The cells were washed twice in PBS, resuspended in 1M NaOH and slowly agitated for 45 minutes at room temperature in a shaker. These cells are labelled Alk-1 cells. One-third of the cells was washed three times in PBS, formalin-treated, washed again and resuspended to 1 X 10^6/ml in PBS. The other two-thirds of the Alk-1 cells were submitted to a more drastic extraction with alkali. The cells were resuspended in 0.6M NaOH and heated at 85°C for five periods of 4h each, with new solvent being using during each period. This was followed by overnight incubation at room temperature, using the same medium. These cells were labelled Alk-2 cells. One-half of these cells were washed three times in PBS, formalin-treated as usual, and resuspended in PBS to 1 X 10^6/ml. The other half were submitted to acid extraction, by treatment with 0.5M acetic acid for 12h at 85°C. The cells were washed with PBS every 4h. These cells were labelled Alk+Acid, were formalin-treated and resuspended to a concentration of 1 X 10^6/ml in PBS.

Con A pretreatment of extracted cells.

An aliquot of cells from each stage in the extraction procedure and from the control was pretreated with Con A at 10 micrograms per ml

of PBS, following the exact procedure as described in article 2 under "Lectin pretreatment of the yeasts".

RESULTS

Effect on Adherence of Incorporating Other Saccharides into the Medium Prior to the Adherence Test

Table 1 shows that no inhibition of adherence resulted from the inclusion of the two sugars at different times incubation medium prior to the adherence test. These results differ from those obtained previously with α-D-methylmannopyranoside. The sugars tested in this experiment are important because they constitute the monomers that are found in chitin and glucan, the other two main polysaccharides found in the cell wall of C. albicans.

Adherence of Various Concentrations of Non-Germinated Yeasts to Buccal Cells as Compared to the Adherence of Germinated Yeasts.

The adherence of non-germinated yeasts to buccal cells, at concentrations much higher than 1 X 10^6/ml used for germinated yeasts, was always lower than the adherence of the latter. This is shown in Table 2. These results point to the importance of germination in the process of adherence.

Table 1: Effect of additional saccharides on the adherence of Candida albicans to buccal cells.

Treatment	Percent Adherence	Probability[b]
Control[a]	100	
N-acetyl-D Glucosamine	88.3	> 0.2
α-D-methyl Glucopyranoside	100	> 0.9

[a]PBS was added instead of a sugar solution. Typical controls in these experiments represented approximately 300 adhering yeasts per 200 buccal cells.

[b]As compared to control.

Table 2: Adherence of non-germinated yeast cells to buccal cells as compared to the adherence of germinated yeast cells.

Candida albicans	Percent Adherence	Probability[b]
Germinated[a]		
1 X 10^6/ml	100	
Non-germinated		
1 X 10^6/ml	1.93	< 0.001
1 X 10^7/ml	3.44	< 0.001
1 X 10^8/ml	63.6	< 0.001
1 X 10^9/ml	91.3	> 0.2

[a]Control

[b]As compared to germinated yeasts.

Adherence of Alkali and Acid-Extracted Candida albicans to Buccal Cells and the Effect of Con A on the Process

A procedure that extracts the α-mannan and a small amount of soluble β-glucan from the cell wall of Candida albicans rendered the yeast cells unable to adhere to buccal cells, as shown in Table 3. The inhibition of adherence was significant for yeast cells in all stages of the extraction process. Con A was used as a probe to pretreat aliquots of cells from each extraction step before mixing with buccal cells. This was done to test whether or not the extracted substance, no longer available on the surface of the non-adhering yeast cells, is what Con A recognizes and binds to in the cell wall of C. albicans. Con A-pretreatment of non-extracted control cells led to a significant decrease in adherence, but pretreatment with Con A of cells from any of the steps in the extraction process resulted in no further decrease in the number of adhering yeasts.

Table 3. Effect of alkali and acid extraction, and of Con A, on the adherence of germinated Candida albicans to buccal cells.

Treatment	Number of adhering yeast per 200 buccal cells ± SEM (% adherence)	Probability
Control	305 ± 0.63 (100)	
Control pretreated with Con A[a]	54 ± 0.61 (17.7)	< 0.001[b]
Alk-1 cells	6 ± 0.23 (1.96)	< 0.001[b]
Alk-1 cells pretreated with Con A	10 ± 2.74 (3.28)	> 0.4[c]
Alk-2 cells	6 ± 1.41 (1.96)	< 0.001[b]
Alk-2 cells pretreated with Con A	4 ± 0.29 (1.31)	> 0.3[d]
Alk + Acid cells	3 ± 1.16 (0.98)	< 0.001[b]
Alk + Acid cells pretreated with Con A	4 ± 2.18 (1.31)	> 0.7[e]

[a]Con A (10 micrograms/ml).

