MECHANISM OF ADHERENCE OF *CANDIDA ALBICANS* TO EPITHELIAL CELLS

Ian A. Critchley B.Sc.(Hons.)

Presented for the degree of Doctor of Philosophy in the Faculty of Science, University of Glasgow.

Department of Microbiology

February 1986

Page

INDEX OF FIGURES

INDEX OF TABLES

ACKNOWLEDGEMENTS

I am extremely grateful to my supervisor, Dr. Julia Douglas, for her guidance and supervision throughout this research and for her constructive criticism of the manuscript. I would also like to thank my industrial supervisor, Dr. John Ryley, for his help and suggestions with the animal experiments.

I am also grateful to the Science and Engineering Research Council for the award of a SERC CASE studentship in collaboration with ICI Pharmaceuticals Division. Thanks are also due to ICI for providing additional financial support.

I would also like to thank Professor A.C. Wardlaw for reading this manuscript and members of the Candida albicans group for their advice and valuable discussion. Gratitude is also expressed to Sandra McGregor of ICI Pharmaceuticals Division for her expert technical assistance with animal experiments. Finally, I would like to thank Mrs. Anne Strachan for her fast and efficient typing of this thesis.

This thesis is the original work of the author. Some of the results described here have been published in FEMS Microbiology Letters.

IAN A. CRITCHLEY B.Sc.(Hons.)

SUMMARY

The research in this thesis was aimed at determining the chemical nature of the surface structures on Candida albicans and on host epithelial cells that are involved in yeast-epithelial adhesion. From previous studies it was known that C. albicans from active infections (I strains) were more adherent to buccal and vaginal epithelial cells after growth in medium containing 500 mM galactose than after growth on 50 mM glucose.

Extracellular polymeric material (EPM) was isolated from culture supernates of C. albicans after growth in medium containing 500 mM galactose. EPM was composed of carbohydrate (70%), protein (10%) and phosphorus (0.05%) and was therefore considered to be glycoprotein. When used to pretreat buccal epithelial cells, EPM inhibited adherence, which suggested that it contains an adhesin that binds to, and blocks, epithelial-cell receptors.

Fractionation of EPM by gel filtration and ion-exchange chromatography gave five components, all of which were capable of inhibiting adherence but to different extents. An index, the adherence inhibition index (AII) was devised to compare activities of the various components. Fractionation of EPM by affinity chromatography on Con A-Sepharose and application of Con A-bound material to a DEAE-cellulose column yielded material which was 30 times more active as an inhibitor than crude EPM. Attempts to resolve EPM by SDS-polyacrylamide gel electrophoresis proved unsuccessful even though a variety of buffer and staining procedures was investigated.

Chemical and enzymic treatment of EPM indicated that the protein portion of the (postulated) glycoprotein was more important than the carbohydrate at inhibiting adherence. Proteolytic enzymes, mild acid and heat treatment of EPM abolished its ability to inhibit adherence. Periodate and α-mannosidase had no effect, whereas papain and mild alkaline treatment of EPM enhanced its ability to inhibit adherence.

The nature of the epithelial cell receptor for C. albicans was investigated with potential receptor analogues such as sugars and lectins and inhibitors. The adhesion of C. albicans GDH 2346 to buccal cells was inhibited by L-fucose and pretreatment of the buccal cells with winged-pea lectin (which is specific for L-fucose). However, the adhesion of another C. albicans strain (GDH 2023) to buccal cells was inhibited by N-acetyl-D-glucosamine and by pretreatment of the buccal cells with wheat-germ agglutinin (which is specific for N-acetyl-D-glucosamine). These experiments indicated that glycosides function as epithelial cell receptors for C. albicans, but with the sugar residue varying between strains.

Samples of EPM from various strains of C. albicans bound to affinity columns of Sepharose containing coupled sugars. The bound fractions were capable of inhibiting adherence, suggesting that they contained an adhesin component. These results indicate that 'lectin-like' interactions may be involved in yeast adherence to host epithelial cells.

The ability of EPM, isolated from different strains, to inhibit adherence was dependent upon the source of the strain. Samples of EPM from I strains were efficient adherence inhibitors, whereas EPM isolated from C strains (obtained from asymptomatic carriers) were poor inhibitors. C. albicans strains GDH 2346 and GDH 2023 produced EPM which inhibited adherence of the strain from which it was isolated, but had no effect on the other strain. The EPM samples from these strains also differed immunologically.

The adherence of different Candida species to epithelial cells and inert surfaces was compared quantitatively. C. albicans, after growth in medium containing a high concentration of galactose, was the most adherent species to buccal epithelial cells and to acrylic surfaces.

One strain of C. tropicalis (the Glasgow strain) showed enhanced adherence to buccal cells, but not to acrylic, after growth in galactose. C. tropicalis (London strain), C. stellatoidea, C. parapsilosis, C. pseudotropicalis, C. guilliermondii and S. cerevisiae did not show enhanced adherence to either epithelial cells or acrylic after growth on galactose. However, C. tropicalis and C. parapsilosis were more adherent to acrylic than was C. albicans after growth in medium containing 50 mM glucose.

The role of adherence of C. albicans in vaginal and systemic infections was explored with mouse models. Vaginal infections were induced with high-galactose grown Candida. These infections could be partially inhibited by pretreating mouse vaginas with EPM from C. albicans GDH 2346. Systemic infections were produced in mice by intravenous inoculation of C. albicans strains GDH 2346 and B 2630. Here, infection was monitored by following mortality patterns and performing yeast counts on the kidneys of infected mice. C. albicans GDH 2023 was not a virulent strain in the mortality tests and gave only low kidney yeast-counts, suggesting less good adherence to the kidney than the other strains. Age and sex of mice did not affect the virulence of these strains in systemic infections; however, the nature of the growth medium did affect the extent of the subsequent infection.

Further work would be needed to establish whether EPM has any therapeutic value in treating Candida infections as it may be potentially useful as a vaccine or as a prophylactic agent. Characterization of the mechanism of adherence to endothelial surfaces in the kidney would lead to a better understanding of systemic infections resulting in more satisfactory preventative measures.

ABBREVIATIONS

AII	adherence inhibition index
CMC	chronic mucocutaneous candidosis
Con A	concanavalin A
DEAE cellulose	diethylaminoethyl cellulose
E_{280nm}	extinction of a solution in a cell of 1 cm light path at a wavelength of 280 nm
EDTA	ethylenediaminetetraacetate
EPM	extracellular polymeric material
M	molar
mA	milliamp
N	normal
NS	not significant
P	probability value
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate-buffered saline
SDA	Sabouraud dextrose agar
SDS	sodium dodecyl sulphate
SEM	standard error of the mean
tris	tris (hydroxymethyl) aminoethane
UV	ultraviolet
v/v	volume per volume
w/v	weight per volume
WGA	wheat-germ agglutinin
YNB	yeast nitrogen base

INTRODUCTION

I HISTORICAL BACKGROUND OF CANDIDA ALBICANS

Candida albicans is a pathogenic yeast of considerable importance because of its involvement in candidosis. The commonest manifestations of this disease are superficial lesions, particularly of mucosal surfaces in the oral cavity and vagina (Odds, 1979). Acute pseudomembranous candidosis and vaginal forms of candidosis are commonly referred to as 'thrush'.

Thrush has been described in several ancient manuscripts. In particular, two cases of oral aphthae with severe underlying diseases were described by Hippocrates, circa 460-377 B.C. (Adams, 1939). The disease was also mentioned in the diary of Samuel Pepys for 17th June 1665 (Winner and Hurley, 1964). However, the organism responsible for these infections was not discovered until 1839 when Langenbeck described the yeast in buccal aphthae in a case of typhus. Although the role of the yeast as a factor contributing to typhus was incorrectly postulated, Langenbeck was one of the first researchers to associate a microorganism with a pathological process (Odds, 1979).

In 1751 the genus Monilia was introduced in the classification of some fungi isolated from vegetation, and by the end of the nineteenth century it became associated with the yeast responsible for thrush. In 1923 the taxonomic position was reviewed by Berkhout (Kreger-van Rij, 1984). Here the medically important Monilia species which differ morphologically and physiologically from the fruit and leaf rotting moulds also called Monilia were given the generic name Candida, which has been internationally adopted as the botanical nomen conservandum.

II BIOLOGICAL ASPECTS OF CANDIDA SPECIES

A. Classification

A yeast may be defined as a unicellular fungus capable of reproducing by budding or fission (Kreger-van Rij, 1984). The yeasts are taxonomically diverse and include ascomycetes and basidiomycetes. A third group, the imperfect yeasts have both ascomycetous and basidiomycetous affinities. The genus Candida accommodates a heterogeneous collection of asporogenous yeast species which do not qualify for classification in any of the more homogenous genera of the imperfect yeasts (Kreger-van Rij, 1984). Yeasts of the genus Candida are members of the Cryptococcaceae family.

Species within the genus Candida are separated by their physiological properties such as carbohydrate assimilation and fermentation tests. Two simple and rapid tests are available for identifying C. albicans. In the germ-tube test, C. albicans produces hyphae from yeast cells after incubation in serum for three hours at $37^{o}C$ (Taschdjian et al, 1960a). The test for chlamydospore production by C. albicans is performed by growing on corn meal agar (Benham, 1931); the refractile nature of the chlamydospore is easily observed by phase contrast microscopy. Two culture media have been widely used in the isolation and identification of C. albicans. One type devised by Nickerson (1953), contains bismuth polysulphite which is reduced to a brown-black sulphide by C. albicans. The other medium utilizes the inability of C. albicans, as distinct from other Candida species, to reduce triphenyl tetrazolium chloride incorporated into the medium.

Serological tests also have been applied to the taxonomy of pathogenic yeasts. Hasenclever and Mitchell (1961a) found that there were two antigenic types among strains of C. albicans. One type, named serotype A possesses the same complement of antigens as serotype B plus two extra antigens. A test for presumptive identification of

C. albicans and differentiation of strains within the species was devised by Odds and Abbott (1980). Isolates were tested for acid and salt tolerance, proteinase production, resistance to 5-fluorocytosine and safranine and assimilation of urea, sorbose, citrate and glycine. Results for these tests were arranged in three groups allowing designation of types by 3-digit numbers. A different method was used by Warnock et al (1979) in which strain variation in resistance to six chemicals in agar media were assessed for different strains of C. albicans.

B. Ecological distribution

Yeasts which are considered principal agents of human candidosis have a restricted natural distribution in comparison to their more ubiquitously occurring relatives. C. albicans has been recovered from a far wider range of animal hosts than any other yeast species (Odds, 1979). This observation, when added to the high prevalence of C. albicans among human yeast isolates suggests that this species is the principal opportunistic yeast pathogen in warm blooded animals (Gentles and La Touche, 1969). C. albicans is rarely recovered from samples of soil, plants or the atmosphere. The yeast has been recovered from the bedding of hospital patients, hospital wash basins and the air of hospital wards (Odds, 1984).

C. Morphogenesis

(i) Different morphological forms

Depending on conditions, yeasts grow as elongated cells or as true mycelia; conversely, some grow as budding yeast cells which is the normal form of growth. The study of the morphogenetic process in dimorphic fungi is important because it helps not only in the diagnosis and treatment of the disease but also in the understanding of morpho-

genesis and its role in the pathologic process. Among *Candida* species mycelium production is the only reproducible property known to belong exclusively to *C. albicans*. The different morphological forms of *C. albicans* have been described by Odds (1979). Cells sometimes appear as ovoid, budding yeasts or as mould-like hyphae, occasionally with large refractile spores which are termed chlamydospores. The ovoid budding yeasts are often referred to as blastospores, which divide by a specific mode of mitotic cell division, budding. Hyphae are long microscopic tubes which comprise multiple fungal cell units, divided by septa. They may arise as branches of existing hyphae or by germination of spores, in which case the new cellular material is termed the germ-tube. Pseudohyphae are similar to true hyphae except they are produced by a budding process whereby the bud remains attached to the parent.

(ii) Correlation between dimorphism and pathogenicity

There are many accounts of experimental studies which associate mycelium formation with infection. These reports are based on the fact that mycelia are seen in smears or scrapings and that budding yeast cells usually produce mycelium in tissues within hours of inoculation into experimental animals. *In vivo*, however, both mycelial and yeast forms are usually seen together. The rapid tissue conversion of *C. albicans* from budding yeast cells to mycelium suggests that there is a greater invasive potential of this form of the organism.

Other studies have shown that there are no differences in invasive potential between the two morphological forms (Russell and Jones, 1973a). Evans and Mardon (1977) claimed that the lethality in mice and rabbits is more evident with yeast-like cells than with pseudohyphal preparations. Yeast-like cells were claimed to be more pathogenic because of the less effective host defence mechanisms against the yeast phase (Evans, 1980).

One serious objection to postulating that yeast-to-mycelium conversion is necessary for infection is that mycelium-deficient variants of C. albicans occur spontaneously in vitro or can be isolated from cases of candidosis (Savage and Balish, 1971). The prevalence of C. albicans strains incapable of forming germ tubes (germ-tube-negative strains) and strains which form pseudohyphae in human and oral candidosis have been investigated by Martin et al (1984). Both types of variant failed to induce palatal candidosis in rats in contrast to a germ-tube positive control strain. The authors concluded that the pathogenic potential of C. albicans appears to be dependent on germ tube formation. Sobel et al (1984) also found that a variant strain of C. albicans which was incapable of hyphal formation at 37^{o}C was less likely to colonize the rat vagina than the wild-type strain. Therefore, the capacity of C. albicans to produce hyphae appears to be an important but not essential virulence factor.

III CELL SURFACE OF CANDIDA ALBICANS

A. Cell wall components

The yeast Saccharomyces cerevisiae has been the subject for most studies on the structure of yeast cell walls. Yeast cell walls are composed of glucans (β1,3 and β1,6 polymers of glucose) mannoprotein and chitin (Phaff, 1971). The location of chitin is thought to be restricted to the bud scars, whereas glucan and mannoproteins are present all around the cell surface.

Evidence that mannose polymers are located at the external surface has been obtained from studies with anti-mannan antibodies

(Ballou, 1976) and concanavalin A (Tkacz and Lampen, 1972). However, it is not yet known how deeply the mannoprotein reaches into the cell wall. The β1,3 linked glucan appears to be responsible for the structural integrity of the cell wall. Therefore, a purified β1,3 glucanase can be used almost entirely to solubilize isolated cell walls (Zlotnik et al, 1984). Mannoproteins can, however, be extracted without affecting the shape of the cell; this implies that it may be a filling material which is enmeshed in the glucan structural network and may have a role in controlling wall porosity.

(i) Chitin

Chitin is less evident in cell walls of yeasts than in the cell walls of many filamentous fungi. It was first identified in S. cerevisiae cell walls by its characteristic X-ray diffraction pattern in residues prepared by acid or combined acid and alkaline treatments of cell wall preparations (Phaff, 1971). Chitin is a β1,4 polymer of N-acetyl-D-glucosamine and its physical nature in the wall is only partially understood.

(ii) Glucans

The term glucan covers a large group of D-glucose polymers which vary in their type and proportion of individual glycosidic bonds. Glucans in the yeast cell wall are usually β D-glucans with β1,3 linkages which may occasionally be interrupted by β1,6 sequences (Peat et al, 1961). Methylation analysis and periodate oxidation of the alkali-extracted glucan of C. albicans has shown that it is a highly branched molecule with 73% β1,6 linkages, the remainder constituting β1,3 bonds (Bacon and Farmer, 1968). The physicochemical properties of β-glucan, its

insolubility and high degree of crystallinity, indicates that its synthesis probably occurs *in situ* i.e. in the cell wall or at the outer surface of the plasmalemma (Sentandreu *et al*, 1975).

(iii) Mannan/Mannoprotein

Yeast mannan was first discovered by Salkowski in 1894 and was termed 'yeast gum' (Phaff, 1971). Many chemical investigations of yeast mannans have been performed on preparations extracted from intact cells with dilute alkali (2% KOH, 100°C) and then precipitated as the insoluble copper complex (Arnold, 1983). Peat *et al* (1961) examined mannan extracted by autoclaving intact yeasts at a neutral pH. A structure in which a backbone of α1,6 linked residues had attached to it short chains containing α1,2 and α1,3 linked residues of mannose was proposed from the products of partial acid hydrolysis. Mannoproteins are attacked readily by dilute alkali, dithiothreitol and pronase which probably degrade the protein that holds the polymannose chains together. Alkali treatment also cleaved small oligosaccharides from crude mannoprotein preparations (β-elimination) releasing fragments linked to serine and threonine in the protein (Figure 1). The majority of carbohydrate is attached to asparagine units in the protein by di-N-acetylchitobiose linkage (Ballou, 1976). Nakajima and Ballou (1974) confirmed that the linkage between mannan and protein is identical with that found in many glycoproteins. The second carbohydrate moiety of mannan consists of both α1,2 and α1,3 linked oligosaccharides attached at their reducing ends by an O-glycosidic linkage to serine or threonine.

B. Cell surface ultrastructure

The cell wall of *C. albicans* is important for several reasons. The cell surface appears to be relevant to pathogenesis of *Candida* infections as

Figure 1 Structure of mannoprotein from Saccharomyces cerevisiae X2180, proposed by Nakajima and Ballou (1974)

Abbreviations used:-

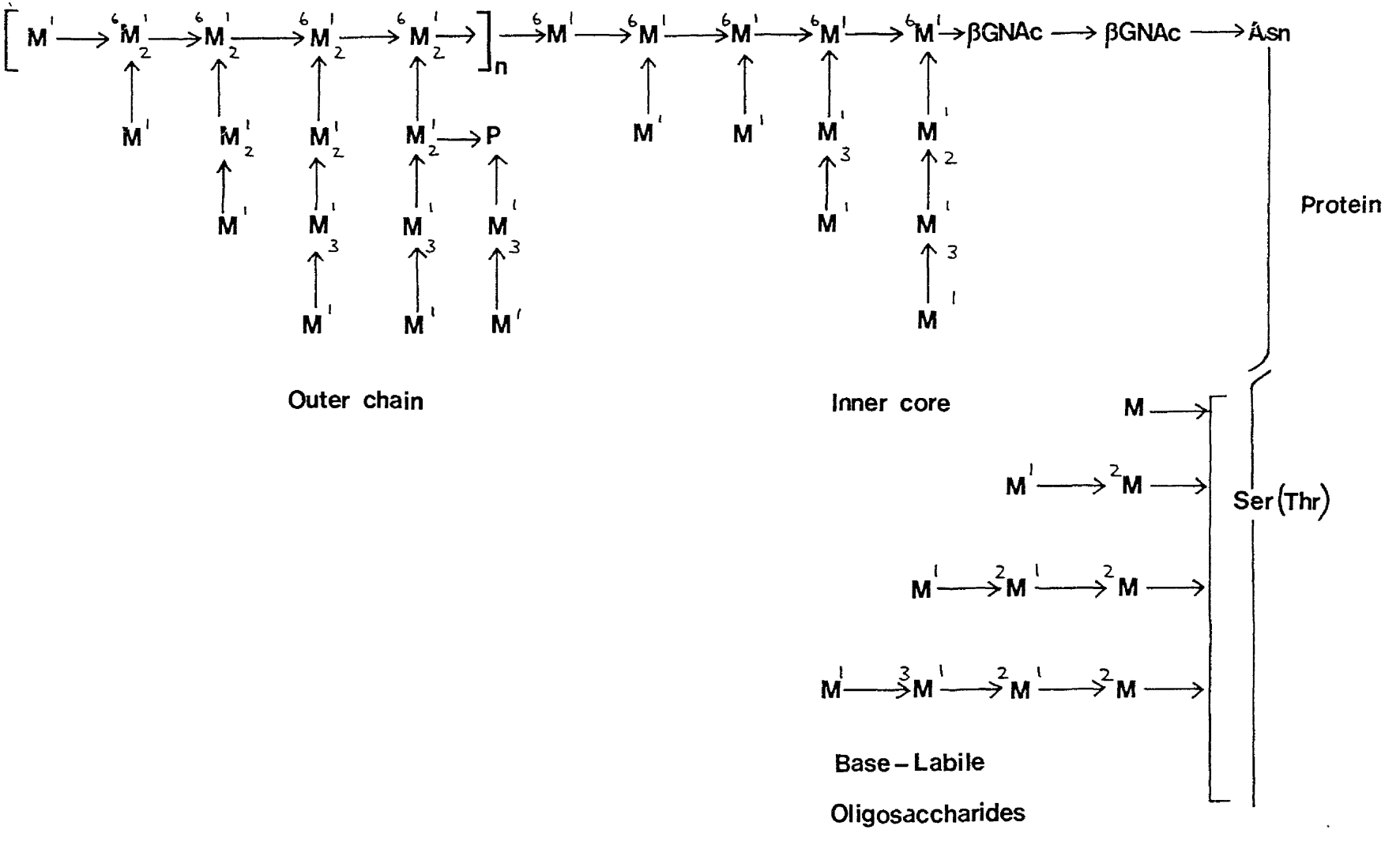
M - Mannose

GNAc - N-acetyl-D-glucosamine

Asn - asparagine

Ser - serine

Thr - threonine


Outer chain
Inner core
Protein
Ser (Thr)
Base – Labile
Oligosaccharides

it constitutes the interface for the host parasite interaction. It also plays a role in determining the cell's immunity to certain drugs such as polyene antibiotics (Gale et al, 1975) and contains antigenic structures which are involved in serotyping (Yu et al, 1967). The structure of the cell wall of C. albicans is complex relative to S. cerevisiae and our understanding of its architecture and biosynthesis is still primitive. Studies on the ultrastructure of the wall of a yeast cell of C. albicans have revealed that it is composed of five (Djaczenko and Cassone, 1972) or eight (Poulain et al, 1978) distinct layers. According to Cassone et al (1973) the cell wall of C. albicans is organized in a multilayer structure with amorphous, granular and fibrous components of various electron densities. Howlett and Squier (1980) used transmission electron microscopy to observe a fibrillar-floccular external layer on the yeast cell surface and also noted five distinct layers of the cell wall. Tronchin et al (1984) used electron microscopy to examine thin sections of yeasts adhering to epithelial cells. Staining by the periodic acid thiocarbohydrazide-silver proteinate technique (PATAg) clearly revealed a fibrillar layer of filaments arranged perpendicularly to the cell surface.