[b]As compared to control.

[c]As compared to Alk-1 cells.

[d]As compared to Alk-2 cells.

[e]As compared to Alk + Acid cells.

DISCUSSION

The results of the experiments that were reported in articles 1 and 2 clearly demonstrated the involvement of Con A-binding moieties on the surfaces of both C. albicans and buccal cells as mediators of adherence in these cells. It was further concluded that Con A was inhibiting adherence by binding to and blocking mannose-containing surface moieties. This was concluded by correlating the known carbohydrate-binding properties of Con A with the following results: only Con A, among all the tested lectins, could inhibit adherence; α-methyl-mannopyranoside could inhibit adherence if added to the adherence medium; and studies performed by other researchers on the cell wall of C. albicans showed that glucan and chitin are found buried too deeply inside the cell wall fabric to be available for surface adhesion phenomena. The results of the latest experiments and the discussion that follows point beyond a doubt to the involvement of the α-mannan component of the cell wall of C. albicans as the entity responsible for adhesion to buccal cell surfaces.

The inclusion of other sugars, one at a time, in the medium prior to the adherence test failed to decrease adherence significantly. These sugars included N-acetyl-D-glucosamine and α-D-methylglucopyranoside, which are monomers of chitin and glucan. These two polymers are present in the cell wall of C. albicans in addition to mannan. A procedure which extracts the α-mannan and a small amount of soluble β-glucan from the cell wall of C. albicans rendered the cells unable

to adhere to buccal cells and this decrease in adherence was significant for cells in all three stages in the extraction process (Alk-1, Alk-2, and Alk+Acid). The last two stages extracted most if not all of the mannan which left the glucan and chitin components exposed on the surface of the yeast cells. Were these components to be the mediators of adherence in C. albicans, the cells at the Alk+Acid stage should have adhered in high numbers to buccal cells, rather than in such low numbers. Furthermore, pretreatment with Con A of the extracted and non-extracted (control) cells before mixing with buccal cells only decreased the adherence of unextracted cells in a significant manner. There was no further decrease in adherence for extracted cells of all stages treated with Con A. These experiments show that Con A inhibits yeast adherence by binding to an alkali-soluble, extractable component which is present in control cells but absent from extracted cells. This component, the α-mannan, must be the entity responsible for adherence. That this compound must somehow be concentrated on the germ tube surface is shown by the finding that non-germinated yeasts adhere poorly to buccal cells when compared to germinated yeasts, even when used at concentrations that are ten or a hundred times higher than the one used with germinated yeasts.

Classical biochemical studies on the cell wall components of C. albicans and on the carbohydrate-binding properties of Con A give full support to our experimental findings and conclusions. Glucan and chitin in the cell wall of C. albicans are both β-linked polymers

(7). For polysaccharides to be bound by Con A, they must be ramified and contain terminal non-reducing α-D-mannopyranosyl or α-D-glucopyranosyl units (32). Other units, like β-fructofuranosides, are also bound by Con A (33) but these are not found in the cell wall of C. albicans. Studies show that yeast mannan is perhaps the most tenaciously bound polysaccharide whereas yeast glucan is not bound at all, even at such high lectin concentrations as 3 and 25 mg/ml (32, 33). Even α-glucans are not bound as tenaciously as α-mannans (91). When various genera of yeasts were pretreated with fluorescein isothiocyanate (FITC)-labelled Con A, it was found that Saccharomyces cerevisiae, a genus with α-mannan in the cell wall, was labelled very strongly (96). Yet, Schizosaccharomyces pombe or Rhodotorula glutinis were not significantly stained, even when the latter possesses an extracellular β-mannan. Neither has α-mannan in their cell walls (96). Another study that should be mentioned is one in which various lectins were used as probes for the detection of fungal opportunistic pathogens. Whereas other fungi were detected by the use of various other lectins, only Con A detected the presence of C. albicans (94).