Takamiya et al (1984) used post-embedding immunoferritin tests with ultra-thin sections of C. albicans embedded in styrene-methacrylate resins which permit the demonstration of mannan antigens on the exterior cell wall surface as well as in the cytoplasm near the cytoplasmic membrane. This technique was used to show that mannan antigens are transported from the cytoplasm via channel-like organelles to the final deposits in the exterior of the cell wall layer (Figure 2).

Montes and Wilborn (1968) reported that the floccular material on the cell wall of hyphae which had penetrated host epithelial cells

Figure 2 Wall architecture of Candida albicans with channels of mannan transportation

Scheme based on that of Poulain et al (1978) and modified by Takamiya et al (1984)

Key to layers

1 mannans, glucans

2 mannans, glucans

3 mannans, glucans

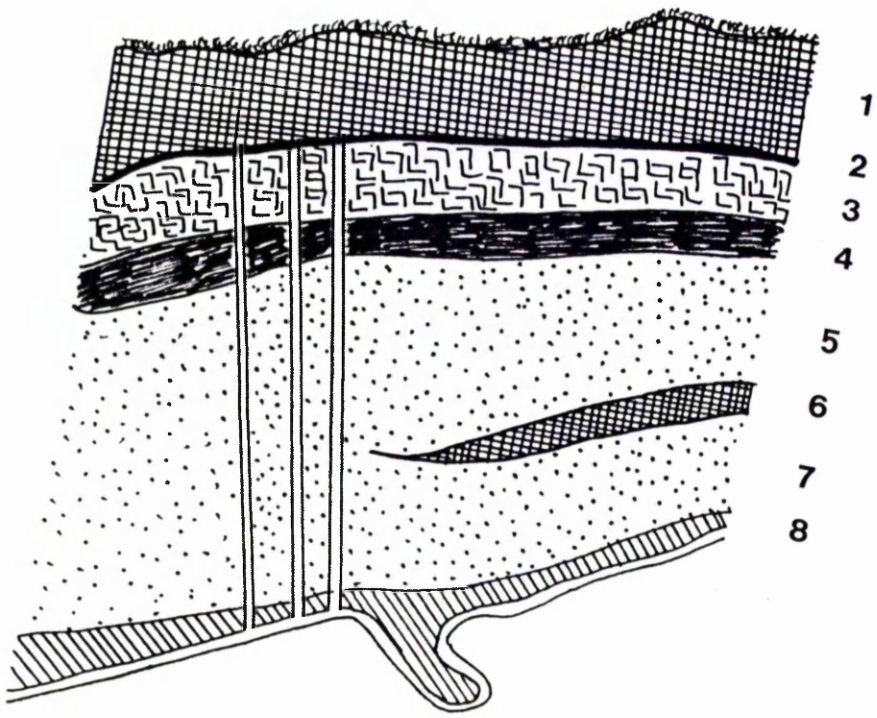
4 mannans, glucans

5 glucans, chitins

6 mannans?, proteins

7 chitins, glucans

8 chitins, proteins, polysaccharides


1
2
3
4
5
6
7
8

was detached during invasion. This material was much condensed along a certain length of the hyphae and diffuse at the hyphal tips (Rajasingham and Cawson, 1982). Pugh and Cawson (1978) suggested that cell walls of hyphae and yeasts were covered by a mucus layer which was secreted by the cell.

C. Extracellular polymeric material

C. albicans excretes polysaccharide-protein complexes into the nutrient medium (Masler et al, 1966). They found this material was composed of 74-86% mannose, 21-31% glucose, 1-1.5% glucosamine and 11-14% protein and that it was immunologically active. Kessler and Nickerson (1959) reported that a polysaccharide-protein complex in the yeast cell wall could be dissociated into a highly soluble glucomannan protein and highly insoluble glucan protein on treatment with weak alkali at room temperature.

Diedrich et al (1984) characterized a low molecular-weight extracellular material produced by metabolically active C. albicans. They showed that three strains of C. albicans produced a low molecular-weight extracellular substance which could be fractionated into two components by ion-exchange chromatography and suggested that these components represent the remnants of proteolytic turnover of cell-wall glycoproteins. This material was also generated by treating cells with papain.

Biological activities have been associated with extracellular polymeric material from C. albicans. Such activities have included growth inhibition in newborn mice and lethality of newborn mice (Mankowski, 1968). Sikl et al (1969) found that glycoprotein from C. albicans released histamine from mast cells. Intravenous injection of rats with C. albicans glycoproteins caused a decrease in cardiac output from 20 seconds to 240 minutes, followed by recovery at 360 minutes (Nosalova et al, 1979).

Saltarelli (1973) isolated morphological mutants from a strain of C. albicans capable of synthesizing complex organic molecules from simple salts, glucose and biotin. Extracellular glycoproteins were released into the culture medium. These substances were found to inhibit or stimulate cell growth and mycelium production, and to influence reductive and enzymic abilities of the other cells. Saltarelli and Coppola (1980) found that extracellular polysaccharides from C. albicans were immunologically active and have been used to successfully treat solid Sarcoma 180 tumor cells implanted subcutaneously in Swiss female mice.

Extracellular polymeric material from C. albicans inhibited attachment of yeasts to neutrophils (Diamond et al, 1980) and adherence to buccal epithelial cells (McCourtie and Douglas, 1985a). Extracellular glycoproteins were part of the outer fibrillar layer which was readily released from the cell surface (McCourtie and Douglas, 1981). This material contained yeast adhesin components which were capable of interacting with epithelial cell receptors.

IV CANDIDOSIS

Candidosis varies in severity from superficial localized oral infections to severe systemic disease. Localized infections may become chronic but do not result in serious sytemic involvement unless the patient is compromised. Although antifungal compounds are useful, recurrences are common if treatment is stopped.

A. Superficial candidosis

In this context superficial candidosis refers to surface infections of the oral, aural and vaginal cavities as well as of the skin and nails.

(i) Oral candidosis

Oral candidosis has been grouped into four descriptive categories (Epstein et al, 1984).

(a) Acute pseudomembranous candidosis (thrush)

Clinical manifestations are usually symptomless white papules or cotton wool-like exudates which can be rubbed off leaving an erythematous mucosa. The disease is seen in infants and debilitated adults (Odds, 1979), especially in diabetics and adults with malignant diseases. Antibiotics, corticosteroids and immunosuppressive drugs enhance infection.

(b) Acute atrophic candidosis

This condition may follow acute pseudomembranous candidosis and is commonly associated with broad spectrum antibiotic therapy; it is sometimes referred to as 'antibiotic-sore tongue' (Roitt and Lehner, 1983). Symptoms include a smooth erythematous tongue, with angular cheilitis and, less often, inflamed lips and cheeks.

(c) Chronic atrophic candidosis (denture stomatitis)

The infection occurs as a diffuse erythema of the palate, limited to the denture-bearing mucosa. The denture covering the palatal mucosa predisposes to proliferation of Candida. The lesion is usually free of painful symptoms but may be associated with angular cheilitis (Odds, 1979).

(d) Chronic hyperplastic candidosis (candidal leukoplakia)

This type of infection is less common and occurs as a firm white patch which cannot be rubbed off and commonly affects the tongue, cheeks and lips.

Diagnosis of candidosis depends upon the presence of the organism in a direct smear, the culture of significant numbers of organisms and indirectly, the effectiveness of antifungal medications (Epstein et al,

1984). Culturing of Candida from whole, unstimulated saliva may be the best method of distinguishing the carrier from the non-carrier state (Epstein et al, 1980).

(ii) Vaginal candidosis (vaginal thrush)

Symptoms of vaginal candidosis range from very mild to severe, abundant vaginal discharge with inflammation and intense pruritis (Oriel et al, 1972). The classical sign of vaginal thrush is the presence of white, curd-like patches, analogous to those in oral thrush, on the vulval, vaginal or cervical epithelium (Odds, 1979).

The incidence of vaginal candidosis is higher in certain susceptible groups of women, an incidence of over 50% for women post antibiotic treatment and 30% for oral contraceptive users (Segal et al, 1984a). Vaginal candidosis is most often seen in pregnancy especially during the third trimester when the vaginal pH is low. The condition may affect women of all ages; however, it is more common during the fertile period, decreasing in postmenopausal women (Segal et al, 1984a).

(iii) Chronic mucocutaneous candidosis

Chronic mucocutaneous candidosis (CMC) is an uncommon superficial candidal infection of skin, nails and oral and genital mucosa. Clinically, it usually occurs within the first year of life resulting in chronic and occasionally granulomatous, candidal infections. Jorizzo (1982) suggests that CMC is not a single disease but rather a final common pathway for multiple predisposing abnormalities of the immune system which range from severe, life-threatening immunodeficiency syndromes to subtle deficiencies, usually of cell-mediated immunity. Many different subgroups of CMC have been reported associated with pleomorphism of inheritance patterns, immunodeficiency states and associated medical conditions (Sams et al, 1979). Oral thrush is

usually the first lesion to appear in cases of CMC. Angular cheilitis and lip fissures may develop and infection may spread to the larynx and oesophagus (Odds, 1979).

B. Systemic candidosis

Systemic infections may occur in the abnormal host, and autopsy data reveal a marked increase in the incidence of invasive candidosis recently (De Repentigny and Reiss, 1984). Candidosis affecting deep organs are unusual in comparison with infections of mucous membranes. Infections of deep organs may arise as localized primary diseases with only one organ affected or as disseminated candidosis where Candida is spread by the bloodstream to invade several organs (Odds, 1979). Patients undergoing therapy for haematologic malignancies or receiving allogenic bone marrow transplantation are especially susceptible to disseminated candidosis and C. tropicalis has become the leading etiologic agent for this condition (Meunier-Carpentier et al, 1981).

A particular strain of C. albicans was responsible for an outbreak of systemic candidosis by cross-infection (Burnie et al, 1985). As well as infecting patients in an intensive care unit, this outbreak strain was also isolated from oral cavities of nurses working in the unit. This strain also survived well on hands and was resistant to Hibiscrub washing.

The diagnosis of systemic candidosis is difficult as there is no typical clinical picture. Antibody tests have been used for the immunodiagnosis of systemic candidosis; however, these may fail either because the patient is incapable of mounting an immune response or because serum is taken before an immune response has developed (Burnie and Williams, 1985). A variety of different assays have been used, including whole

cell-agglutination, agar-gel diffusion, counterimmunoelectrophoresis (CIE), latex-agglutination, indirect immunofluorescence, radioimmunoassay (RIA), passive haemagglutination (HA) and enzyme immunoassay (EIA). De Repentigny and Reiss (1984) suggested that results from these tests are difficult to compare because of differences in patient population, case definitions and the prospective or retrospective design employed.

(i) Renal candidosis

Renal candidosis usually occurs in middle-aged and elderly patients. Fever may occur at the onset of infection suggesting that renal candidosis arises as a consequence of the haematogenous spread of yeasts (Odds, 1979). The organ most susceptible to infection with C. albicans in man appears to be the kidney (Barnes et al, 1983). Blood-borne C. albicans cells are rapidly cleaved from the circulation by the liver, lung, spleen and kidney. Viable yeasts are eliminated from all these tissues except the kidney, where C. albicans proliferates extensively (Lee and King, 1983b).

(ii) Gastrointestinal candidosis

Systemic infections are thought to arise endogenously, most likely through the gastrointestinal tract. The gastrointestinal mucosa is an important portal of entry into the body for C. albicans as yeasts are capable of crossing the human bowel wall (Pope and Cole, 1981). Symptoms of gastrointestinal candidosis are dysphagia which is often accompanied by severe retrosternal pain, nausea, vomiting and occasionally gastro-intestinal bleeding (Odds, 1979).

(iii) Candida endocarditis

Symptoms of candida endocarditis are similar to those of subacute bacterial endocarditis and the patient may suffer fever, chills, anorexia, nausea, vomiting and heart failure. The condition was rarely seen before the introduction of antibiotics, steroids and cardiac surgery.

The condition is increasing in frequency and is usually found in three clinical settings: after prosthetic valvular insertion, in intravenous drug abusers and as a compilation of prolonged intravenous antibiotic administration or hyperalimentation (Scheld et al, 1983).

C. Factors and conditions permitting Candida infections

(i) Natural

Natural factors which predispose the host to candidosis include other microbial infections, endocrine dysfunctions and defects in cell-mediated immunity. Vaginal candidosis has been described as a common presenting symptom of diabetes mellitus (Segal et al, 1984a). Diabetics have high numbers of yeasts present in samples from the vagina, rectum, urine and oropharynx. It is thought that high blood and tissue glucose levels, or low skin lactate levels favour growth of C. albicans in diabetics. Segal et al (1984a) found that vaginal epithelial cells from fecund women who were pregnant or diabetic had a greater propensity to bind C. albicans than did epithelial cells from non-diabetic controls. During pregnancy the vaginal glycogen content rises and is converted to lactic acid by lactobacilli which favours the growth of Candida species (Cruickshank, 1934).

Maternal vaginal candidosis also appears to be responsible for neonatal oral thrush. Presumably, the infant ingests some of the vaginal contents during its passage through the colonized vagina (Davidson et al, 1984). Other predisposing factors include prematurity, low birth-weight, previous antimicrobial therapy, prolonged labour with resuscitative procedures and contact with nipples and bottles contaminated with the yeast. The increased susceptibility of infants to candidosis was also due to the immaturity of the normal antimicrobial defences.

(ii) Dietary

Gentles and La Touche (1969) suggested that a carbohydrate-rich diet may predispose the host to an infection. Feeding rats on carbohydrate-rich diet promoted carriage and persistence of Candida species in the oral cavity (Russell and Jones, 1973b). A hypothesis describing the mechanism by which high concentrations of carbohydrate might influence the pathogenesis of inflammatory lesions in mucosal candidosis was proposed by Samaranayake and MacFarlane (1985). They suggested that commensal yeasts on mucosal surfaces and high concentrations of sugars in surface secretions result in proliferation and release of yeasts into these secretions. The yeasts then adhere to, and multiply on fresh surfaces. C. albicans produces acidic metabolites which initiate a cytotoxic response, and activate acid proteinases and phospholipases which are responsible for inflammation of mucosal surfaces. Vitamin deficiency may also predispose the host to candidosis, which is more prevalent in rats fed on diets deficient in vitamins B_1 and B_2. Iron-deficiency has been proposed as an important factor in the aetiology of chronic mucocutaneous candidosis (Jorizzo, 1982).

(iii) Mechanical

Mechanical factors which predispose the host to candidosis include trauma and local occlusion or maceration of tissues. Several cases of Candida peritonitis and septicaemia have been reported in patients following accidental injury (Odds, 1979). Burn patients are particularly susceptible to colonization and deep-seated infections with yeasts (Fader et al, 1985). C. albicans is rarely found on skin; however, it may occur in areas such as axillae, groins and toe webs, so maceration of skin or membranes may raise the local humidity predisposing to yeast overgrowth and infection.

(iv) Medical

Medical factors which render the host more susceptible to Candida infections include certain drugs and various surgical procedures such as the introduction of mechanical devices and prostheses into vessels or tissues. Patients receiving antibiotic or corticosteroid treatment are more susceptible to candidosis. These drugs exert their effect by altering the composition of the endogenous flora or suppressing the host defense mechanisms against infection. The effect of antibacterial therapy was investigated by Liljemark and Gibbons (1973), who showed that colonization by C. albicans in gnotobiotic mice was inhibited by mixed human salivary bacteria and strains of Streptococcus salivarius and S. miteor. These results suggest that indigenous oral flora may interfere with adherence and colonization by C. albicans. Immunosuppressive drugs may also be a precipitating event that creates an environment favourable for host invasion by C. albicans. Cyclophosphamide can selectively deplete the immune system in experimental animals (Bistoni et al, 1983). Corticosteroids have a wide and complex range of actions. They depress inflammatory responses and cell-mediated immunity and lower the host's resistance to infection (Odds, 1979). Oral contraceptives may also predispose the host to vaginal candidosis (Lopez-Martinez et al, 1984). There is now evidence which suggests that oral contraceptives containing oestrogen enhance the susceptibility of the vagina to yeast overgrowth and lead to symptoms of vaginal candidosis.

Indwelling catheters and intravenous feeding are also more likely to increase infection in the host. Catheter-associated sepsis is one of the most serious complications of intravenous therapy. These infections increase with the time that the catheter has been in place.

Several types of catheter have been used to deliver fluid and nutrition to patients. Peripheral veins are normally used; however, central veins such as the subclavian vein may be used for total parenteral nutrition. Marrie and Costerton (1984) suggested that microorganisms in a liquid medium may multiply on the surfaces of catheters. Marrie et al (1983) examined Tenckhoff peritoneal catheters by scanning and transmission electron microscopy to study the morphology of microbial attachment. Catheters were removed from a patient with C. albicans peritonitis and yeast cells were found to be embedded in an amorphous background.

D. Antifungal agents for Candida infections

Antifungal agents which are currently used in candidosis therapy include the polyene macrolides, nystatin and amphotericin B; the imidazole derivatives clotrimazole and miconazole; and 5-fluorocytosine. Nystatin is a tetraene and was one of the first polyenes to be used in the treatment of candidosis. In vitro, nystatin causes leakage of intracellular potassium, sodium and magnesium (Odds, 1979). It also causes a decrease in the dry cell mass and intracellular amino acid pools and has been implicated as an inhibitor of mycelial transformation. The toxicity and insolubility of nystatin has restricted its use to superficial infections.

Amphotericin B is also widely used in the topical therapy of candidosis. Polyenes bind to sterol components of the yeast cell membrane affecting its permeability properties, resulting in leakage of cytoplasmic chemicals, metabolic disruption and cell death. Manjeet et al (1979) in a study of the lipid composition and polyene antibiotic sensitivity of C. albicans found that several other lipids besides sterol

in the membrane may be involved in polyene antibiotic sensitivity. Amphotericin B is not absorbed in the gut and may be given orally; however, it may be toxic to the kidneys resulting in uraemia and hypokalemia.

5-Fluorocytosine inhibits *in vitro* growth of yeasts and many fungi. It is well absorbed from the gut and may be given orally for the treatment of systemic infections. However, not all yeasts are susceptible and resistant strains have been described (Odds, 1979). 5-Fluorocytosine is transported into the cell by a cytosine permease and is deaminated to 5-fluorouracil, which is phosphorylated and is incorporated into cellular RNA which disrupts protein synthesis (Polak and Grenson, 1973).

Clotrimazole was one of the first imidazoles to be approved as an antifungal. It has a broad spectrum of activity and is effective against many filamentous fungi and dermatophytes. The mechanism of action is poorly understood, however, it appears to alter the permeability of susceptible yeasts. It has various side effects including nausea, vomiting, anorexia and fatigue (Odds, 1979). Germ tube-forming cells are more sensitive to the fungicidal action of clotrimazole than yeast cells (Niimi *et al*, 1985).

Miconazole was the second imidazole derivative to be accepted for antifungal use. It is poorly absorbed from the gastrointestinal tract. However, an intravenous formulation is available. Miconazole has been successful in treating superficial infections (Odds, 1979).

The development of new antifungal drugs has been dominated by molecular modifications of the imidazole class. Ketoconazole is one such recently marketed imidazole derivative which has a broad spectrum of activity and low toxicity (Dixon *et al*, 1978). Oral ketoconazole has

been successful in reducing the severity of C. albicans keratitis in rabbits and appears to be less toxic than other antifungal agents (Ishibashi and Matsumoto, 1984). Serotype B strains of C. albicans are more sensitive to ketoconazole than serotype A strains (Auger et al, 1984). Ketoconazole has been reported to work synergistically with sulphamethoxazole against C. albicans (Beggs, 1982). Amphotericin B-susceptible yeasts become resistant to the drug after growth in the presence of ketoconazole (Sud and Feingold, 1983). The activity of ketoconazole against C. albicans is markedly influenced by pH (Minagawa et al, 1983).

Ryley et al (1984) investigated two isolates of C. albicans from chronic mucocutaneous infections which were resistant to ketoconazole. These strains showed unusual responses in several in vitro and animal models of vaginal or systemic infections. Johnson et al (1984) also found differences between imidazole-resistant strains of C. albicans and other non-resistant strains by means of an agar incorporation MIC method, a broth microdilution procedure and by assessing hyphal elongation.

E. Pathogenesis of candidosis

(i) Virulence of different species of Candida

The pathogenic Candida species may be ranked in order of descending virulence as follows:- C. albicans, C. tropicalis, C. stellatoidea (C. albicans), C. parapsilosis, C. pseudotropicalis, C. krusei and C. guilliermondii (Odds, 1979). There have been relatively few studies comparing the pathogenicity of the various species of Candida under well controlled and reproducible experimental conditions. Bistoni et al (1984) found that only C. albicans, C. tropicalis and C. viswanthii were pathogenic for normal mice, with C. albicans being the most pathogenic followed by C. tropicalis and C. viswanthii. C. parapsilosis, C. krusei

and C. guilliermondii were considered non-pathogenic as revealed by absence of mortality and low transient colony forming unit counts in brain and kidney which are usually the principal target organs in disseminated candidosis. The same authors also found that strong immunodepression enhances the pathogenicity of the species of Candida which are already endowed with significant infective potentials for unmodified hosts, but has little effect on those species which are non-pathogenic for unmodified hosts.