A very interesting point surfaced in the course of research when yeast cells at different stages of extraction were submitted to agglutination by Con A. This is different from the yeast-buccal cell interactions which are the subject of this thesis, since it involves only yeast cells which are placed on glass slides rather than submitted to continual agitation in the presence of buccal cells. Yet, it shed

some light on the possibility that more than one "type" of mannose-containing moiety exists in the mannan of the C. albicans cell wall. It has been reported by Cassone et al. (10), and confirmed by the current research, that agglutination of blastospores by Con A occurs in direct proportion to the amount of mannan present in the wall. Thus, Alk-1 cells were still very agglutinable by Con A and light microscopy confirmed the presence of the outer electron dense layer. Alk-2 cells were much less agglutinable, and Alk+Acid cells were agglutinated only slightly or not at all with the outer electron-dense layer being completely absent. Yet, these results are very different from those obtained when extracted cells are submitted to adhesion onto buccal cell surfaces. Alk-1 cells were unable to adhere to buccal cells as shown by a very significant decrease in adherence when compared to controls. The low adherence obtained with Alk-1 cells was basically as low as, and not lower than, that obtained with cells in the later stages of extraction. It appears that the mannose-containing entity involved in adherence to epithelial cells is extracted readily during the mild alkali 1 treatment, while there is still a large amount of some other category of mannan that remains behind in the cell wall and renders the yeast agglutinable by Con A. We could speculate on the existence of "structural" mannan and "functional" mannan. Structural mannan would be the one involved in cell wall layering and that remains behind in the outer cell wall layers following mild alkali treatment. Functional mannan would be the mannose containing cell wall moiety

involved in adhesion to buccal cells. This mannan might simply be located more superficially than the rest of the polymer or be more labile to denaturation by alkali. The importance of this functional mannan is related to the fact that it could be the mannose moiety of an indigenous mannoprotein lectin associated with the yeast cell surface and which mediates adherence to epithelial cells. It is probably this mannan which Con A binds to and blocks in non-extracted (control) cells which renders them unadherent to buccal cells but which is no longer present in extracted cells for Con A to decrease adherence further.

Our model is a reasonable one if we consider that there is recent evidence for the involvement of surface mannoproteins in adherence of C. albicans to epithelial cells (47), and that in most, if not all, microbial systems the lectin is found on the surface of the microorganism. The mannose, or mannose-related, buccal cell surface moiety that was found to be involved in adherence has also been implicated as a possible receptor for indigenous lectins found in other microorganisms (68). The postulated surface lectin in C. albicans would be both mannose-containing and mannose-specific.

The field of candidal adherence can be advanced greatly by the design of future experiments that probe deeper into the role of the germ tube structure in adherence. Antisera could be prepared against germinated yeasts, adsorbed with non-germinated yeasts, and tested to see if significant inhibition of adherence occurs. It could be labelled with FITC or ferritin to light up germ tube antigens. Mutants

that form incomplete germ tubes could be used as probes. Ultimately, the extraction, partition, purification and characterization of a surface moiety involved in adherence that is found in greater amounts in the germ tube structure than in the blastospore surface will give greater validity to the hypothesis that the adhesin is concentrated on the germ tube. These purified extracts, or even culture suspensions, could be put to clinical use in the form of vaccines or as competitors to intact cells if used to flush the affected surfaces.

A last question should be left in the mind that deserves a keen answer. What leads an organism to preferentially colonize some body surfaces and not others? It first comes to mind that the receptors on different body surfaces vary and that this might be the reason for the observed differences in adherence. Studies with FITC-labelled lectins do show that the carbohydrate components of the cell surfaces of rat small intestine vary widely (22). Yet, other studies show that organisms that bind differentially to different types of intestinal cells _in vivo_, bind in equal quantities to those cells _in vitro_ (69). The intact immune system might be the single most important factor in determining whether organisms adhere or not onto surfaces _in vivo_. Further research should place all the contributing elements in the right perspective.

Appendices

APPENDIX A

Hyaluronidase Pretreatment of Buccal Cells

Hyaluronic acid is one of the main components of the ground substance of connective tissue (50). It is an amorphous mucopolysaccharide. We had thought at the beginning of the research that perhaps it was the hyaluronic acid found between the buccal cells that was responsible for the adherence of yeast cells to the oral epithelium, since it has a mucous consistency *in vivo*. For this reason, hyaluronidase was used to carry out an experiment with buccal cells.