Pope and Cole (1982) made comparative studies of gastrointestinal colonization and systemic spread by C. albicans and the non-pathogenic C. guilliermondii and S. cerevisiae. Scanning electron microscopic examination of the gastrointestinal tracts of infant mice inoculated with strains of C. albicans showed yeasts associated with the entire length of the villus while gastrointestinal tracts of animals receiving C. guillermondii or S. cerevisiae were devoid of adherent yeast cells. Barrett-Bee et al (1985) compared phospholipase activity, adherence and pathogenicity in C. albicans, C. parapsilosis and S. cerevisiae. C. albicans isolates which were highly adherent were the most pathogenic in mice and had high phospholipase activities. In contrast, C. parapsilosis and S. cerevisiae were not very adherent, did not kill mice and had lower activities of phospholipase.

C. tropicalis has been isolated in pure culture from several cases of systemic candidosis and these species have been shown to cause fatal disseminated infection on intravenous injection into rabbits and mice (Odds, 1979). Wingard et al (1979) reported C. tropicalis as the aetiologic agent in 15 of 18 cases of disseminated candidosis in patients undergoing treatment for haematologic malignancies or receiving bone marrow transplants. Hurley and Winner (1962) found that a strain of

C. tropicalis which was pathogenic for mice when injected intravenously did not show any definite evidence of pathogenicity when the organism was injected by the intradermal, intraperitoneal or intrapulmonary routes. Hasenclever and Mitchell (1961b) compared the pathogenicity of C. albicans and C. tropicalis in mice and found that strains of C. tropicalis were less virulent than strains of C. albicans. The strain of C. tropicalis used by Hurley and Winner (1962) was more virulent for mice than two strains of C. albicans; however, it was less virulent than other strains of C. albicans.

C. pseudotropicalis has been isolated from the bloodstream and other sites in cases of systemic candidosis; however, fungaemia and invasive disease caused by C. pseudotropicalis are extremely rare. Morgan et al (1984) reported a case of C. pseudotropicalis fungaemia and invasive disease in an immunocompromised patient, the source of the infection being the urinary tract.

C. parapsilosis does not appear to be a normal human commensal. However, it has been isolated from hospital patients with various illnesses such as diabetes, chronic pulmonary disease and genitourinary tract infections. In such patients it has been recovered from the oral cavity, rectum and vagina in the absence of clinical signs of infection. C. parapsilosis has often been incriminated in systemic candidosis, in particular in Candida endocarditis (Marrie et al, 1984).

(ii) Host defence mechanisms

Reviews of host resistance to candidosis have been written by Epstein et al (1984) and by Smith (1985). It has become apparent that host defence against C. albicans involves several mechanisms which, when acting together, represent a formidable barrier to candidosis. In the oral cavity there is a variety of non-specific host-defence mechanisms.

The epithelium is a physical barrier and epithelial-cell turnover contributes to defence. Saliva has a diluting and washing-out effect and contains antimicrobial factors such as lysozyme, lactoferrin and lactoperoxidase. Oral bacteria may also compete with or inhibit growth of yeasts. The effect of other bacteria on colonization or growth of C. albicans is one of antagonism. This effect is well illustrated in the germ-free mouse model. Helstrom and Balish (1979) showed that the gastrointestinal tract of germ-free mice is more easily colonized by C. albicans than that of mice with a normal gastrointestinal flora. Therefore, the indigenous bacterial flora can decrease colonization by Candida by competing for adherence receptor sites on epithelial cells (Marrie and Costerton, 1981). This means that some alterations in the microbial flora associated with systemic illness, hormonal changes and use of medications such as antibiotics or corticosteroids, may be significant in clinical candidosis (Rogers and Balish, 1980).

In the oral cavity lysozyme originates from saliva, gingival crevice fluid and polymorphonuclear leukocytes. It can agglutinate and kill C. albicans in nonionic solutions and increases the fungicidal activity of amphotericin B in culture media (Epstein et al, 1984). Salivary glycoproteins may also form part of the host's non-specific defence mechanism. These glycoproteins may be similar to those on the host cell surface and may bind to the yeast cell surface preventing adherence to the mucosal epithelium. Human parotid-saliva, rich in histidine polypeptides, has been reported to have antifungal activity as it inhibits the growth and viability of C. albicans at concentrations similar to the known antifungal activity of the imidazole antibiotics (Pollock et al, 1984). The candidastatic and candidacidal activities

were thought to be elicited by poly-L-histidine, as histidine itself has an imidazole-containing moiety and may be a key amino acid in the body's defences.

The stratified squamous epithelium of the skin is also an effective barrier against microbial invasion and it is not usually colonized by C. albicans. The regular sloughing and replacement of skin cells represents an effective method of eradicating attached organisms, and skin lipids are thought to inhibit the growth of C. albicans (Smith, 1985).

After the mucosal and skin barriers have been broken by C. albicans, the next line of defence is mediated by factors in the serum. Usually, humoral or serum factors are of less importance in host resistance than are phagocytic and cellular immune responses. Nevertheless, Candida antibodies have a direct inhibitory effect on C. albicans. Grappel and Calderone (1976) found that the inhibition of oxygen consumption and retardation of germ tube formation occurred with the purified gamma-globulin fraction of the antiserum. The binding of antibodies to antigens in general is known to activate the serum complement system, one of whose functions is to facilitate phagocytosis. Epstein et al (1982) found that anti-Candidal IgA antibodies in saliva can inhibit attachment of C. albicans to epithelial cells. However, they also noted that salivary levels of IgA and other anti-Candidal antibodies were higher in subjects with candidosis than in non-infected controls. Secretory IgA isolated from human breast milk also inhibits adherence of C. albicans to human oral epithelial cells, the action being due to its specific candidal antibody (Vudhichamnong et al, 1983).

There is considerable controversy regarding the importance of anti-candida immunoglobulins as opsonins for phagocytosis of yeast walls.

Diamond et al (1978) found that low levels of anti-candida IgG in normal serum enhanced the phagocytosis of pseudohyphae by human neutrophils. However, serum from patients with disseminated candidosis has been shown to inhibit phagocytosis of C. albicans by human neutrophils (La Force et al, 1975).

The increasing incidence of vaginal candidosis has led to interest in the origin and role of anti-C. albicans IgA and IgG in human cervicovaginal secretions. Gough et al (1984) measured the levels of anti-C. albicans IgA and IgG in the genital tract secretions of serum of non-pregnant women with vaginal candidosis as compared with uninfected women. There was no significant difference between the mean levels of specific IgA or IgG in secretions from women from the two groups.

Hector et al (1982) suggested that resistance to systemic candidosis is dependent upon a combination of innate factors, such as an intact complement system and an immune response, most likely of a cell mediated type. Phagocytic cells such as neutrophils, eosinophils, monocytes and other macrophage-like cells are an important part of the host defence mechanism against Candida species. Evidence for an important role of cell-mediated immunity (CMI) in defence against Candida infections is provided by animal studies and by recognition of specific defects of CMI in patients with chronic mucocutaneous candidosis (Smith, 1985). Diamond and Krzesicki (1978) showed that candidacidal activity was associated with a neutrophil oxidative mechanism which depends on the interaction of hydrogen peroxidate and myeloperoxidases and possibly also on the release of lysosomal enzymes from specific granules. Monocytes are also important in the host defence system against C. albicans. Normal human peripheral blood monocytes have the ability to phagocytose and kill C. albicans in vitro (Diamond and Haudenschild, 1981).

(iii) Attributes of virulence

(a) Toxins

Toxic substances from C. albicans were biologically and chemically characterized by Cutler et al (1972) who compared fractions of C. albicans prepared by mechanical and chemical methods and investigated them for toxicity in three animal models. They found that only cell-wall glycoproteins of C. albicans had toxic properties. These cell-wall materials were pyrogenic in rabbits and were lethal to mice which had been pretreated with actinomycin D. Cell-wall glycoproteins from C. albicans are known to be major antigenic determinants and are found in culture fluids and cytoplasmic extracts under certain conditions of preparation. In this respect they resemble Gram-negative bacterial endotoxins with respect to their localization at the cell surface, their pyrogenicity and immunogenicity. However, their biological activities are otherwise quite different and their potency as toxins is much lower (Cutler et al, 1972).

Cell-wall glycoproteins from C. albicans may be the attachment site for the yeasts to interact with epithelial cells. McCourtie and Douglas (1985a) found that extracellular glycoproteins isolated from culture supernatants of C. albicans inhibited adhesion of yeasts to buccal epithelial cells when epithelial cells were pretreated with the extracellular glycoprotein. It has been shown that Candida hyphae liberate substances which inhibit attachment of hyphae to neutrophils (Diamond et al, 1980).

(b) Enzymes

Pathogenic fungi produce a variety of toxic metabolites and extracellular enzymes which have been implicated in fungal infections. Patients suffering from candidosis have high titres of specific antibodies in

their serum and the localization of Candida proteinase in the vicinity of the yeast cells which are invading the host tissues suggests that the enzyme is important in the pathogenesis of Candida infections (MacDonald and Odds, 1980). Staib (1965) grew yeasts on agar containing bovine serum albumin as sole source of nitrogen and found that certain strains produced acid proteinases. Acid proteinases are secreted by the majority of C. albicans, C. tropicalis and C. parapsilosis strains; this correlates with the sequence of virulence of these species in man (Rüchel, 1984; MacDonald, 1984). Therefore, an inducible proteinase secreted by C. albicans is likely to be a virulence factor of the yeast. MacDonald and Odds (1980) found that antibodies to proteinases were present in serum from patients with systemic candidosis but could not be detected in serum from normal patients. Strains of C. albicans which are proteinase-deficient are less virulent in mice than strains which secrete the enzyme (MacDonald and Odds, 1983).

Proteinases from C. albicans and C. tropicalis have been partially purified by ion-exchange chromatography or totally purified using affinity chromatography with a pepstatin ligand (Rüchel, 1981). All these enzymes were aspartic proteinases, consisting of a single polypeptide chain with a molecular weight above 40,000 (Rüchel, 1981). Proteinases which are capable of degrading keratin are important in superficial infections of C. albicans. Hattori et al (1984) found that C. albicans produced a keratinolytic proteinase after growth in medium containing human stratum corneum as a nitrogen source. Once parasitic on the stratum corneum the fungi are able gradually to invade nails, possibly with the help of keratinolytic proteinases induced by the stratum corneum. This enzyme had a molecular weight of 42,000, an isoelectric point at pH 4.5 and an optimal pH value of 4.0. From

inhibitory profiles the enzyme was grouped as a carboxyl proteinase (Negi et al, 1984).

C. albicans produces phospholipases which may be associated with the pathogenicity of the fungus. In stationary-phase cells, Pugh and Cawson (1975) demonstrated the localization of phospholipase activities in cells and culture medium by a cytochemical method. Phospholipase has been detected quantitatively by a plate method; this has shown large variations in phospholipase in clinical isolates of C. albicans (Price et al, 1982). Several phospholipases secreted by C. albicans have been identified by pure substrates for detailed analysis of the hydrolytic products (Banno et al, 1985). Three fractions with phospholipase activity were obtained when concentrated culture filtrates were applied to DEAE-Sephadex columns. These enzymes were lysophospholipase, lysophospholipase-transacyclase and a phospholipase B.

Phospholipase A and lysophospholipase activities have been localized in yeast cells and hyphae of C. albicans infecting chick chorioallantoic membranes using cytochemical techniques (Pugh and Cawson, 1977). Yeast cells with high levels of phospholipase which were invading tissues made a path enabling other yeasts to follow and invade and colonize with the production of hyphae. Very high levels of phospholipase activity were present at the hyphal tip which was in contact with the host tissue.

Samaranayake et al (1984) determined phospholipase activity in 41 oral isolates of Candida species by the plate assay of Price et al (1982). They found that 79% of C. albicans isolates were capable of producing the enzyme; the enzyme was not detected in C. tropicalis, C. glabrata or C. parapsilosis isolates. In vitro, the enzyme was only produced within a pH range of 3.6 to 4.7 and production was decreased if

high concentrations of sucrose or galactose were included in the media. Barett-Bee et al (1985) found that there was a correlation between phospholipase activity, pathogenicity and adherence. C. albicans strains which were highly virulent in mice and highly adherent had high levels of phospholipase.

(c) Filamentation

The transition from the yeast to the hyphal-form is important in the expression of fungal pathogenicity, although no evidence has been provided on the relative pathogenicity of the different forms of growth. It has been suggested that hyphal forms are endowed with in vivo resistance to host phagocytic defences by a variety of mechanisms (Saltarelli et al, 1975). Comparison of the activity of different murine effectors against yeast and hyphal forms has shown the latter to be killed to a similar, if not greater extent than the former (Baccarini et al, 1985). Cockayne and Odds (1984) suggested that hyphal forms of C. albicans are more susceptible to killing by human polymorphonuclear leukocyte effectors than yeast forms. These recent results seem to contradict the commonly held belief that hyphal forms of C. albicans are endowed with a greater invasiveness and resistance to host defences than the yeast forms.

Extracts of rabbit brain and kidney stimulate more hyphal formation in C. albicans than other organ extracts and this observation may explain the predilection of C. albicans for kidney tissue (Odds, 1979). Therefore, it is possible that hyphae are more likely than yeast cells to form emboli as thrombembolic sequalae and fungus balls in the urinary tract are probably encountered more often in infections due to C. albicans than other species.

V. ADHERENCE OF MICROORGANISMS AS A VIRULENCE DETERMINANT

A. Role of adherence

Virulent microorganisms have the ability to proliferate and maintain themselves on mucosal surfaces while resisting the mechanical flow of fluids, gases and particulate materials through the habitat. Beachey (1980) suggested that adherence was the first stage in the pathogenicity of bacterial infections, leading to colonization and eventual invasion. To colonize a mucosal surface, a bacterium must be able to make contact (actively or passively) with the mucosal surface and penetrate the mucus gel. After adhering to the epithelial cell surface the organisms may be capable of multiplying either on the surface or after penetration (Freter and Jones, 1983). In deeper tissues attachment of bacteria to phagocytic cells may result in their ingestion and destruction (Beachey, 1980). Organisms with surfaces which are not recognized by antibody and complement or by phagocytic cells may multiply unimpeded to produce a systemic infection. The role of adherence as an ecological determinant is suggested by the relationship between *in vivo* adherence of oral bacteria to different surfaces and their proportional distribution in the mouth (Gibbons and Van Houte, 1971).

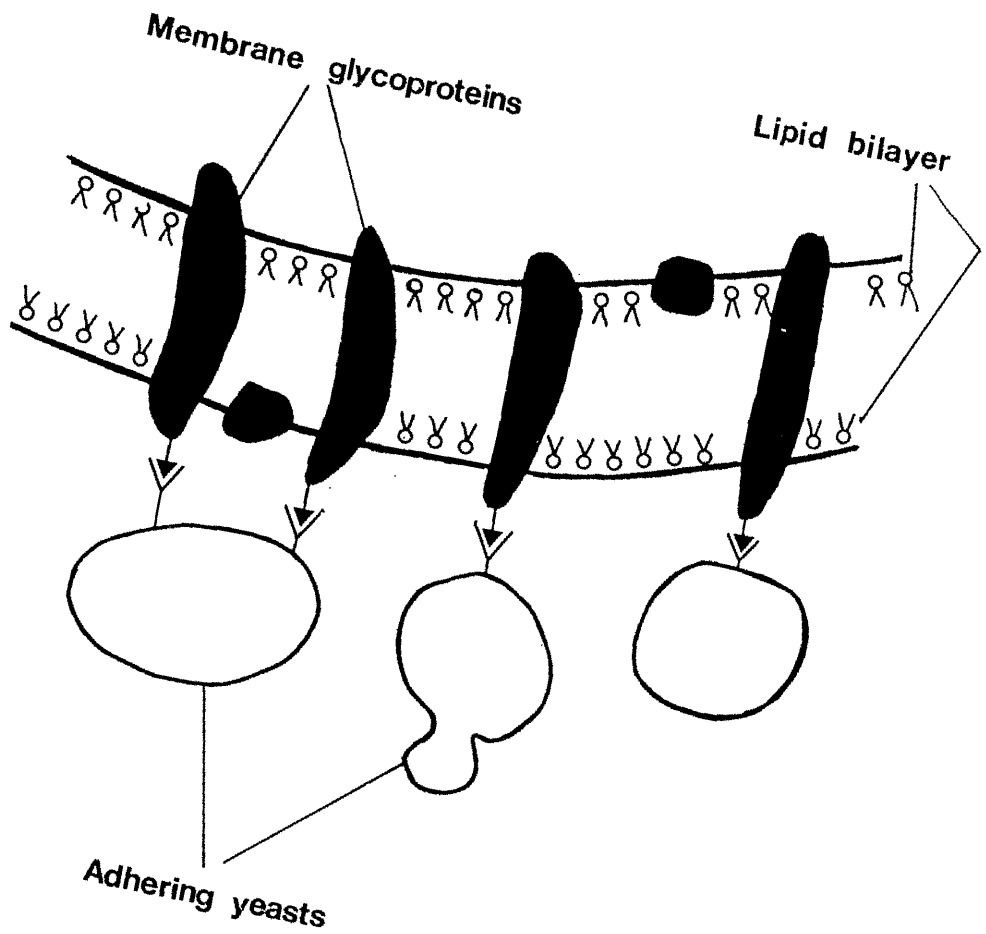
Although yeast adherence has been less well investigated than that of bacteria, *Candida* species have been shown to adhere to buccal epithelial cells (Kimura and Pearsall, 1978), the gastrointestinal epithelium of mice (Pope and Cole, 1981) and materials used in dentures (McCourtie and Douglas, 1981). All these surfaces are areas where yeasts may cause infections, and the fact that yeasts have been demonstrated adhering to these surfaces suggests a role of adherence in colonization.

B. Proposed mechanisms of microbial attachment to epithelial surfaces

A major problem which must be overcome for all microorganisms to associate with mucosal surfaces results from the charge attraction and repulsion between the bacterial cell and surface. Bacteria and eukaryotic cells have net negative charges and as a result should never come into physical contact. The Darjaguin-Landau and Verway-Overbeek (DLVO) theory is useful in interpreting the interactions between two negatively-charged bodies (Rutler and Vincent, 1980). The theory states that, as two rigid bodies of like charge approach each other, they are subject to the forces of attraction and repulsion which are additive, but vary independently with the distance of separation between the bodies. Filamentous structures on the surfaces of bacterial cells such as pili or fimbriae, are capable of overcoming the forces of repulsion and may come into contact with the substratum. Beachey (1980) suggests that hydrophobic molecules on the surface of bacteria allow bacteria to approach the negatively-charged epithelial cell and thereby enable special binding on each of the cell surfaces to interact with each other forming specific bonds of affinity.

Recently, there has been much evidence to suggest that microorganisms possess molecules on their surfaces which are capable of binding in a stereospecific fashion with complementary receptors on the surfaces of tissues and host cells. The binding molecules on the surface of microorganisms are called adhesins and those on host cells are termed receptors (Beachey, 1980). The molecular interaction between adhesins and receptors are probably analogous to receptor-ligand interactions (Sharon and Lis, 1972) (Figure 3) and may be facilitated by hydrophobic and charge interactions. The specificity of adhesin-receptor interactions may be demonstrated by inhibiting adherence with an excess of 'haptens' which are identical to or resemble the adhesin or the

Figure 3 Attachment of microorganisms via specific adhesins (Y) to complementary receptors (↓) on the host cell membrane.


Membrane glycoproteins
Lipid bilayer
Adhering yeasts

receptors (Figures 4a and 4b). Chemical and enzymic treatment of microorganisms or host cells to abolish the specific structures involved in adherence have been used to demonstrate the specificity of the interaction.

The principles described above have been applied to studies on the adherence mechanisms of group A streptococci (Beachey, 1981). There is now evidence to suggest that lipoteichoic acid (LTA) is involved in the attachment of group A streptococci to epithelial cells. LTA is an amphipathic molecule which occurs in most Gram-positive bacteria and consists of a chain of polyglycerol phosphate covalently linked to a glycolipid at one end. The binding of LTA is mediated by the glycolipid end of the molecule which forms complexes with streptococcal surface proteins. This interaction enables LTA to reorientate itself exposing some of its lipid ends towards the surface of the organisms. The lipid moiety of the hydrolysed molecule will inhibit adherence whereas deacylated LTA does not. Host cell membranes contain specific receptors for LTA of Streptococcus pyogenes; the binding sites are saturable with increasing concentrations of LTA and receptor sites appear to be limited to the outer surface of the membrane. Radiolabelled LTA has been used to demonstrate specific binding sites in erythrocytes, isolated erythrocyte membranes, platelets, lymphocytes, neutrophilic leukocytes and oral epithelial cells (Beachey et al, 1982). Simpson and Beachey (1983) suggested that fibronectin on oral epithelial cells serves as a LTA-sensitive receptor for group A streptococci.

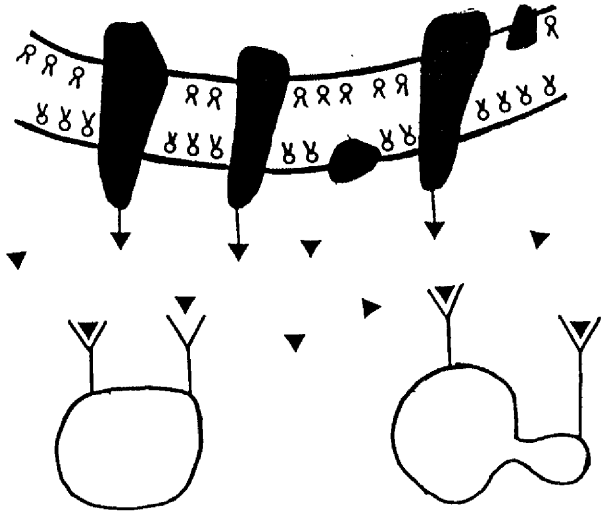
Streptococcus salivarius is commonly found on the dorsal surface of the tongue and attaches preferentially to keratinized oral epithelial cells in vitro (Sklavanou and Germaine, 1980). A fuzzy coat outside the cell wall is seen in thin-sectioned material (Gibbons et al, 1972).

Figure 4 Procedures used to demonstrate the specificity of microbial adherence

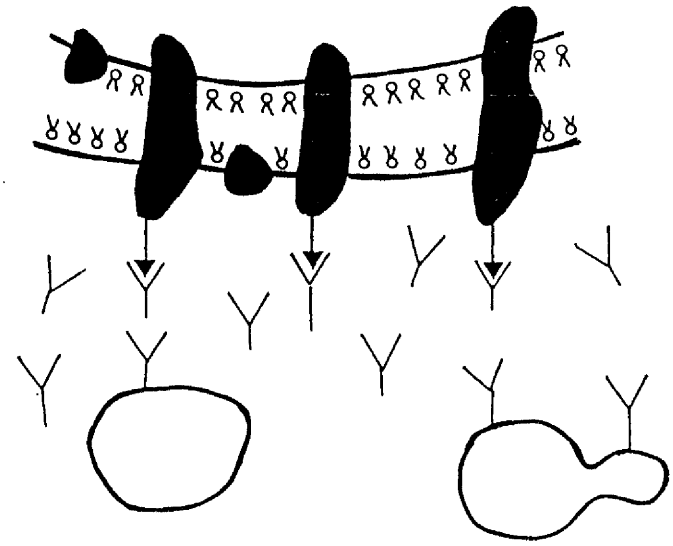
(a) Blocking adherence with an excess of haptens which resembles receptor components

(b) Blocking adherence with an excess of adhesin components.

(a)



(b)



Handley et al (1984) suggested that there are two structural subgroups of S. salivarius strains: K^+ adhesive strains which carry fibrils and K^- non-adhesive strains which carry fimbriae. Strains with long fibrils were unaffected by protease or trypsin; however, short fibrils were completely digested by protease or partially removed by trypsin. Neither type of fibril was affected by pepsin or lipase. Adhesive functions may be located in both types.

Using vaginal epithelial cells, Botta (1979), found that the adherence of group B streptococci was dependent upon the menstrual cycle. Various hormones resulted in a significant reduction in adherence. Broughton and Baker (1983) examined the ability of group B streptococci to adhere to buccal epithelial cells from adults, healthy neonates and infants with group B streptococcal infections. Higher adherence occurred with epithelial cells from healthy neonates than those from adults with type III group B streptococci. No differences in adherence were observed with type Ia or type II strains. Nealon and Mattingly (1984) showed that LTA of serotype III strains of group B streptococci mediated adherence to human embryonic, foetal and adult buccal epithelial cells.

At least nine different adhesins have been demonstrated in Escherichia coli. The mannose-binding activity of several isolates of E. coli has been monitored by aggregometry with mannan-containing yeasts (Ofek and Beachey, 1978). The mannose-specific adhesin of E. coli is thought to be associated with pili (Clegg et al, 1984; Ofek and Silverblatt, 1982). Fimbrial adhesins are thought to be proteinaceous polymers composed of identical subunits and easily visible by electron microscopy (Jones and Isaacson, 1983). Enteropathogenic strains of E. coli attach to HeLa cells, and Scaletsky et al (1984) found two

attachment patterns. In one, bacteria covered the entire surface of the HeLa cell (diffuse adherence) and in the other, bacteria bound to localized areas of the HeLa cell (localized adherence). Faecal isolates of E. coli from healthy humans are excellent colonizers of mouse colonic mucus. Lipopolysaccharide from these strains blocked adhesion of the bacteria to mucus which had been immobilized to polystyrene (Cohen et al, 1985). Purified capsule also blocked adherence; however, adherence was nonspecific. Exogenous human plasma fibronectin binds to human buccal epithelial cells and blocks adherence of Type I-fimbriated strains of E. coli, adherence being inhibited by fibronectin in a dose-dependent manner (Simpson et al, 1985).

Uropathogenic strains of E. coli show mannose-resistant adherence to urinary tract epithelial cells and agglutination of human erythrocytes (Svanborg-Eden et al, 1981). Leffler and Svanborg-Eden (1981) demonstrated that glycolipids were receptors on human uroepithelial cells and erythrocytes for uropathogenic strains of E. coli. The variable carbohydrate chain structures confer the ability of some glycolipids to act as specific receptors.

Colonization of the respiratory tract by Pseudomonas aeruginosa correlated with its adherence to upper respiratory tract epithelium (Johanson et al, 1979). Woods et al (1981b) found that in vitro adherence of P. aeruginosa correlated with loss of fibronectin from the epithelial cell surface. The loss of fibronectin was also related to an increase in salivary protease levels, particularly in seriously ill patients, eg patients undergoing coronary artery bypass (Woods et al, 1981a). The adherence of P. aeruginosa to upper respiratory tract epithelium appears to be mediated by pili on the bacterial cell surface (Woods et al, 1980). Pili also mediate adherence of P. aeruginosa to

buccal epithelial cells. This was demonstrated by preincubating epithelial cells with purified pili which resulted in a significant decrease in adherence of intact organisms (Woods et al, 1983). Cells from ciliated surfaces bind more bacteria than cells from squamous surfaces; therefore, adherence of P. aeruginosa at these respiratory sites may involve a different mechanism (Niederman et al, 1983).

For convenience most bacterial receptors are classified as carbohydrate (including glycoproteins) and as glycolipid (Jones, 1977). Mannose, L-fucose, neuraminic acid and D-galactose have been suggested as receptors for various bacteria. All surface sugars are common constituents of glycoproteins and glycolipids found on animal cell membranes. Approaches to determine the nature of host cell receptors include inhibition of adhesion by soluble carbohydrates which compete with natural receptors for the adhesin (Jones and Isaacson, 1983). Lectins of known carbohydrate specificity have been used to inhibit adherence, the lectin and adhesin both competing for the same receptor. The nature of receptors has also been elucidated by treating host cells with enzymes; any reduction in adherence may suggest that degraded components represent the receptor. Any substance which is considered to be a receptor on the basis of inhibition studies, when linked to an inert carrier, should act as a receptor and promote microbial attachment. Receptors which have been suggested for different bacteria are shown in Table 1.

Fibronectin, a large molecular weight glycoprotein (440,000) has been suggested as a modulator of the oropharangeal bacterial flora (Simpson et al, 1982). Fibronectin has been demonstrated in animal and human sera, in connective tissue and as a component of the extracellular matrix of tissue culture cells. In the oral cavity, fibronectin has

Table 1 Adherence mechanisms of various bacteria

Organism	Adhesin	Receptor	Reference
Escherichia coli (uropathogenic)	Fimbriae	Glycolipid	Leffler and Svanborg-Eden (1981)
Escherichia coli Type I	Fimbriae	D-Mannose	Jones and Isaacson (1983)
Escherichia coli K99	Haemagglutinin	Sialic acid	Jones and Isaacson (1983)
Vibrio cholerae	Haemagglutinin	L-Fucose	Jones and Freter (1976)
Streptococcus pyogenes	Lipoteichoic acid	Fibronectin	Babu *et al* (1983)
Pseudomonas aeruginosa	Fimbriae	Not known	Woods *et al* (1983)
Staphylococcus aureus	Not known	Fibronectin	Vercellotti *et al* (1984)

been detected on superficial epithelial cells distal from connective tissue. The presence of fibronectin only on cells exposed to the oral cavity suggests that cell surface fibronectin on buccal epithelial cells may be derived from saliva (Simpson et al, 1982). S. aureus has been reported to bind fibronectin-coated surfaces (Vercellotti et al, 1984) as has S. pyogenes (Beachey et al, 1983).

C. Adherence of Candida albicans

(i) Adherence to buccal epithelial cells

C. albicans was first reported to adhere to human buccal epithelial cells by Kimura and Pearsall (1978). The degree of attachment was determined in vitro by mixing epithelial cells with the yeasts under standard conditions and counting the mean number of organisms adhering per cell. Adherence of C. albicans was dependent upon yeast cell concentration and was detectable in vitro above 10^4 yeasts ml^{-1} of incubation mixture with a 1:10 yeast/epithelial cell ratio. Germinating yeasts were more adherent than non-germinating yeasts (Kimura and Pearsall, 1980). Cox (1983) investigated the effect of pH of the assay medium on adherence of C. albicans to buccal cells and found that there was no significant change in adherence between pH 4 to 11.

Buccal cells from healthy adult donors showed no significant change in adherence of C. albicans in daily measurements. Buccal cells from adults gave similar adherence values to those from children, suggesting that receptors remain stable and are not affected by age (Cox, 1983). However, Davidson et al (1984) claimed that yeasts were more adherent to buccal cells from adults rather than children.

C. albicans has a greater propensity to adhere to buccal epithelial cells from children with oral candidosis and oral colonization than to

buccal cells from uninfected control children. Adherence to epithelial cells from healthy children receiving antibiotics was measured by Cox (1983). The numbers of adhering bacteria decreased and the numbers of adhering yeasts increased on the second day of antibiotic therapy.

The adherence of C. albicans and other Candida species to buccal cells has been measured by several investigators (King et al, 1980; Macura et al, 1983 and Ray et al, 1984). C. albicans was found to be the most adherent species, while C. tropicalis and C. stellatoidea exhibited moderate adherence capabilities and C. parapsilosis was only slightly adherent to buccal cells. Therefore, there is a relationship between the adherence capabilities of Candida species and their ability to colonize mucosal surfaces, as the most adherent species are those most commonly found colonizing mucosal surfaces.

Strains of C. albicans isolated from active infections show enhanced adhesion to buccal cells after growth in defined medium containing 500 mM galactose as a carbon source (Douglas et al, 1981). In response to high sugar concentrations these strains synthesize surface components which are responsible for enhanced adhesion. Variations in cell-surface features of C. albicans with different carbon source has been reported by Kulkarni et al (1980).

(ii) Adherence to human vaginal epithelial cells

Factors affecting adherence of C. albicans to vaginal epithelial cells have been investigated by Sobel et al (1981). Germinating yeasts were more adherent to vaginal cells than non-germinating yeasts in in vitro assays. Sobel et al (1981) also reported higher adherence to vaginal cells at pH 6 than at pH 3 to 4 which is nearer the normal vaginal pH. Persi et al (1985) concluded that the adhesion of C. albicans to vaginal cells was determined by the strain used in the assay, the side of the

vaginal cell exposed to the yeast, the pH value, and CO_2 levels present in the adhesion assay. King et al (1980) found that yeasts adhered in greater numbers to vaginal cells than to buccal cells isolated from the same individual. In contrast to Segal et al (1982), King et al (1980) found that stationary-phase organisms were more adherent to vaginal cells than exponential-phase yeast cells.

Vaginal cells isolated from fecund women who were pregnant and/or diabetic had a greater propensity to bind C. albicans than did epithelial cells from oral contraceptive users and non-pregnant diabetic controls (Segal et al, 1984b). To assess whether a correlation exists between the hormonal status of women and the adherence capacity of vaginal cells, the hormonal status was assessed cytologically by the Karyopyknotic and Maturation indices (Segal et al, 1984a). Pregnant and diabetic women were found to have low Karyopyknotic values which indicates an increase in the number of intermediate epithalial cells rather than superficial and parabasal vaginal cells.

(iii) Effect of commensal bacteria on adherence of C. albicans

In the natural environment a mixed bacterial flora is present which may compete for adherence sites on epithelial cells. Liljemark and Gibbons (1973) found that C. albicans attached in lower numbers to epithelial cells from the tongue or cheek of conventional rats than to those from germ-free rats. Mouth and gut colonization by C. albicans in gnotobiotic mice was suppressed by mixed human salivary bacteria and strains of S. salivarius and S. miteor. Commensal bacteria such as E. coli and Klebsiella aerogenes enhance C. albicans adherence to HeLa cells. S. sanguis and S. miteor decreased adherence and Streptococcus milleri had no effect on candidal adherence (Makrides and MacFarlane, 1982). Attachment of C. albicans to vaginal cells was also influenced by the

presence of the normal flora (eg lactobacilli), which may block epithelial cell receptors (Sobel et al, 1981).

The effects of D-mannose and D-glucose, E. coli and Klebsiella pneumoniae on adherence of C. albicans to epithelial cells was investigated by Centeno et al (1983). Glucose had no effect on yeast attachment, but mannose decreased adherence of both yeasts and piliated bacteria while it had no effect on the adherence of non-piliated K. pneumoniae. Adherence of C. albicans to epithelial cells was enhanced when the epithelial cells were preincubated with piliated strains of bacteria. Makrides and MacFarlane (1983) also found that increased adhesion of C. albicans to HeLa cells could be mediated by E. coli and concanavalin A. Possibly type I fimbriae of E. coli act as a bridging mechanism between the epithelial cells and C. albicans.

(iv) Effect of antibiotics on adherence of C. albicans

The effect of sub-inhibitory concentrations of antifungal agents on in vitro adherence of C. albicans to vaginal epithelial cells was studied by Sobel and Obedeanu (1983) who failed to demonstrate any direct effect. However, by inhibiting germ tube formation, ketoconazole indirectly reduced attachment of C. albicans to epithelial cells. Growth of the yeast form in the presence of sub-inhibitory concentrations of ketoconazole resulted in clumping and enhanced adherence, although the numbers of yeasts in direct contact with the epithelial cell was no greater.

The ability of cerulenin-resistant mutants of C. albicans to adhere to buccal epithelial cells was investigated by Cihlar et al (1984). The mutant strains were less adherent than the wild type strains. Changes in cell-wall proteins occurred and a new protein appeared in the mutant. The relationship of the protein changes to altered adherence properties remains to be established.

(v) Adherence to fibrin-platelet matrices

Infective endocarditis is characterized by the colonization of heart valves by C. albicans. Maish and Calderone (1980) examined the adherence of different Candida species to fibrin-platelet matrices fromed in vitro. C. albicans and C. stellatoidea were highly adherent, whereas C. krusei, C. guilliermondii and S. cerevisiae adhered less readily. Maisch and Calderone (1981) suggested that surface mannan played a role in the adherence of C. albicans to fibrin-platelet clots.

After yeasts adhere to traumatized heart valves, aggregation of platelets occurs at the surface of the vegetation and yeasts grow within the vegetation. Platelet aggregation responses occurred by a complement-dependent mechanism and platelets promoted C. albicans germination (Skerl et al, 1981). Humoral antibody may protect against C. albicans endocarditis by inhibiting yeast adherence to heart valves (Scheld et al, 1983).

(vi) Adherence to endothelial surfaces

C. albicans and C. tropicalis were capable of adhering to and penetrating endothelial cell surfaces without forming germ tubes. Heat- or formalin-killed yeasts were also capable of adhering but incapable of vascular penetration (Klotz et al, 1983). Therefore, yeast viability appears to be necessary for invasion but not adhesion. A yeast adherence mechanism in the kidney is thought to play a role in initiating renal infections (Lee and King, 1983b). Barnes et al (1983) injected yeasts into the left renal artery of rabbits and within 5 min yeasts had localized within glomerular and pertubular capillaries. Numerous fibrils were observed by transmission electron microscopy, which projected in a radial array from the yeast to the endothelial surface.

(vii) Adherence to inert surfaces

C. albicans adheres to various inert surfaces. Yeasts grown in

mycological peptone containing sucrose were more adherent to acrylic than yeasts grown in peptone alone (Samaranayake and MacFarlane, 1980). This enhancement of adhesion was abolished if yeasts were heat-killed prior to incubation in sucrose-containing medium. McCourtie and Douglas (1981) found that adherence of C. albicans to acrylic in vitro was increased by growing yeasts in defined medium containing high concentrations of sugar, especially galactose, as the carbon source. Samaranayake et al (1980) found that adherence was increased by using serum-coated acrylic strips and by incubating the yeasts in sucrose, glucose and a dialysate of S. salivarius. Adherence was reduced by pre-coating the acrylic with mixed saliva, 2% chlorhexidine and S. salivarius.

The rate of adhesion of C. albicans to acrylic surfaces after growth in medium containing different carbon sources was determined by McCourtie and Douglas (1985b). They devised consecutive adhesion assays in which acrylic strips were removed from the yeast suspension after 15 min and replaced with a fresh strip. Yeasts grown on 500 mM sucrose, 50 mM glucose or 50 mM galactose showed non-linear adhesion rates. Populations of yeasts were isolated from these cultures which showed increased adhesion and increased resistance to spheroplast formation. This suggested an unequal distribution of adhesins among cells in such cultures. Growth of yeasts in medium containing 500 mM galactose showed constant adhesion throughout the assay indicating that the population had a homogenous distribution of adhesins between cells.

Minagi et al (1985) examined the adherence of C. tropicalis a relatively hydrophobic species and C. albicans, a hydrophilic species, to hydrophobic surfaces such as denture base resin materials. With increasing solid surface free energy, the adherence of C. albicans increased and adherence of C. tropicalis decreased because it had a

lower surface free energy than the resin material. These results have been confirmed by Klotz et al (1985). Candida species adhered to PVC and Teflon catheters, adherence being greater to PVC than to Teflon (Rotrosen et al, 1983); C. tropicalis was more adherent to both these surfaces than C. albicans.

D. Relationship between cell surface composition, adherence and pathogenicity of C. albicans

The enhanced adherence of C. albicans, after growth in defined medium containing high concentrations of galactose, sucrose, maltose and fructose as carbon sources, appears to be due to the production of an additional fibrillar surface layer (McCourtie and Douglas, 1981). Yeasts grown on 500 mM galactose were the most resistant to spheroplast formation with Zymolyase-5000 and the most adherent to acrylic. The additional surface layer which may be responsible for enhanced adhesion has been demonstrated by electron microscopy. Douglas and McCourtie (1981) found that yeasts in the exponential phase of growth were poorly adherent to acrylic and sensitive to Zymolyase. The adherence of strains isolated from active infections (I strains) and from asymptomatic carriers (C strains) to epithelial and acrylic surfaces has been compared by McCourtie and Douglas (1984). The adherence of I and C strains after growth in medium containing 50 mM glucose was similar and all strains were sensitive to Zymolyase. Growth in medium containing 500 mM galactose enhanced the adherence of the I strains by 5 to 11-fold with corresponding increases in resistance to spheroplast formation. C-strains showed only small increases in adherence, remaining sensitive to spheroplast formation.

When I strains, after growth on 500 mM galactose, were injected

intravenously into mice they showed a 5 to 24-fold increase in virulence compared with strains grown on 50 mM glucose. McCourtie and Douglas (1984) concluded that I strains were capable of modifying their surface composition in response to growth in media containing high concentrations of sugar. Such cell surface modification results in increased adherence and virulence.

E. Mechanism of adherence of C. albicans to epithelial cells

(i) Nature of the yeast adhesin

Studies on the nature of the yeast adhesin have involved attempts to antagonise or to stimulate adherence and to isolate the actual material involved. Several investigators have treated yeast cell surface components with a variety of enzymes and examined their effect on adherence. Pretreatment of C. albicans with trypsin, chymotrypsin, pronase and papain reduced adherence to vaginal epithelial cells (Lee and King, 1983a). Sobel et al (1981) found that chymotrypsin and trypsin significantly inhibited adherence when either C. albicans or vaginal epithetlial cells were preincubated with these enzymes. Treatment of yeasts with trypsin, but not chymotrypsin, inhibited contact between C. albicans and neutrophils (Diamond and Krzesicki, 1978). In contrast to these results, Rotrosen et al (1985) exposed yeasts to pronase and trypsin and found that adherence to cultured endothelial cells was enhanced.

Lee and King (1983a) treated yeast cells with detergents, salts, urea, glycosidases, lipases and pepsin and found that none of these treatments had any effect on adherence to vaginal epithelial cells. Cell-wall mannoprotein was thought to be an essential component of the yeast adhesin as adherence of cell-wall fragments was reduced after

treatment with α-mannosidase or papain. Tronchin et al (1984) also found that pronase and mercaptoethanol treatments of yeasts significantly reduced adherence.

Other studies on surface components involved in the adherence of microorganisms have consisted of specifically inhibiting in vitro microorganism-cell adhesion, usually with compounds which are structurally analogous to adhesins or host cell receptors. Sugar inhibition tests and lectins have been widely employed in determining the nature of adhesins or receptors. Sobel et al (1981) found that mannose, galactose or αmethyl-D-mannoside had no effect on adhesion, but L-fucose inhibited adhesion of C. albicans to vaginal epithelial cells. Segal et al (1982) found that amino sugars inhibited adhesion to vaginal cells and suggested that the C. albicans adhesin was chitin or a related compound. Amino sugars also inhibited adherence of C. albicans to buccal cells and corneocytes (Collins-Lech et al, 1984).

Lectins which are specific for sugar-containing moieties on the surfaces of yeasts or epithelial cells have been used to pretreat yeasts or epithelial cells prior to their use in adhesion assays. Pretreatment of yeasts with concanavalin A inhibited adherence to buccal cells; however adherence could be restored by pretreating the lectin with methyl-α-D-mannoside prior to use in the assay (Sandin et al, 1982; Sandin and Rogers, 1982). Concanavalin A had no effect on adherence of C. albicans to corneocytes (Ray et al, 1984) or on adherence to neutrophils (Diamond and Krzesicki, 1978).

McCourtie and Douglas (1981) found that the outer-floccular layer produced by yeasts after growth on high concentrations of galactose is largely composed of mannoprotein. This material is readily released from the cell surface and can be recovered from culture supernatant fluid.

This extracellular polymer inhibits adherence of C. albicans to buccal cells after pretreatment of epithelial cells prior to their use in the adhesion assay (McCourtie and Douglas, 1985a). Yeasts treated with the antibiotic tunicamycin at the end of the exponential phase of growth showed decreased adherence to buccal cells (Douglas and McCourtie, 1983). Tunicamycin specifically inhibits mannoprotein synthesis without affecting glucan synthesis; therefore, these results suggest that the extracellular polymer produced by C. albicans contains the yeast adhesin which is mannoprotein in nature.