Buccal cells were collected as usual, washed in PBS and resuspended to 2 X 10^5/ml. Two mls of the standardized suspension of cells were spun down and resuspended in 5 ml of a solution of hyaluronidase in PBS (pH 7.2). Two concentrations of enzyme were used: 150 and 300 National Formulary units. The suspensions were incubated for 30 min. at 37°C on a shaker. Following this incubation, the cells were spun down, washed in PBS and resuspended to 2 ml in PBS. The 1-hour adherence test was then carried out after mixing with yeast cells as usual.

Table 1 shows that no significant inhibition of adherence occurred when buccal cells were pretreated with either concentrations of enzyme. Thus, the experiment provided some evidence against the original postulate.

Table A1: Effect of hyaluronidase pretreatment of buccal cells before the adherence test.

Treatment	Number of adhering yeasts per 200 buccal cells + SEM (% adherence)	Probability[b]
Control[a]	424 ± 4.16 (100)	
Hyaluronidase		
150 N.F. units per ml	362 ± 5.74 (85.4)	> 0.4
300 N.F. units per ml	419 ± 2.93 (98.8)	> 0.9

[a]PBS-treated cells.

[b]As compared to control.

APPENDIX B

Carbohydrate-binding Specificities of Various Lectins.

The following table summarizes the carbohydrates that are bound by the lectins used in the pretreatment of yeast cells.

Table B1: Carbohydrate-binding specificities of various lectins.

Lectin	Specificity (ties)
Concanavalin A	α-D-mannopyranosides, α-D-glucopyranosides, α-N-acetyl-D-glucosaminides, α-D-arabinosides and β-D-fructofuranosides
Soybean Agglutinin	α- and β-N-acetyl-D-Galactosaminides
Ulex europaeus Agglutinin 1	L-fucose N-acetyl-D-glucosamine
Dolichos biflorus Agglutinin	terminal non-reducing residues on α-N-acetyl-D-galactosaminides
Wheat Germ Agglutinin	terminal non-reducing residues of β-N-acetyl-D-glucosamine, N-acetyl-neuraminic acid
Peanut Agglutinin	β-D-galactosides
Ricinus communis Agglutinin 1	terminal non-reducing residues of β-D-galactose
Phytohemagglutinin	N-acetyl-D-galactosamine or its glycosides

APPENDIX C

Statistical Study on the Receptors Available for Candida albicans Adherence on the Surfaces of Buccal Cells.

Following the one-hour incubation period that has been used throughout this research to study yeast adherence to buccal cells, one-hundred buccal cells were selected at random from each of four filters. These filters had been used to collect four adherence systems, as usual. Each buccal cell was classified according to the number of yeasts it possessed adhering onto its surface.

As can be concluded from Table C1, buccal cells are not uniform in their ability to bind yeast cells. Instead of there being an average number of yeast cells adhering per buccal cell, it was found that most buccal cells (approximately 40%) bound no yeast cells at all. A smaller proportion of buccal cells had greater numbers of yeast cells adhering onto their surfaces, a fact that tends to show that receptors for C. albicans are not found uniformly distributed in the buccal cell population. One could speculate that this could be due to the differences in age or stage in the cell cycle among buccal cells, or to the number of other microorganisms occupying receptor sites on the buccal cell surfaces at the same time as the yeasts. Additional research is needed to solve this enigma.

Table C1: Statistical study on the number of Candida albicans bound by cells of the oral epithelium.

System	Percent of buccal cells with the following number of C. albicans on their surfaces												
	0	1	2	3	4	5	6	7	8	9	10	11	12
1	34	20	16	16	8	4	0	2	0	0	0	0	0
2	34	27	17	9	6	4	2	0	2	0	0	0	0
3	43	18	18	7	2	5	1	1	2	0	0	1	1
4	50	16	19	7	2	1	1	3	1	0	0	0	0
Average	40	20	18	10	5	4	1	2	1	0	0	0	0

Table C2 shows that the majority of those adhering yeasts were germinated (i.e. had germ tubes)

Table C2: Ratio of germinated:non-germinated yeast cells adhering to cells of the oral epithelium.

System	Number of yeasts adhering to 100 buccal cells	
	with germ tubes	without germ tubes
1	146	17
2	152	5
3	151	18
4	100	17
Average	549/606 (91%)	57/606 (9%)

It must be mentioned that the results of this statistical study correspond to systems in which yeast and buccal cells were present in 5:1 ratios (i.e., 1 X 10^6/ml yeasts and 2 X 10^5/ml buccal cells). It should also be mentioned that there was great variation in size among the cells of the oral epithelium.