(ii) Nature of the epithelial cell receptor

Epithelial cell membranes may have specific receptors for the surface adhesin components of yeasts. Sobel et al (1981) suggested that the epithelial cell receptor for C. albicans may be a glycoprotein as adherence was partially inhibited by L-fucose and D-fucose and by proteolytic enzymes. Sandin et al (1982) claimed that mannose-containing moieties on the epithelial cell surface may be involved as receptors. Buccal epithelial cells pretreated with concanavalin A had fewer yeasts attached to them than untreated cells. Skerl et al (1984) suggested that fibronectin might be the receptor on epithelial cells for C. albicans. They used indirect fluorescent anitbody techniques to show that fibronectin was present on epithelial cell surfaces. Fibronectin blocked adhesion of pretreated yeasts to both buccal and vaginal cells. The binding of C. albicans to fibronectin was similar to the binding to epithelial cells in that yeasts pretreated with proteolytic enzymes were less adherent to the glycoprotein and mannan blocked binding.

(iii) In vivo studies on the adherence of C. albicans

In vivo attachment of C. albicans to epithelial cells has been studied in an experimental model of murine vaginitis created by inoculating mice

intravaginally with yeasts during the oestrous phase of the cycle (Lehrer *et al*, 1983). Attachment of yeasts was assessed microscopically by examining Gram-stained vaginal smears. Previous studies had shown that chitin soluble extract (CSE) (Segal *et al*, 1982) and anti-*C. albicans* antibodies (Maisch and Calderone, 1980) inhibited *in vitro* adherence of *C. albicans*. Therefore, Lehrer *et al* (1983) attempted to block *in vivo* attachment by pretreating vaginas of mice with CSE or with N-acetyl-D-glucosamine, mannan or PBS. Mice pretreated with CSE or N-acetyl-D-glucosamine had lower infection rates than those pretreated with mannan and PBS. Segal *et al* (1984b) found that CSE from *C. albicans* was effective at a much lower concentration than the preparation of CSE from commercially-prepared chitin. They found that blocking the attachment of the yeasts to the epithelial cells resulted in a reduction of the development of an infection.

OBJECT OF RESEARCH

The mechanism of adherence of *Candida albicans* to mucosal surfaces has yet to be determined. It is thought that yeast adherence involves a specific interaction between fungal adhesins and epithelial cell receptors. Previous work has shown that enhanced adherence of this organism to buccal cells *in vitro* is increased after growth in medium containing a high concentration of galactose. This enhanced adherence appears to be due to the production of an additional fibrillar layer of mannoprotein on the yeast cell surface. Electron microscopy indicates that this layer is released from the cell surface and this polymeric material can be recovered from culture supernatant fluids.

The aims of this project were to:

(i) isolate and fractionate extracellular polymeric material (EPM) and to test components for adherence inhibition

(ii) determine the minimum structure of EPM necessary for adherence inhibition by chemical and enzymic dissection

(iii) characterize the epithelial cell receptor involved in yeast adherence by using sugars and lectins, as receptor analogues, to inhibit adherence.

This information may lead to more efficient procedures in the prevention of infections.

MATERIALS AND METHODS

I ORGANISMS

Twelve different strains of C. albicans, five different species of Candida and one strain of Saccharomyces cerevisiae were used. C. albicans strains GDH 2346 (NCYC 1467) and GDH 2023 (NCYC 1468) were isolated at Glasgow Dental Hospital from patients with denture stomatitis. C. albicans strains GRI 681 (NCYC 1472) and GRI 682 (NCYC 1473) were obtained from routine cervical smears taken from asymptomatic women at Glasgow Royal Infirmary. These strains have been deposited with the National Collection of Yeast Cultures, Food Research Institute, Norwich, England.

C. albicans 'outbreak strain' (serotype A), C. albicans 'systemic strain' (serotype B), C. tropicalis (London strain) and C. parapsilosis were all isolated from patients with systemic candidosis at the London Hospital Medical College, London, England, and were kindly supplied by Dr. J.P. Burnie.

C. stellatoidea, C. tropicalis (Glasgow strain), C. pseudotropicalis and C. guilliermondii were isolated from clinical specimens and were obtained from the Medical Mycology Unit, Anderson College, University of Glasgow. Saccharomyces cerevisiae X2180-1A(a) was obtained from Dr. C.E. Ballou, Department of Biochemistry, University of California, Berkeley, CA.

C. albicans B2630 was a laboratory reference strain, C. albicans AD, KB and Darlington were isolated from patients suffering from chronic mucocutaneous candidosis and were kindly supplied by Dr. J.F. Ryley, ICI Pharmaceuticals Division, Alderley Park, Cheshire. The origin of these strains are summarized in Tables 2 and 3.

The organisms were maintained in slopes of Sabouraud dextrose agar (Difco) and subcultured monthly. Every two months the cultures were

Table 2 Origin of *Candida albicans* strains

Strain	Source of strain	Type of infection	Site of infection/ isolation
GDH 2346	Glasgow Dental Hospital	Denture stomatitis	Oral cavity
GDH 2023	Glasgow Dental Hospital	Denture stomatitis	Oral cavity
MRL 3153	Mycological Reference Laboratory	Denture stomatitis	Oral cavity
MRL 3156	Mycological Reference Laboratory	Not known	Not known
GRI 681	Glasgow Royal Infirmary	None[a]	Cervix
GRI 682	Glasgow Royal Infirmary	None[a]	Cervix
'Outbreak' strain (serotype A)	London Hospital Medical College	Systemic candidosis	Oral cavity
'Systemic' strain (serotype B)	London Hospital Medical College	Systemic candidosis	Oral cavity
B2630	Janssen	Not known	Not known
AD	Not known	CMC[b]	Oral cavity
KB	Not known	CMC[b]	Oral cavity
Darlington	Not known	CMC[b]	Not known

[a]These strains were isolated from routine cervical smears taken from symptomless women

[b]CMC, chronic mucocutaneous candidosis

Table 3 Origin of *Candida* and *Saccharomyces* species

Species	Source of yeast	Type of infection	Site of infection/ isolation
Candida tropicalis (Glasgow strain)	Medical Mycology Unit, Glasgow University	Clinical isolate	Sputum
Candida tropicalis (London strain)	London Hospital Medical College	Systemic candidosis	Blood
Candida stellatoidea	Medical Mycology Unit, Glasgow University	Clinical isolate	Sputum
Candida parapsilosis	London Hospital Medical College	Systemic candidosis	Blood
Candida pseudotropicalis	Medical Mycology Unit, Glasgow University	Clinical isolate	Sputum
Candida guilliermondii	Medical Mycology Unit, Glasgow University	Clinical isolate	Sputum
Saccharomyces cerevisiae X-2180	Dept. Biochemistry, University of California, Berkeley	-	-

replaced by new ones freshly grown from freeze-dried stocks. The identity of isolates were checked using the germ-tube, chlamydospore formation and sugar fermentation tests.

II GROWTH CONDITIONS

In all experiments, organisms were exclusively in the budding yeast phase.

(i) For freeze-drying

For freeze-drying, yeasts were grown in yeast nitrogen base (Difco) containing 500 mM sucrose. Batches of medium (50 ml, in 250 ml Erlenmeyer flasks) were inoculated with overnight yeast cultures (5 ml) and incubated at 37°C in an orbital shaker operating at 150 rev min^{-1}. Cells were harvested after 24 h (MSE bench centrifuge : 5 min, 1200 x g) and washed in sterile 0.15M phosphate-buffered saline (pH 7.2; PBS). Yeast cells were resuspended in a small volume of 2% (w/v) skimmed milk and a drop of suspension was added to a sterile glass ampoule. Ampoules were frozen before being dried in a centrifugal freeze-drier (Edwards High Vacuum Ltd., Sussex).

(ii) For adherence assays

Batches of medium (50 ml, in 250 ml Erlenmeyer flasks) containing yeast nitrogen base (YNB) and 50 mM glucose or 500 mM galactose were inoculated from stock cultures and incubated overnight at 37°C on an orbital shaker operating at 150 rev min^{-1}. The overnight culture (5 ml) was used to inoculate YNB medium (50 ml) containing the same carbon source as before. This culture was incubated at 37°C on an orbital shaker at 150 rev min^{-1} for 24 h so that yeast cells reached the stationary phase of growth. Cells were harvested by centrifugation (MSE bench centrifuge : 5 min, 1200 x g) and washed twice in 0.15M PBS (pH 7.2).

(iii) For isolation of extracellular polymeric material

Yeasts were grown in YNB medium containing 500 mM galactose as the carbon source. Batches of medium (500 ml, in 2 l Erlenmeyer flasks) were inoculated with 50 ml of an overnight culture and incubated at 37°C on an orbital shaker operating at 150 rev min^{-1} for 5 d.

III ADHERENCE ASSAYS

A. Adherence to human buccal epithelial cells

(i) Collection and preparation of buccal cells

Buccal epithelial cells were collected from a single donor by gently swabbing the inside of the cheeks with a sterile swab. The swab was agitated in PBS in a universal. Epithelial cells were harvested by centrifugation (MSE bench centrifuge : 5 min, 1200 x g) and washed twice in PBS to remove unattached microorganisms. After the second washing epithelial cells were standardised using an improved neubauer haemocytometer before resuspending in PBS at a concentration of 1×10^5 cells ml^{-1}. Buccal cells were always collected at the same time of day to minimise variability.

(ii) Preparation of yeast cells

After the second washing of the yeasts in PBS, yeast suspensions were standardized using an improved Neubauer haemocytometer. The amount of PBS added was adjusted so that a final yeast cell concentration of 1×10^7 cells ml^{-1} was obtained.

(iii) Adherence assay

The method used was based on that described by Douglas et al (1981). Epithelial and yeast cell suspensions were vortexed using a whirlimixer

before being used in adherence assays. Standardized suspensions of buccal epithelial cells (1×10^5 cells ml^{-1} in PBS; 0.1 ml) and yeasts (1×10^7 yeasts ml^{-1} in PBS; 0.1 ml) were mixed in small screw-capped bottles and incubated at 37°C, with gentle shaking, for 45 min. Control mixtures were set up containing epithelial cells and PBS. After incubation, 2 ml of PBS was added to each bottle to stop any further attachment. The epithelial cells were collected on polycarbonate filters (12 μm pore size, 25 mm diameter; Nucleopore Corp., Pleasanton, CA) and washed with PBS (30 ml) to remove unattached yeasts. The washed filters were placed on labelled slides and left for a few minutes to air dry. Epithelial cells on the filters were fixed with absolute methanol and stained using the Gram procedure. After drying, filters were mounted under coverslips using DPX mountant (BDH Chemicals Ltd). The numbers of adherent yeasts on each of 100 epithelial cells were counted on every filter. Triplicate filters were prepared for each assay. All adherence values quoted represent mean figures from 3 independent assays.

B. Adherence to human vaginal epithelial cells

Vaginal mucosal epithelial cells were obtained from a healthy female volunteer. Swabs were gently agitated in universals containing PBS. Epithelial cells were standardized to a concentration of 1×10^5 cells ml^{-1} using an improved Neubauer haemocytometer. Vaginal cells were washed twice in PBS and extensively vortexed using a whirlimixer before use in adherence assays. Yeast cells were standardized to a concentration of 1×10^7 cells ml^{-1} and adherence assays were performed as for buccal epithelial cells.

C. Adherence to vaginal epithelial cells from mice

Twenty female mice (six weeks old) were swabbed for vaginal epithelial cells using small sterile cotton wool swabs. The swabs were gently agitated in universals containing PBS (5 ml). The vaginal epithelial cells were harvested by centrifugation (MSE bench centrifuge : 5 min, 1200 x g) and resuspended in PBS (1 ml) to increase cell concentration. The cells were examined by light microscopy in a counting chamber. Mice from which high yields of epithelial cells were obtained were concluded to be in the oestrous stage of the oestrous cycle. These mice were then re-swabbed 5 d later for epithelial cells, the cycle having returned to the oestrous stage. Vaginal epithelial cells from these mice were pooled, by agitating the swab in a universal containing PBS (5 ml), so that sufficient numbers of epithelial cells were present for use in adherence assays. Vaginal epithelial cells were washed twice in PBS and standardized using an improved Neubauer haemocytometer so that a final epithelial cell concentration of 1×10^5 cells ml^{-1} was obtained. Yeasts were used at a concentration of 1×10^7 cells ml^{-1} and adherence assays were performed as described for human buccal epithelial cells.

D. Adherence to acrylic

(i) Preparation of acrylic

The method used was that described by Samaranayake and MacFarlane (1980). Acrylic monomer (methyl methacrylate) and polymer (Simplex Rapid; Howmedica International, 622 Western Ave, London) were mixed on aluminium foil-covered glass slides (0.5 g acrylic powder and 0.5 ml monomer liquid). The aluminium foil was used to aid recovery of acrylic from between the glass slides. After 45 s another glass slide was placed on top of the polymerizing mixture and the slides were secured at the edges with

crocodile clips. The acrylic was left to polymerize in warm water (40°C) for 5 min; this reduces bubbles within the acrylic and increases its clarity. The acrylic sheet was then washed for 4 h in running tap water to remove any monomer and the sheet was cut into 5 by 5 mm square strips.

(ii) Preparation of yeast suspension

Yeasts were grown in the same way as for adherence assays with buccal epithelial cells. A yeast cell concentration of 1×10^7 yeasts ml^{-1} was used.

(iii) Adherence assay

Acrylic strips were placed vertically in wells of a flat-bottomed serology plate (Nunc). Yeast suspension (0.4 ml) was added to each well and the plate was shaken gently for 60 min at room temperature on an orbital shaker. The strips were removed and washed for a total of 75 s in 5 changes of PBS. Yeasts were fixed in 95% methanol for 10 min, and rehydrated for 5 min in 50% methanol and then in water before staining. Yeasts were stained by suspending the strips in crystal violet for 10 min and then in Grams iodine for 5 min before washing with water. After drying the strips were mounted under coverslips on glass slides using DPX mountant. The numbers of yeasts adhering to 1 mm^2 of acrylic was determined by light microscopy. All adherence values quoted were obtained counting 10 fields on each acrylic strip which corresponds to an area of 1.1 mm^2 using a Vickers microscope at X400 magnification. All adherence assays were performed three times in triplicate.

IV ISOLATION AND CHARACTERIZATION OF ADHESIN COMPONENT(S)

A. Isolation of extracellular polymeric material

After growth for 5 d cells were harvested by centrifugation and the culture

supernatant fluid (500 ml) was extensively dialysed in cold distilled water (12 l) for a week. The distilled water was changed at least 5 times during the week. The dialysed culture supernatant fluid was then freeze-dried (Multi-dry freeze-drier, FTS Systems Inc). To determine the yield of EPM, the dry weight of cells was determined by collecting cells (1 ml) on preweighed cellulose acetate membrane filters (Oxoid, 0.45 μm). The filters were placed in petri dishes and left to dry to constant weight at 37°C for 24 h; the filters were then reweighed to give an estimate of cell dry weight. The weight of freeze-dried EPM was also recorded so that the yield of EPM could be calculated.

B. Biochemical analysis of extracellular polymeric material

(i) Carbohydrate

Carbohydrate was estimated by the method of Dubois et al (1956) using mannose as a standard (see appendix IA).

(ii) Protein

Protein was determined using the method of Lowry et al (1951) with bovine serum albumin as standard (see appendix IB).

(iii) Phosphorus

Phosphorus content of EPM was estimated according to the method of Chen et al (1956) using KH_2PO_4 as standard (see appendix IC).

(iv) Glucose

Glucose content of EPM was determined using a Boehringer GOD-Perid test combination kit (Boehringer, W. Germany). Prior to glucose analysis, samples (0.2 ml of a 2 $mgml^{-1}$ solution of EPM) were hydrolysed in 1 N HCl (0.2 ml) in sealed ampoules at 100°C for 5 h and neutralised with 0.5N NaOH, as described by McCourtie and Douglas (1985a). Enzyme solution (5 ml) was added to the hydrolysate (0.2 ml). The samples were incubated

in a water bath at 20-25°C, avoiding direct exposure to sunlight. After 20 to 50 min the extinction of samples was measured at 610 nm using a Unicam SP6-550 spectrophotometer. A standard curve was produced using glucose.

C. Column chromatography

(i) Gel filtration

A column with bed dimensions 58 cm x 2.6 cm was used. The column was packed with Sephadex G-200 (Sigma) and equilibrated with 0.15M phosphate buffer (pH 7.2) containing 0.1% sodium azide by extensively washing the gel with this buffer. Blue dextran 2000 (Pharmacia; 2 $mgml^{-1}$) was applied to the column to determine the void volume. Blue dextran was eluted with buffer and the volume of buffer collected before its elution was regarded as the void volume.

Freeze-dried EPM (50-100 mg in 2 ml buffer) was applied to the column and eluted with phosphate buffer, pH 7.2. Flow was in an upward direction, achieved by means of a peristaltic pump (LKB instruments). Fractions (3.2 ml) were collected at a flow rate of 12 mlh^{-1} using an LKB Ultrarac 7000 fraction collector. The absorbance of all fractions at 280 nm was measured using a Unicam SP6-550 spectrophotometer. Fractions were analysed for protein, carbohydrate and phosphorus. After biochemical analysis, appropriate fractions were pooled, dialysed against distilled water and concentrated using aquacide IIA (Calbiochem).

To determine the molecular weight of components eluted from the Sephadex G-200 column, standards (10 mg in 2 ml of buffer) of known molecular weight were applied to the column and eluted with phosphate buffer. Fractions (3.2 ml) were collected at a flow rate of 12 mlh^{-1} and monitored at 280 nm using a Unicam SP6-550 spectrophotometer.

(ii) Ion-exchange chromatography

A column with bed dimensions 58 cm x 2.6 cm was used. The column was packed with DE52, a preswollen, pre-cycled diethylaminoethyl-cellulose derivative (Whatman Ltd) and equilibrated with several litres of 0.15M phosphate buffer (pH 7.2). Freeze-dried EPM (100 mg) in 0.15M phosphate buffer was applied to the column. Fractions (3.2 ml) were eluted with a linear gradient (0-0.5M) NaCl in the starting buffer at a flow rate of 20 mlh^{-1} and collected using an LKB Ultrarac 7000 fraction collector. Absorbance of eluted fractions was monitored at 280 nm using a Unicam SP6-550 spectrophotometer. Fractions were biochemically analysed and concentrated as for gel filtration samples.

(iii) Affinity chromatography with Concanavalin A-Sepharose

A column with bed dimensions 1.5 cm x 10.7 cm was used. The column was packed with Concanavalin A-Sepharose (Sigma) and equilibrated with at least 10 column volumes of PBS (0.15M; pH 7.2) as starting buffer. When not in use the column was washed with preservation buffer which was 0.1M acetate buffer (pH 6.0) containing 1 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$ and 0.02% (w/v) merthiolate. Freeze-dried EPM (30 mg in 5 ml PBS) was applied to the column and eluted with PBS until the absorbance at 280 nm of the eluate was negligible. Bound substances were eluted using 0.5M methyl-α-D-mannoside. The flow rate was 34 mlh^{-1} and 3.0 ml fractions were collected. After biochemical analysis appropriate fractions were pooled, dialysed against distilled water and concentrated using aquacide IIA (Calbiochem).

D. Electrophoresis

(i) Laemmli method (Laemmli, 1970)

The procedures for preparing stock solutions are given in appendix II.

Gel plates were prepared by sealing three sides with special adhesive tape and sealing the corners by dipping the plates in molten paraffin wax. The separating gel solutions containing 11% acrylamide, 0.375M Tris-HCl (pH 8.8), 0.1% SDS, 0.025% TEMED and 0.08% ammonium persulphate, was poured into the gel plates to about 5.5 cm from the bottom edge. Tris-HCl buffer (0.375M; pH 8.8) containing 0.1% SDS was used to overlay the separating gel which was allowed to polymerise. After polymerization, the overlay solution was removed from the separating gel. Stacking gel solution containing 5% acrylamide, 0.125M Tris-HCl (pH 6.8), 0.1% SDS, 0.025% TEMED and 0.08% ammonium persulphate, was used to fill the gel plates to within 3 to 4 mm of the top. Sample combs were suspended in the stacking gel solution and any remaining space was filled with gel solution. This was allowed to polymerize and combs were then removed under running buffer. Gels were also prepared with 5 and 7.5% acrylamide in the resolving gels.

Tris-glycine buffer (pH 8.3) containing 0.1% SDS was used to fill the lower electrode vessel. The sealing tape was removed from the gel plates and the plates were inserted into the top electrode vessel using a liberal amount of grease around the gasket to prevent leakage. The electrode vessel was placed into position and filled with buffer. Samples of EPM (2, 3.5 and 5 $mgml^{-1}$; 25-50 µl) treated with solubilizing buffer (see appendix II) were carefully pipetted into the sample wells. Gels were run at constant current (15 mA per gel) for approximately 2.5 h. When tracking dye had reached the end of the gel the power was switched off.

(a) Coomassie blue staining (Weber and Osborn, 1969)

Gels were carefully removed from the plates and immersed in fixing solution (see appendix II) for 60 to 90 min. Gels were destained by

several changes of destaining solution (see appendix II). Molecular weights of unknown bands could be estimated by interpolation on the scale provided by marker proteins or glycoproteins.

(b) Periodic acid-Schiff staining (Huang and Evans, 1973)

Gels were fixed in 12% (w/v) tricarboxylic acid (TCA) and left to soak overnight in 5% (v/v) acetic acid. They were then immersed in 1% periodic acid (dissolved in 3% acetic acid) for 90 min and washed repeatedly with distilled water to remove the acid. Gels were finally soaked in Schiff's reagent for 1 h to visualize bands.

(c) Silver staining (Oakley et al, 1980)

Silver stain was used to detect carbohydrate components. Gels were handled with rinsed plastic gloves and gels were agitated at most of the stages of the staining procedure. Gels were prefixed in 50% methanol and 10% acetic acid for 30 min, then in 5% methanol and 7% acetic acid for 30 min. Glutaraldehyde (10%) was used to fix gels for 30 min. After fixing, gels were rinsed in a large volume of distilled water overnight and then left to soak in dithiothreitol (5 $\mu g ml^{-1}$) for 30 min without shaking. This solution was poured off and 0.1% (w/v) silver nitrate was added without rinsing and was left for 30 min. Gels were rinsed rapidly in distilled water and then twice rapidly in developer, which consisted of 37% formaldehyde (50 μl) in 100 ml of 3% Na_2CO_3, before being left to soak in the third volume of developer until the desired level of staining was reached. Development was stopped by adding 2.3M citric acid (5 ml) and mixing for 10 min. Gels were washed in distilled water several times for 30 min, then soaked in 0.03% Na_2CO_3 for 10 min before drying.

(d) Silver stain adapted for staining lipopolysaccharide (LPS)

EPM samples run on gels were stained using the LPS silver stain method (Tsai and Frasch, 1982). To prevent staining artefacts on the gel surface,

the gel plates were washed with detergent, thoroughly rinsed with water and wiped clean with paper tissues soaked in ethanol. The staining trays were cleaned using concentrated nitric acid and were thoroughly rinsed with distilled water. Rinsed plastic gloves were worn when handling gels to prevent fingerprints. Mild agitation (40 rev min^{-1}) on an orbital shaker was applied to each step in the staining procedure except the overnight fixation step. Gels were fixed in 40% ethanol, 5% acetic acid overnight. The fixing solution was replaced with 0.7% periodic acid in 40% ethanol and 5% acetic acid and left for 5 min; the gels were then washed three times for 15 min in distilled water. The staining reagent was prepared as described in the appendix. Staining reagent (150 ml) was added and gels were shaken vigorously for 10 min. After staining, the gels were washed in water for 10 min at least three times. The water was replaced by formaldehyde developer (50 mg citric acid and 0.5 ml formaldehyde per litre). Gels stained dark brown within 2-5 min and development was terminated when the desired level of staining had been attained.

(ii) SDS Gel electrophoresis using a continuous buffer system

The procedures used for preparing stock solutions are given in appendix II. The method used was that of Segrest and Jackson (1972). The samples were loaded directly onto the resolving gel. EPM samples were dissolved in 1% SDS containing 0.01M dithiothreitol (for sulphydryl bond reduction) (0.5 ml) and incubated for 1 h at 37°C. Before electrophoresis, an equal volume of 0.002M sodium phosphate buffer (pH 7.1) in 8M urea was added to give a final SDS concentration of 0.5% (0.5 ml). Samples of EPM, 2, 3.5 and 5 $mgml^{-1}$, were applied to the gels and the tracking dye was electrophoresed to approximately 1 cm from the bottom of the gel.

E. Immunological studies

(i) Preparation of antisera

Freeze-dried EPM (20 mg) from C. albicans GDH 2023 was dissolved in sterile saline (0.5 ml). This was then mixed thoroughly with 0.5 ml Bayol F (kindly supplied by Dr. D.E.S. Stewart-Tull) containing 250 μg freeze-dried Mycobacterium tuberculosis and Arlacel A (Honeywell-Atlas, Carshalton, Surrey). EPM in adjuvant (1 ml) was injected intramuscularly into the inner aspect of the hind limb of a rabbit. After 28 d the rabbit was boosted with EPM (2 ml) without M. tuberculosis (incomplete adjuvant) by injecting intraperitoneally. After a further 10 d the rabbit was bled from the ear and serum was tested for antibody production by the Ouchterlony method and by slide agglutination. After these tests proved positive, the rabbit was bled by cardiac puncture and serum collected by centrifugation at 1200 x g (MSE bench centrifuge).

(ii) Double diffusion tests

The Ouchterlony slide method was used (Ouchterlony, 1958). For further details of the procedure used see appendix III. Microscope slides were covered in 2% (w/v) agarose (2.5 ml) which was then allowed to set. Wells were cut using a punch (2 mm in diameter and 4 mm apart). Wells were filled with antiserum or antigen (10 $mgml^{-1}$). Slides were incubated in a moist atmosphere (a petri dish containing dampened Kleenex tissues) for 48 h at $37^{o}C$.

(iii) Agglutination reactions

C. albicans GDH 2023 and C. albicans GDH 2346 were grown for 24 h in YNB containing 500 mM galactose. Antiserum was prepared to EPM from galactose-grown C. albicans GDH 2023. The yeast cells were washed in saline and standardized so that they contained 1×10^{7} yeasts ml^{-1} using

an improved Neubauer haemocytometer. Doubling dilutions of antiserum in saline (50 µl) were prepared using a microtitre tray (Sterilin, Middlesex). *C. albicans* (50 µl) suspended in saline was added to each well. A control was set up containing 50 µl yeast suspension and 50 µl saline. The microtitre trays were shaken by sideways agitation and incubated at 37°C for 1 h. The endpoint of the titration was recorded as the highest dilution of antiserum which gave visible agglutination of yeast cells. All agglutination assays were performed in duplicate and the mean titre determined. The microtitre trays were then incubated at 4°C overnight to check for any increases in titre.

V. ADHERENCE STUDIES

A. Use of inhibitors to block in vitro adherence

(i) Extracellular polymeric material

Epithelial cell suspensions (1 ml, containing 1×10^5 cells ml^{-1}) were centrifuged in a Beckman microfuge B at 8740 g for 1 min and the supernatant discarded. Crude EPM samples (10 mg in 1 ml PBS) were added to the cell pellets and the cells were mixed using a whirlimixer. Epithelial cells were incubated with the EPM solution for 30 min at 37°C on an orbital shaker. After this treatment, epithelial cells were recovered by centrifugation, resuspended in PBS (1 ml) and used in adherence assays with *C. albicans*.

(ii) Lectins

All lectins were used at a concentration of 100 $\mu g ml^{-1}$ in 0.05M Tris-saline (pH 7.2), containing 0.002M $CaCl_2$, $MnCl_2$ and $MgCl_2$. Concanavalin A (Con A), *Arachis hypogea* lectin (Peanut lectin), *Lens culinaris* lectin (Lentil lectin), *Lotus tetragonolobus* lectin (Winged pea lectin) and

Triticumvulgaris lectin (Wheat germ agglutinin) (all Sigma) were the lectins used to pretreat epithelial cells in this study. The sugar specificities of each lectin are given in Table 4.

Epithelial cells (1 ml, containing 1 x 10^5 cells ml^{-1}) were harvested in a Beckman microfuge B at 8740 x g for 1 min. Cells were suspended in lectin solution (1 ml) and incubated for 30 min at 37°C on an orbital shaker operating at 150 rev min^{-1}. After incubation, epithelial cells were centrifuged and resuspended in 0.15M PBS (1 ml, pH 7.2). The lectin-pretreated epithelial cells were used in adherence assays as described for buccal epithelial cells.

(iii) Human plasma fibronectin

Fibronectin (kindly supplied by Mrs. A. Lancaster and Mr. M. Achison, Microbiology Dept., Glasgow University) isolated from human plasma by affinity chromatography using gelatin-Sepharose was used to pretreat epithelial cells prior to their use in adherence assays. Epithelial cells (1 ml, containing 1 x 10^5 cells ml^{-1}) were harvested by centrifugation using a Beckman microfuge B at 8740 x g for 1 min. Fibronectin (1 ml) in 0.05M Tris-saline buffer (pH 7.0) was added to the cell pellet. Epithelial cells were incubated in the fibronectin solution (in microfuge tubes) for 30 min at 37°C on a rotating wheel. After incubation epithelial cells were centrifuged and resuspended in 0.15M PBS (pH 7.2).

(iv) Sugars

All sugars were used at a concentration of 25 $mgml^{-1}$. Sugars used were: N-Acetyl-D-glucosamine, D-Fucose, L-Fucose, D-Galactose, D-Glucose, D-Glucosamine and D-Mannosamine; all were obtained from Sigma. Epithelial cells (1 ml, containing 1 x 10^5 cells ml^{-1}) were centrifuged using a Beckman microfuge B at 8740 g for 1 min and the supernatant removed. Sugar solution (1 ml, containing 25 $mgml^{-1}$) was added to the cell pellet.

Table 4 Lectins used to pretreat epithelial cells and their sugar specificities

Lectin	Sugar specificity
Arachis hypogeae (Peanut)	D-Galactose
Canavalia ensiformis (Jack bean)	D-Mannose, D-Glucose
Lens culinaris (Lentil)	D-Mannose, D-Glucose
Lotus tetragonolobus (Winged pea)	L-Fucose
Triticum vulgaris (Wheat germ)	N-Acetyl-D-glucosamine

Yeast cells (1 ml, containing 1 x 10^7 cells ml^{-1}) were centrifuged using a Beckman microfuge B for 1 min. Sugar solution (1 ml) was added to the yeast pellet. All cells were vortexed using a whirlimixer before using in adherence assays. Adherence assays were performed as described previously, using yeasts and epithelial cells suspended in sugar solutions.

B. Determination of the minimum structure of EPM required for inhibition of adherence

(i) Physical treatment

Samples of EPM (10 $mgml^{-1}$ in PBS) were heated at 50°C, 80°C and 100°C for 15 min. Heat-treated samples were used to pretreat buccal epithelial cells for 30 min at 37°C as described above in section A(i). After pretreatment, buccal cells were removed by centrifugation, resuspended in PBS and used in adherence assays as previously described.

(ii) Chemical treatment

(a) Mild alkali

Crude EPM (20 mg) was added to 0.1N NaOH (1 ml) and incubated at 25°C for 24 h. The sample was neutralized with 0.1N HCl (1 ml) to give a final concentration of 10 $mgml^{-1}$.

(b) Mild acid

Crude EPM (20 mg) was added to 0.01N HCl (1 ml), sealed in an ampoule and incubated in a water bath at 100°C for 30 min. The sample was neutralized by adding 0.01N NaOH (1 ml) to give a final concentration of 10 $mgml^{-1}$.

(c) Sodium periodate

Crude EPM samples were dissolved in 0.05M sodium acetate buffer (pH 4.5) to a concentration of 5 $mgml^{-1}$. Two solutions were prepared and sodium periodate was dissolved in each to give a final concentration of 0.02M

and 0.05M respectively. These solutions were incubated in the dark at $4^{o}C$ for 30 min. The activity of the periodate was inhibited by the addition of ethylene glycol (10 ml). An excess of acetone was added to each solution to precipitate EPM. The precipitate was recovered by centrifugation (MSE bench centrifuge) and the supernatant discarded. Precipitation and centrifugation was repeated to remove traces of ethylene glycol. The precipitate was left to dry in a dessicator. For adherence assays a concentration of 10 $mgml^{-1}$ was used to pretreat epithelial cells.

(d) Dithiothreitol

Crude EPM (10 mg) was dissolved in 0.05M Tris buffer (pH 7.2; 2 ml). Dithiothreitol (Sigma) was added to give a final concentration of 0.012M. The sample was incubated at $37^{o}C$ for 60 min before being used to pretreat buccal epithelial cells prior to adherence assays.

(iii) Enzymic treatment

(a) Endoglycosidase H

EPM preparations (1 $mgml^{-1}$) were treated with 1 μg of endoglycosidase H, since 1 mg of glycoprotein requires 1 μg endoglycosidase H. The pH value of the samples was adjusted to 5-6, the optimum pH of the enzyme, and the samples were treated with endoglycosidase H for 2 h at $37^{o}C$.

Enzyme-treated samples were applied to a column of Concanavalin A-Sepharose. Protein components cleaved from the EPM were eluted with 0.15M PBS (pH 7.2). The bound carbohydrate components of EPM were eluted from the column with 0.5M methyl-α-D-mannoside. These fractions were freeze-dried (Multi dry freeze-dryer, FTS Systems Inc.) and reconstituted in PBS before being used to pretreat buccal epithelial cells prior to their use in adherence assays.

(b) Proteolytic enzymes

Trypsin, chymotrypsin, protease (from Streptomyces griseus; Pronase E)

and papain (Sigma) were used at a concentration of 0.1 $mgml^{-1}$ in 0.01M phosphate buffer (pH 7.2), except papain which was prepared in 0.01M phosphate buffer (pH 6.2). EPM samples (10 mg in 1 ml PBS) were treated with enzymes for 30 min at 25°C. After incubation an equivalent concentration of enzyme inhibitor was added (trypsin inhibitor for trypsin; chymotrypsin inhibitor for chymotrypsin; α_2-macroglobulin for papain and pronase). All inhibitors were assessed separately for their effects on adherence, by incubating EPM samples with the enzyme inhibitor alone, which were found to be negligible. EPM samples treated with the proteolytic enzymes were used to pretreat buccal epithelial cells prior to their use in adherence assays with C. albicans.

(c) α-Mannosidase and bromelain

Crude EPM (10 mg) was treated with α-mannosidase (1 $mgml^{-1}$) in 0.04M acetate buffer (pH 4.5) for 60 min at 37°C. The same procedure was used for bromelain treatment. Enzyme-treated EPM samples were used to pretreat buccal epithelial cells prior to their use in adherence assays. Controls were set up which involved pretreating epithelial cells with α-mannosidase alone.

(iv) Isolation of 'lectin-like' components from EPM

Sugars which are known to be present on the surfaces of mammalian cells were coupled to epoxy-activated Sepharose (Sigma). The coupling procedure involved swelling epoxy-activated Sepharose (1 g) in distilled water for 15 min. The gel was washed on a sintered glass filter (G-3) with distilled water (100 ml). The ligand solution (50 $mgml^{-1}$ of sugar; 3 ml) was mixed with the gel suspension and incubated for 16 h at 25-40°C using a shaking water bath. Excess ligand was washed away on a sintered glass filter with distilled water (100 ml), bicarbonate buffer (0.1M; pH 8.0; 100 ml) and acetate buffer (0.1M; pH 4.0; 100 ml). Excess groups were

blocked with 1M ethanolamine (5 ml) overnight. The ligand spacer-conjugate was then washed with buffer (PBS) and stored at 4-8^{o}C until ready for use.

Crude EPM (10 mgml^{-1}; 1 ml) was applied to a mini column of epoxy-activated Sepharose coupled to a particular sugar. Sugars coupled to the Sepharose were: D-mannose, L-fucose, D-galactose, N-acetyl neuraminic acid (sialic acid), N-acetyl-D-glucosamine and N-acetyl-D-galactosamine (Sigma). The protein contents of crude EPM applied to the column and material eluted before the addition of the sugar solution were determined using the Lowry technique to give a measure of the proportion of protein applied to the column which binds. The eluted material was then discarded after the protein determination. Bound material was eluted using the sugar solution (25 mgml^{-1}; 5 ml) corresponding to the sugar coupled to the gel. The eluted samples were dialysed for 24 h against cold PBS (2 l) to remove the eluting sugar. The sample was then used to pretreat buccal epithelial cells prior to their use in adherence assays.

VI MOUSE MODELS

(i) Systemic infections

Yeasts were grown in YNB medium containing 50 mM glucose as described for *in vitro* adherence assays. The yeast cells were washed in sterile saline and standardized to a given concentration using an improved Neubauer haemocytometer. Suspensions of yeasts were prepared containg 10^7, 6×10^6, 3×10^6, 1×10^6, 3×10^5 and 1×10^5 in 0.2 ml. Mice (in groups of 20) of either sex weighing 25-35 g (Alderley Park strain) were injected intravenously with 0.2 ml of yeast suspension via the tail vein. Systemic infections were assessed by following mortality patterns over a time span

of several weeks or by performing kidney counts. Kidney counts were usually performed on mice injected with yeast suspensions containing 1×10^5 or 3×10^6 yeasts one week after infecting. Kidneys were removed by dissection and added to sterile saline (5 ml). The kidneys were homogenized and diluted by factors of 1/10 and 1/100 using sterile saline. The diluted samples (50 µl) were plated out on to SDA plates, containing 0.1% penicillin and streptomycin, using a spiral plater (Don Whitney Scientific Instruments Ltd.). All plates were incubated at 37°C for 24 h. The total number of yeast colonies on each plate was counted to assess the extent of systemic infection.

(ii) Vaginal infections

For vaginal infections yeasts were grown in YNB medium containing 50 mM glucose or 500 mM galactose as described for *in vitro* adherence assays. Female mice (10 per group) weighing 25-35 g (Alderley Park strain) were injected subcutaneously with 0.5 mg oestradiol benzoate (Sigma). Oestradiol was used to maintain the oestrous stage of the oestrous cycle. To prepare oestradiol, β-oestradiol-3-benzoate was made up to a concentration of 10 $mgml^{-1}$ in 0.5% Tween 80 and ball-milled overnight. This concentrate was then diluted 1 in 5 with 0.5% Tween 80 giving a final concentration of 2 $mgml^{-1}$. Each mouse was injected with 0.25 ml (ie 0.5 mg). Two days later the mice were inoculated intravaginally using a suspension of a 24 h culture of *C. albicans* at a concentration of 1×10^7 yeasts ml^{-1} in saline; the yeast suspension was squirted into the vagina using a blunt-ended Pasteur pipette until liquid was seen to run out. One week later the mice were killed and vaginas were removed and added to sterile saline (5 ml) in a universal. The vaginas were homogenized and samples diluted 1/10 and 1/100 with sterile saline. The diluted samples (50 µl) were plated out onto SDA plates, containing 0.1% penicillin and streptomycin, using a

spiral plater. All plates were incubated at 37°C for 24 h. The total number of yeast colonies on each plate was counted to assess the extent of vaginal infection.

(iii) Attempt to block vaginal infections

Four hundred and eighty mice weighing 25-35 g (Alderley Park strain) were injected subcutaneously with 0.5 mg oestradiol benzoate. The mice were divided into twelve groups of forty. Two days later, EPM (20 $mgml^{-1}$, in saline) isolated from C. albicans GDH 2346 was used to treat vaginas of six groups of mice by squirting the EPM solution into the vagina with a blunt-ended Pasteur pipette until liquid was seen to run out. The remaining six groups of mice were pretreated with saline. All mice were left for 30 min before inoculating intravaginally with yeast suspensions. C. albicans GDH 2346 and C. albicans B2630 were grown to the stationary phase in YNB medium containing 50 mM glucose. Mice were inoculated with suspensions containing 10^3, 10^5 and 10^7 yeasts ml^{-1}. Vaginal counts were performed on groups of ten mice after 4, 7, 14 and 21 days as previously described.

VII STATISTICS

Students' t test was used to detect significant differences between adherence values and the results are presented as p-values. Standard errors of the mean are quoted for all adherence assay and animal experiment results.

VIII CHEMICALS

Sabouraud dextrose agar and yeast nitrogen base were from Difco. Acrylic was obtained from Simplex Rapid, Howmedica International. Concanavalin A,

Arachis hypogea (Peanut lectin), Lens culinaris lectin (lentil lectin), Lotus tetragonolobus lectin (winged pea lectin) and Triticum vulgaris lectin (wheat germ agglutinin) were obtained from Sigma. Sugars used in adherence studies were from Sigma. Glucose and galactose used in growth media were from BDH Chemicals Ltd. Sodium periodate, dithiothreitol, α-mannosidase and all proteolytic enzymes were obtained from Sigma. Endoglycosidase H was from Miles Laboratories Ltd.

RESULTS

I ADHERENCE OF CANDIDA ALBICANS

A. Adherence of nine strains of C. albicans to human buccal epithelial cells

The adherence of nine strains of C. albicans to buccal epithelial cells was determined after growth of the yeasts to stationary phase in defined medium containing 50 mM glucose or 500 mM galactose as the carbon source. C. albicans GDH 2346 was more than eight times more adherent to epithelial cells after growth in medium containing galactose than after growth in medium containing glucose. The adherence of C. albicans GDH 2023 and C. albicans MRL 3153 was also promoted by growth in medium containing 500 mM galactose (Table 5). All of these strains (I strains) were isolated from active infections. C. albicans GRI 681 and C. albicans GRI 682, on the other hand, were isolated from symptomless carriers (C strains). After growth in medium containing 50 mM glucose adherence of C. albicans strains GRI 681 and GRI 682 was similar to that of strains isolated from active infections. However, these strains only showed a two-fold increase in adherence after growth in medium containing 500 mM galactose.

C. albicans B2630 and C. albicans strains AD, KB and Darlington also showed enhanced adherence after growth in galactose containing medium, although not to the same extent as C. albicans GDH 2346. C. albicans strains AD, KB and Darlington were ketoconazole-resistant isolates and were used in animal models to study the role of adhesion in vivo. The results to these experiments will be described later.

B. Adherence of C. albicans GDH 2346, C. albicans GDH 2023, C. albicans GRI 681 and C. albicans 'outbreak' strain to human vaginal epithelial cells

Table 5 Adherence of nine strains of *C. albicans* to human buccal epithelial cells after growth in YNB medium containing 50 mM glucose or 500 mM galactose as the carbon source

Strain	Carbon source	Mean no. of adherent yeasts/epithelial cell ± SEM	Relative adherence[a]
GDH	Glucose	1.65 ± 0.11	1.00
2346	Galactose	13.65 ± 0.23	8.27
GDH	Glucose	1.74 ± 0.05	1.00
2023	Galactose	10.58 ± 0.52	6.08
MRL	Glucose	1.61 ± 0.10	1.00
3153	Galactose	8.28 ± 0.16	5.14
GRI	Glucose	1.75 ± 0.04	1.00
681	Galactose	3.58 ± 0.01	2.04
GRI	Glucose	2.08 ± 0.06	1.00
682	Galactose	4.38 ± 0.28	2.11
B2630	Glucose	1.11 ± 0.08	1.00
	Galactose	6.21 ± 0.22	5.59
AD	Glucose	2.58 ± 0.06	1.00
	Galactose	11.14 ± 0.81	4.32
KB	Glucose	2.58 ± 0.11	1.00
	Galactose	11.98 ± 0.23	4.63
Darlington	Glucose	2.32 ± 0.23	1.00
	Galactose	9.43 ± 0.83	4.06

[a]Adherence relative to that of 50 mM glucose-grown cells of the same strain

The adherence of these strains of C. albicans to vaginal epithelial cells was determined after growth of the yeasts to stationary phase in defined medium containing 50 mM glucose and 500 mM galactose as carbon sources. C. albicans 'outbreak' strain grown in medium containing 500 mM galactose showed more than an 11-fold increase in adherence to vaginal cells than yeasts grown in 50 mM glucose-containing medium. As with adherence to buccal epithelial cells, the adherence of C. albicans GDH 2346 and C. albicans GDH 2023 was also promoted after growth in medium containing 500 mM galactose while C. albicans GRI 681 showed only a 2-fold increase. The relative adherence values for these strains are similar for both types of epithelial cells (Table 6).

C. Effect of yeast cell concentration on adherence of C. albicans GDH 2346 to buccal epithelial cells

Yeasts were grown to stationary phase in YNB medium containing 500 mM galactose. Yeast cell concentration ranging from 10^3 to 10^8 cells ml^{-1} was used in adherence assays. Figure 5 illustrates the results obtained from these experiments. Considerable yeast attachment was observed when a yeast cell concentration ranging between 10^6 and 10^7 cells ml^{-1} was used. No increase in yeast adherence was observed if yeast cell concentration greater than 10^8 cells ml^{-1} was used. A yeast cell concentration of 1×10^7 cells ml^{-1} was used in later adherence assays which permitted accurate counting of yeasts adhering to epithelial cells.

D. Effect of cations on adherence of C. albicans GDH 2346 to buccal epithelial cells

Various cations were examined for their effect on adherence of C. albicans GDH 2346 to buccal epithelial cells (Table 7). For adherence assays

Table 6 Adherence of four strains of C. albicans to human vaginal epithelial cells after growth in defined medium containing 50 mM glucose or 500 mM galactose as the carbon source

Strain	Carbon source	Mean no. of adherent yeasts/vaginal epithelial cell ± SEM	Relative adherence[a]
Outbreak strain	Glucose	1.29 ± 0.14	1.00
	Galactose	14.63 ± 0.54	11.34
GDH 2346	Glucose	1.20 ± 0.08	1.00
	Galactose	10.35 ± 0.65	8.65
GDH 2023	Glucose	1.68 ± 0.15	1.00
	Galactose	10.13 ± 0.36	6.03
GRI 681	Glucose	1.29 ± 0.13	1.00
	Galactose	3.20 ± 0.15	2.48

[a]Adherence relative to that of 50 mM glucose-grown cells of the same strain

Figure 5 Effect of yeast cell concentration on adherence of *C. albicans* GDH 2346 to buccal epithelial cells, after growth in defined medium containing 500 mM galactose

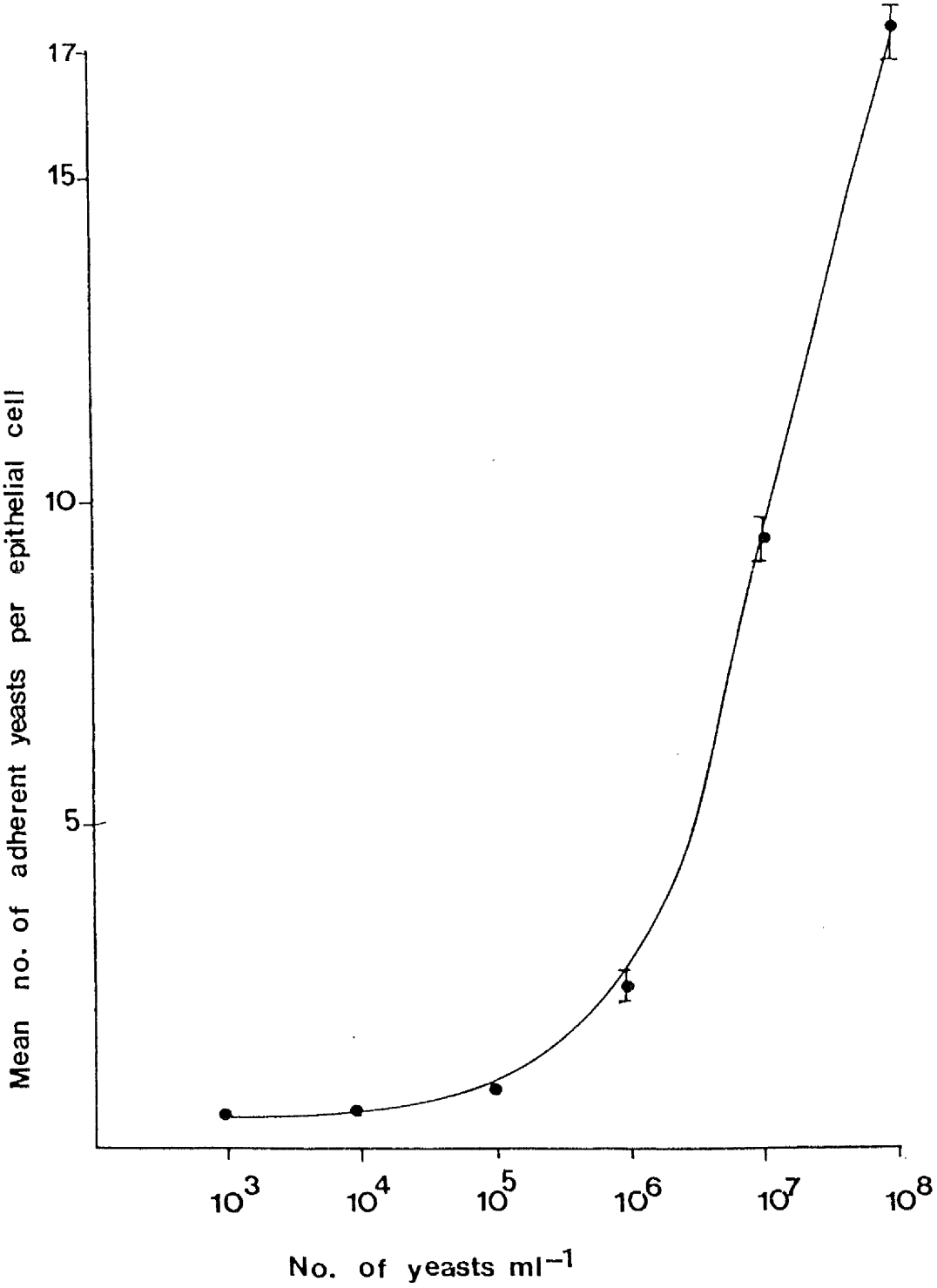

17
15
10
5
Mean no. of adherent yeasts per epithelial cell
10^3
10^4
10^5
10^6
10^7
10^8
No. of yeasts ml^{-1}

Table 7 Effect of cations on adherence of *C. albicans* GDH 2346 to buccal epithelial cells

Suspending solution[a]	Mean no. of adherent yeasts/epithelial cell ± SEM	Relative adherence[c]	P[d]
Deionised water	8.12 ± 0.07	1.00	-
0.1 mM $CaCl_2$	10.21 ± 0.41	1.26	<0.001
0.04 mM $FeCl_2$	9.80 ± 0.50	1.21	<0.01
0.1 mM $MnCl_2$	9.25 ± 0.65	1.14	<0.01
0.2 mM EDTA	9.14 ± 0.47	1.13	<0.05
0.1 mM $MgCl_2$	9.00 ± 0.22	1.11	<0.01
0.1 mM $ZnCl_2$	8.81 ± 0.26	1.10	<0.05
PBS	7.78 ± 0.32	0.96	<0.05
0.2 mM EDTA + 0.1 mM $CaCl_2$	7.14 ± 0.17	0.88	NS[b]
0.1 mM KCl	6.86 ± 0.32	0.84	<0.001
0.1 mM NaCl	6.54 ± 0.75	0.81	<0.001

[a] Adherence was measured as described in materials and methods except that cells were suspended in the solutions indicated

[b] NS, not significant

[c] Adherence relative to that of cells suspended in deionised water

[d] Probability values comparing yeast adherence to buccal cells in the presence of cations with yeast adherence in the presence of deionised water

yeasts were grown to the stationary phase in YNB medium containing 500 mM galactose. Divalent cations significantly increased yeast adherence whereas monovalent cations decreased adherence slightly. Calcium ions enhanced adherence to the greatest extent. The effect of calcium ions was abolished when 0.2 mM EDTA was included in the incubation mixture; however, EDTA on its own had little effect on adherence (Table 7). PBS decreased adherence slightly when compared with deionised water as the medium for suspending cells.

II ISOLATION AND PURIFICATION OF EXTRACELLULAR POLYMERIC MATERIAL (CRUDE ADHESIN)

C. albicans GDH 2346 has previously been shown to synthesize an additional fibrillar cell surface layer after growth to stationary phase in medium containing high concentrations of galactose (Douglas and McCourtie, 1981). These fibrils have been shown by electron microscopy to be readily released from the cell surface (McCourtie and Douglas, 1981). This material contains C. albicans adhesin components, as material isolated by acetone precipitation is capable of blocking yeast attachment to epithelial cells (McCourtie and Douglas, 1985a).

A. Yield of EPM

Growth of the yeast for 5 d in 1 litre of YNB medium containing 500 mM galactose gave 8.23 $\pm$ 0.62 g dry weight of cells and 1.8165 $\pm$ 0.08 g dry weight of EPM, ie, an EPM yield of 22%. The figures quoted here represent the mean $\pm$ SEM.

B. Analysis of crude EPM

The extracellular polymeric material (EPM) produced by C. albicans GDH 2346

contained carbohydrate and protein in a ratio of 7:1 (Table 8) together with a small amount of phosphorus.

EPM isolated from the culture supernatant fluids of four other strains of C. albicans were also analysed biochemically. C. albicans GRI 681 and C. albicans GRI 682 produced EPM with slightly more protein (Table 9). EPM from C. albicans GRI 682 contained less carbohydrate than EPM from yeasts isolated from active infections.

C. Fractionation of EPM

(i) Gel filtration

C. albicans GDH 2346 was grown for 5 d in YNB medium containing 500 mM galactose. Yeast cells were recovered by centrifugation and the culture supernatant extensively dialysed and freeze-dried to isolate EPM. This material was applied to a column of Sephadex G-200 which has a molecular weight fractionation range of 1000-200,000 for dextrans. Five components were eluted from the column (indicated by the bars, Figure 6) and these were analysed for carbohydrate and protein. Component 3 contained only protein; all other components contained carbohydrate and protein in different proportions. Therefore, it seems likely that EPM which is released into the culture medium by C. albicans GDH 2346 is glycoprotein in nature and can be separated into fractions with different carbohydrate and protein contents. Fractions corresponding to each component were pooled and tested for their ability to inhibit adherence; these results are described later.

(ii) Ion-exchange chromatography

C. albicans GDH 2346 EPM was applied to an ion-exchange column of DEAE-cellulose (DE52). Samples of EPM were eluted with a linear gradient of sodium chloride (0-0.5M) in 0.15M phosphate buffer. No components were

Table 8 Analysis of EPM isolated from culture supernatants of *C. albicans* GDH 2346 grown for 5 days in YNB medium containing 500 mM galactose

Component	Percentage composition ± SEM
Protein	10.0 ± 0.7
Carbohydrate	70.0 ± 2.6
Phosphorus	0.49 ± 0.04

Results represent means ± SEM of three independent determinations where assays were done in triplicate

Table 9 Biochemical analysis of EPM isolated from different strains of C. albicans grown in YNB medium containing 500 mM galactose

Strain	Percentage composision ± SEM	
	carbohydrate	protein
GDH 2346	88.2 ± 3.1	9.0 ± 0.8
GDH 2023	78.4 ± 1.9	14.8 ± 0.7
GRI 681	82.3 ± 2.8	15.5 ± 0.7
GRI 682	74.0 ± 2.2	10.5 ± 0.5
MRL 3153	76.2 ± 2.2	7.5 ± 0.3

Results represent the mean of three independent determinations performed in duplicate

Figure 6 Fractionation of EPM from C. albicans GDH 2346 by gel filtration on Sephadex G-200

Yeast cells were grown for 5 days in YNB medium containing 500 mM galactose. Cells were harvested by centrifugation and EPM was isolated by dialysing and freeze-drying culture supernatant fluids.

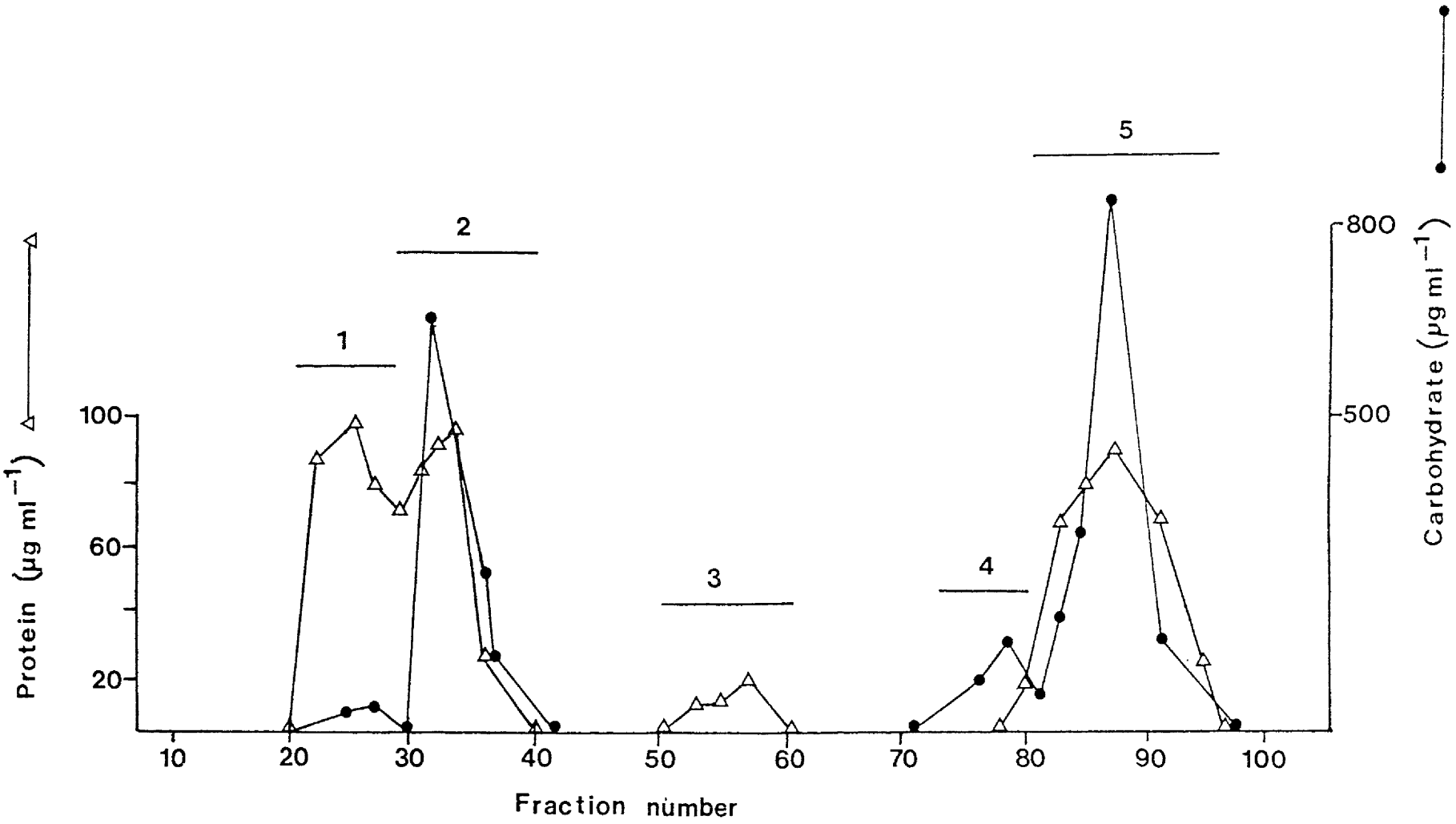
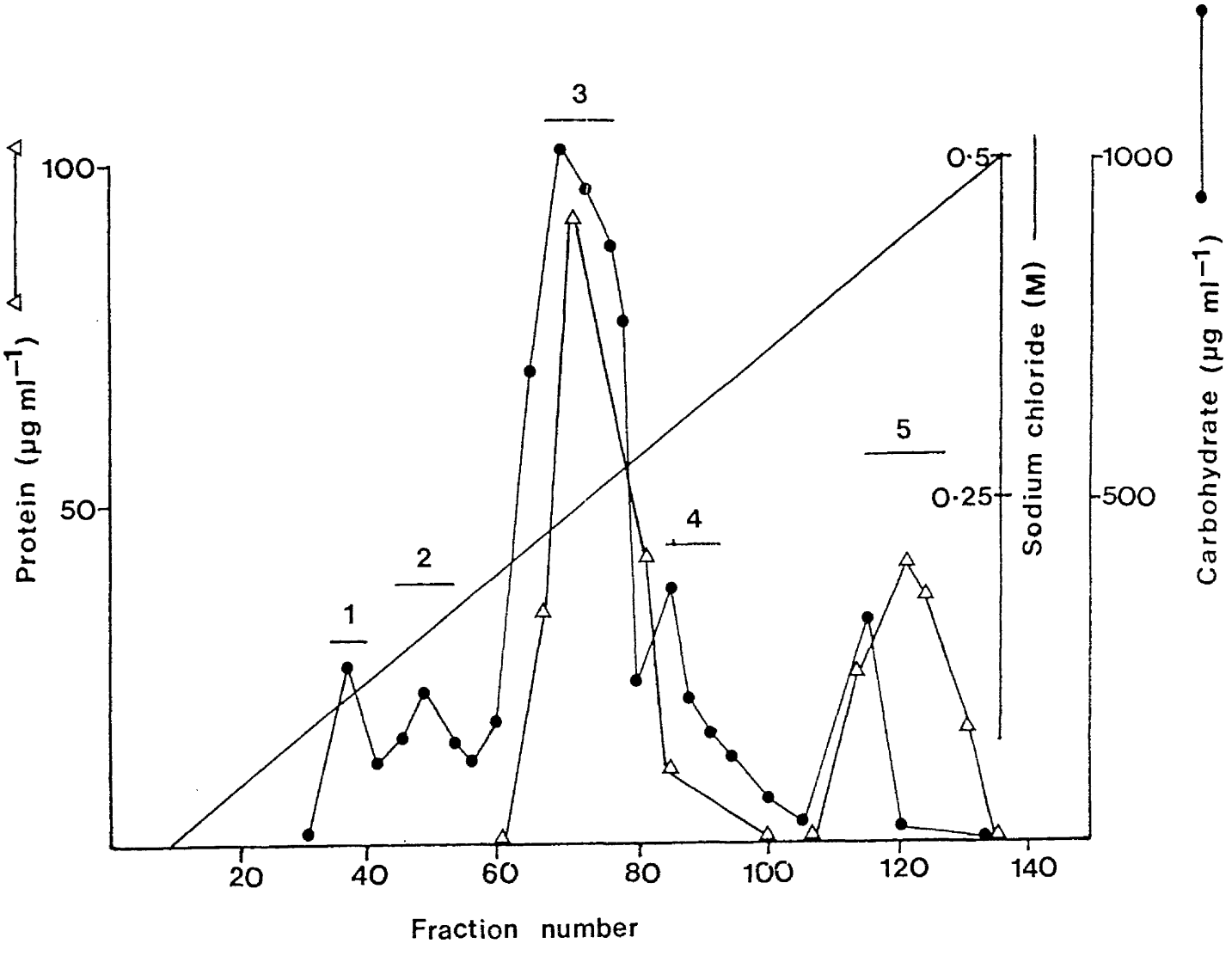

Protein (μg ml⁻¹)
Carbohydrate (μg ml⁻¹)
Fraction number
1
2
3
4
5
100
60
20
800
500
10
20
30
40
50
60
70
80
90
100

Figure 7 DEAE-Cellulose chromatography of EPM from C. albicans GDH 2346

Yeast cells were grown for 5 days in YNB medium containing 500 mM galactose. Cells were harvested by centrifugation and EPM was isolated by dialysing and freeze-drying culture supernatant fluids.


Protein (µg ml⁻¹)
100
50
Sodium chloride (M)
0·5
0·25
1000
500
Carbohydrate (µg ml⁻¹)
1
2
3
4
5
20
40
60
80
100
120
140
Fraction number

eluted before the gradient was applied indicating that all components were adsorbed to the anion-exchange resin under the conditions used. Five components were eluted after the application of the sodium chloride gradient (Figure 7). Components 1 and 2 contained only carbohydrate, whereas components 3, 4 and 5 contained both carbohydrate and protein. All of these fractions were tested for their ability to inhibit yeast adherence; the results obtained are described later.

(iii) Affinity chromatography with Concanavalin A-Sepharose

Crude EPM isolated from C. albicans GDH 2346 was applied to a column of Concanavalin A-Sepharose 4B. Concanavalin A (Con A) binds molecules which contain αD-mannopyranosyl and sterically related structures which are present in the yeast cell wall as mannoproteins. Samples of EPM were eluted with PBS until no components were detected in fractions. Material which bound to the column was eluted with 0.5M methyl-α-D-mannoside in PBS. Eluted components were monitored by carbohydrate and protein analysis of column fractions. Two components were eluted with PBS, before the addition of methyl-α-D-mannoside (Figure 8). These components contained both carbohydrate and protein. The components indicated by the bars were tested in adherence inhibition assays; the results obtained are described later. All the material which was eluted after the addition of methyl-α-D-mannoside was pooled. This material was then applied to a DE52 ion-exchange column to see whether further purification could be achieved.

(iv) Ion-exchange chromatography of components eluted from Con A-Sepharose after the addition of methyl-α-D-mannoside

The material which bound to the Con A-Sepharose column was eluted with methyl-α-D-mannoside. This fraction was dialysed to remove methyl-α-D-mannoside and then applied to an ion-exchange column containing DE52. This material was eluted with a linear gradient of sodium chloride (0-0.5M)

Figure 8 Affinity chromatography of crude EPM from C. albicans GDH 2346 on Concanavalin A-Sepharose

Yeast cells were grown for 5 days in YNB medium containing 500 mM galactose. Cells were harvested by centrifugation and EPM was isolated by dialysing and freeze-drying culture supernatant fluids.

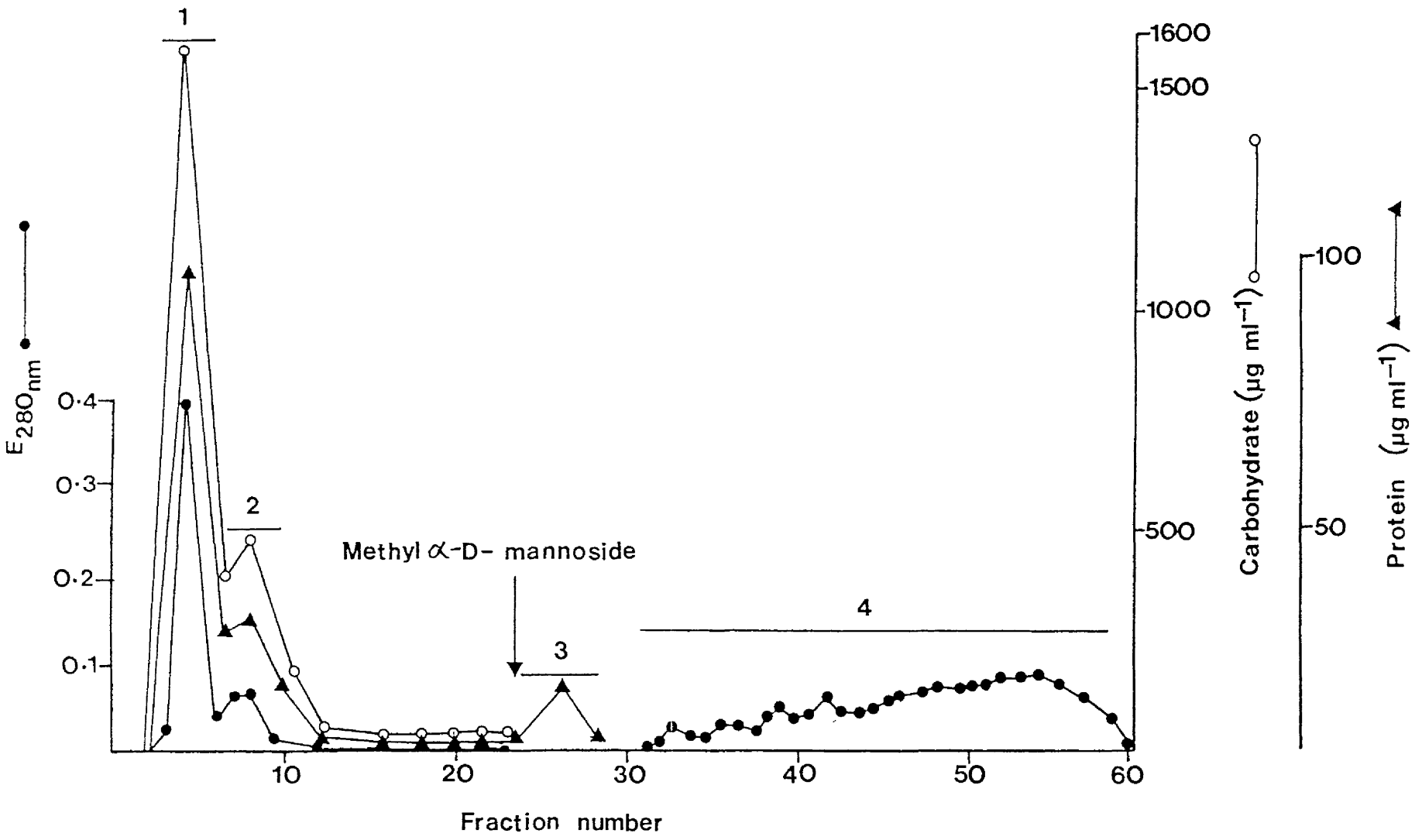
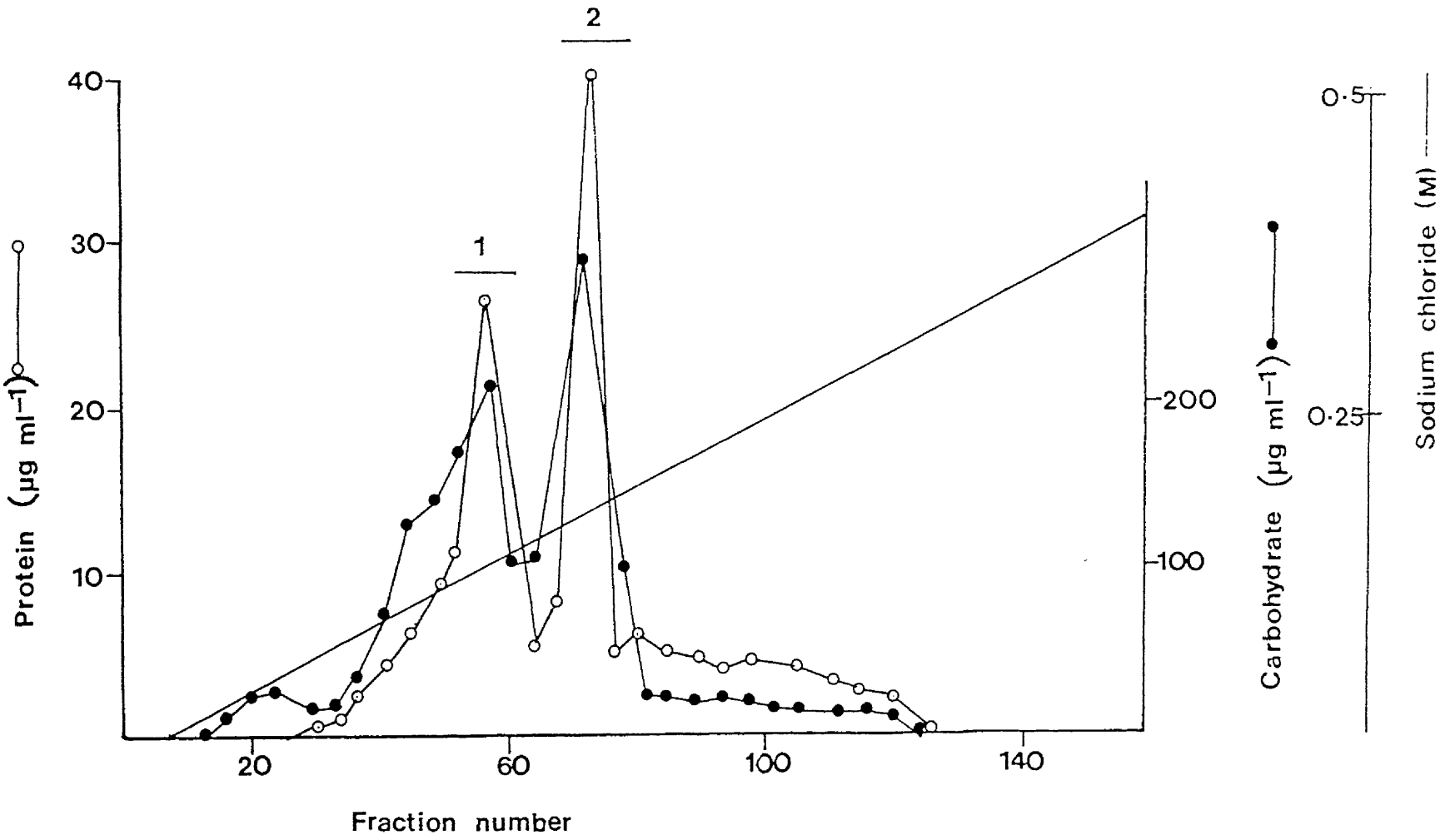

1
2
3
4
Methyl α-D- mannoside
$E_{280_{nm}}$
0·4
0·3
0·2
0·1
1600
1500
1000
500
100
50
Carbohydrate (μg ml^{-1})
Protein (μg ml^{-1})
10
20
30
40
50
60
Fraction number

Figure 9 DEAE-Cellulose chromatography of Concanavalin A-Sepharose bound fraction of EPM from *C. albicans* GDH 2346

Components which were eluted after the addition of methyl α-D-mannoside to the Con A-Sepharose column (see Figure 8) were applied to a DE52 ion-exchange column.


2
1
40
30
20
10
Protein (µg ml⁻¹)
200
100
Carbohydrate (µg ml⁻¹)
0·5
0·25
Sodium chloride (M)
20
60
100
140
Fraction number

in 0.15M phosphate buffer. Two components were eluted which contained 6 to 7 times more carbohydrate than protein (Figure 9). These results suggest that the mannoprotein which bound to the Con A-Sepharose could be separated into two further components. From the appearance of peak 1 (Figure 9), it is likely that it could be further resolved although this was not attempted because of insufficient material. Components 1 and 2 were tested in adherence inhibition assays; the results are described later.

(v) Isolation of 'lectin-like' components by affinity chromatography

Yeast adherence may be mediated by sugar residues on the epithelial cell surface which interact with sugar-binding components on the yeast surface. The sugars used in this investigation were chosen as they have been shown to be present on the surfaces of mammalian cells. EPM samples from five different strains of C. albicans were investigated for sugar-binding properties by applying to affinity columns with sugar coupled to Sepharose. The samples of EPM were eluted with PBS and then with the appropriate sugar to remove bound components. The bound fractions were dialysed and analysed for protein. The bound fraction from each affinity column was tested for its ability to inhibit adherence by preincubating it with buccal epithelial cells prior to their use in adherence assays (results are described later). L-Fucose coupled to Sepharose bound the largest amount of protein from C. albicans GDH 2346 EPM (Figure 10). D-Mannose and N-acetyl-D-glucosamine Sepharose columns also bound some protein but not as much as L-fucose. Samples of EPM isolated from C. albicans strains MRL 3153, GRI 681 and GRI 682 also showed similar sugar-binding specificities to GDH 2346 EPM.

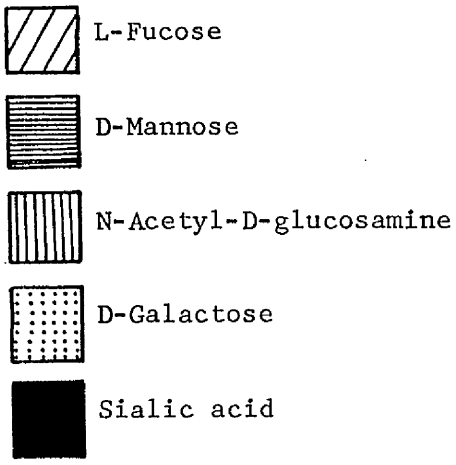
EPM components from C. albicans GDH 2023 showed a strikingly different pattern of sugar-binding specificities (Figure 10). Affinity

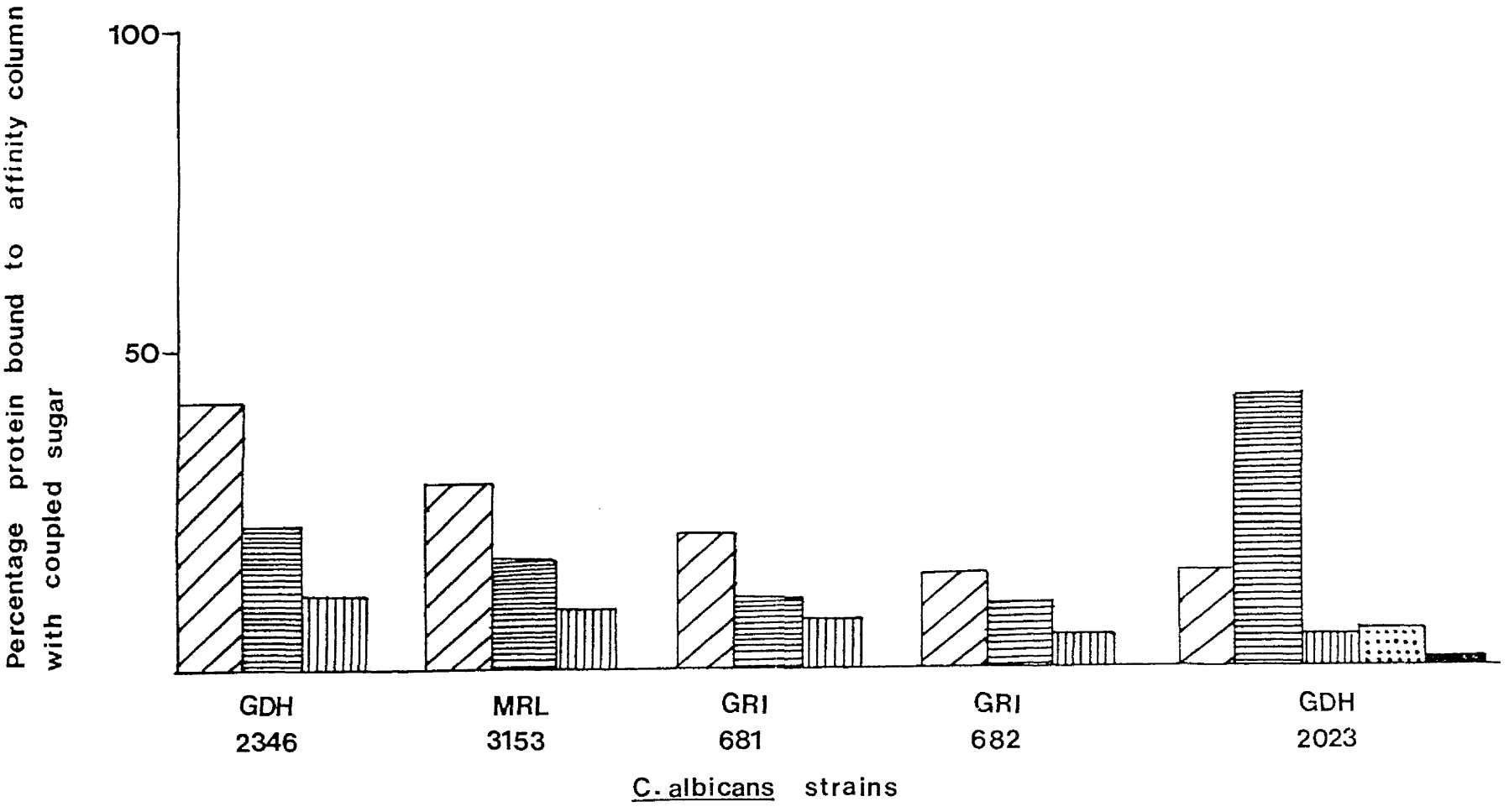
Figure 10 Percentage of EPM protein which bound to affinity columns for five strains of C. albicans

EPM was isolated from each strain as described in materials and methods.

EPM (10 $mgml^{-1}$) was applied to affinity columns of Sepharose with coupled sugar. Material was eluted with PBS to remove unbound material. Bound material was eluted with a solution of appropriate sugar (25 $mgml^{-1}$). This fraction was dialysed and analysed for protein to calculate the percentage of protein bound to the column.

Sugar coupled to Sepharose which binds EPM protein




Percentage protein bound to affinity column with coupled sugar
100
50
GDH 2346
MRL 3153
GRI 681
GRI 682
GDH 2023
C. albicans strains

columns with L-fucose, D-mannose, N-acetyl-D-glucosamine, D-galactose and sialic acid coupled to Sepharose all bound EPM components to different extents. These results suggest that EPM contains 'lectin-like' components and that different strains have components with different sugar specificities.

Biochemical analysis of bound fractions from affinity columns

For C. albicans strains GDH 2346, MRL 3153 and GRI 681 the bound fractions from D-mannose, L-fucose and N-acetyl-D-glucosamine Sepharose affinity columns were analysed for total carbohydrate, glucose and protein. The components in the bound fractions were glycoproteins with an approximate ratio of 7:1 carbohydrate to protein (Table 10). Glucose was also shown to be present in these bound fractions.

For C. albicans GDH 2023 the bound fractions from D-mannose, L-fucose, N-acetyl-D-glucosamine and D-galactose Sepharose columns were biochemically analysed. These fractions were also glycoproteins containing a small amount of glucose (Table 10).

(vi) SDS-polyacrylamide gel electrophoresis

An attempt was made to determine whether components present in crude EPM and various column fractions could be resolved by SDS polyacrylamide gel electrophoresis. Samples of crude EPM (2.0, 3.5 and 5.0 $mgml^{-1}$) were applied to gels. Results for different separation protocols and staining techniques of SDS-polyacrylamide gels are summarized in Table 11. No bands were detected with Coomassie blue R250, presumably because EPM contains only 10% protein. Results from periodic acid-Schiff staining suggests EPM components are high in molecular weight as they did not migrate very far into the resolving gel. Decreasing the percentage of acrylamide did not increase resolution as thick diffuse bands were obtained.

The silver stain techniques of Tsai and Frasch (1982) and Oakley

Table 10 Biochemical analysis of bound fractions of EPM from affinity columns of Sepharose with coupled sugar for four strains of C. albicans

Strain	Coupled sugar on affinity column	Percentage of bound material from crude EPM[a]		
		Protein	Carbohydrate	Glucose
GDH 2346	D-Mannose	18.1±2.1	17.9±2.6	7.5±0.3
	L-Fucose	32.0±4.3	27.3±2.6	12.6±0.8
	N-Acetyl-D-glucosamine	6.9±0.8	8.01±0.5	2.4±0.3
MRL 3153	D-Mannose	21.7±0.9	16.5±1.4	8.1±0.5
	L-Fucose	25.7±2.3	19.8±1.9	9.4±0.4
	N-Acetyl-D-glucosamine	12.8±1.8	11.6±0.6	5.5±0.4
GRI 681	D-Mannose	13.9±2.6	18.2±0.5	8.6±0.3
	L-Fucose	16.3±3.1	22.4±3.1	10.1±0.5
	N-Acetyl-D-glucosamine	4.9±0.8	5.2±0.3	2.2±0.4
GDH 2023	D-Mannose	32.7±3.1	44.7±8.2	22.8±3.1
	L-Fucose	8.1±0.9	10.9±2.0	5.6±0.5
	N-Acetyl-D-glucosamine	15.5±1.5	15.5±1.4	15.2±0.8
	D-Galactose	10.4±0.6	13.7±0.5	7.3±0.6

[a]Samples of EPM (10 $mgml^{-1}$) were applied to nine columns of Epoxy-activated sepharose with coupled sugar. Material which bound to the column was eluted with the appropriate sugar. Samples were dialysed and analysed as described in materials and methods

Results represent means ± SEM of two independent determinations where assays were done in duplicate

Table 11 SDS polyacrylamide gel electrophoresis of EPM from C. albicans GDH 2346: results obtained with different separation protocols

Separating gel	Stacking gel	Buffer system	Stain	Reference	Results obtained with EPM
11% (w/v)	5% (w/v)	Tris-glycine	Coomassie blue	Laemmli (1970)	No bands detected even with 5 $mgml^{-1}$ EPM
11% (w/v)	5% (w/v)	Tris-glycine	Periodic acid - Schiff	Laemmli (1970)	Difficult to detect single bands
7½% (w/v)	3% (w/v)	Tris-glycine	Silver stain	Laemmli (1970)	Thick diffuse band. Intensity of staining greater towards side of lane
7½% (w/v)	3% (w/v)	Phosphate	Silver stain or LPS silver stain	Segrest and Jackson (1972)	Both result in diffuse bands. Intensity of staining greater towards side of lane

et al (1981) were used to detect extremely low concentrations of protein which could not be vizualized by Coomassie blue. A thick diffuse band with a greater intensity of staining towards the sides of the lane was observed using both procedures. The results from these various separation protocols suggest that EPM does not resolve well on electrophoresis.

III MECHANISM OF ADHERENCE

A. Adherence inhibition studies to identify the nature of the yeast adhesin component

(i) Effect of crude EPM on adherence

A range of concentrations of crude EPM (5-20 $mgml^{-1}$) was examined for their effect on adherence of C. albicans GDH 2346. Samples of EPM were used to preincubate buccal epithelial cells for 30 min at 37°C. Buccal cells were then centrifuged and resuspended in PBS for use in adherence assays. At EPM concentrations of 15-20 $mgml^{-1}$ there was 60% inhibition of adherence. However, the percentage inhibition did not increase further at higher concentrations of EPM. As a result of this investigation 10 $mgml^{-1}$ of crude EPM, which gives approximately 50% inhibition, was used in subsequent experiments (Figure 11).

(ii) Effect of crude EPM on adherence of C. albicans to mouse vaginal epithelial cells

The strains used here were selected because they represent a range of strains of varying virulence. Yeasts were grown in defined medium containing 500 mM galactose. EPM samples used in this investigation were isolated from each of the three strains of C. albicans and were used to block attachment of each respective strain. Mouse vaginal epithelial cells were pretreated with a solution of EPM (10 $mgml^{-1}$) for 30 min at 37°C

Figure 11 Effect of crude EPM on adherence of C. albicans GDH 2346 to buccal epithelial cells

C. albicans GDH 2346 was grown for 5 days in YNB medium containing 500 mM galactose. Cells were harvested by centrifugation and EPM was isolated by dialysing and freeze-drying the culture supernatant fluid.

Epithelial cells were pretreated with a solution of EPM (2-20 $mgml^{-1}$) for 30 min at $37^{o}C$. Epithelial cells were recovered by centrifugation and used in adherence assays as described in materials and methods.

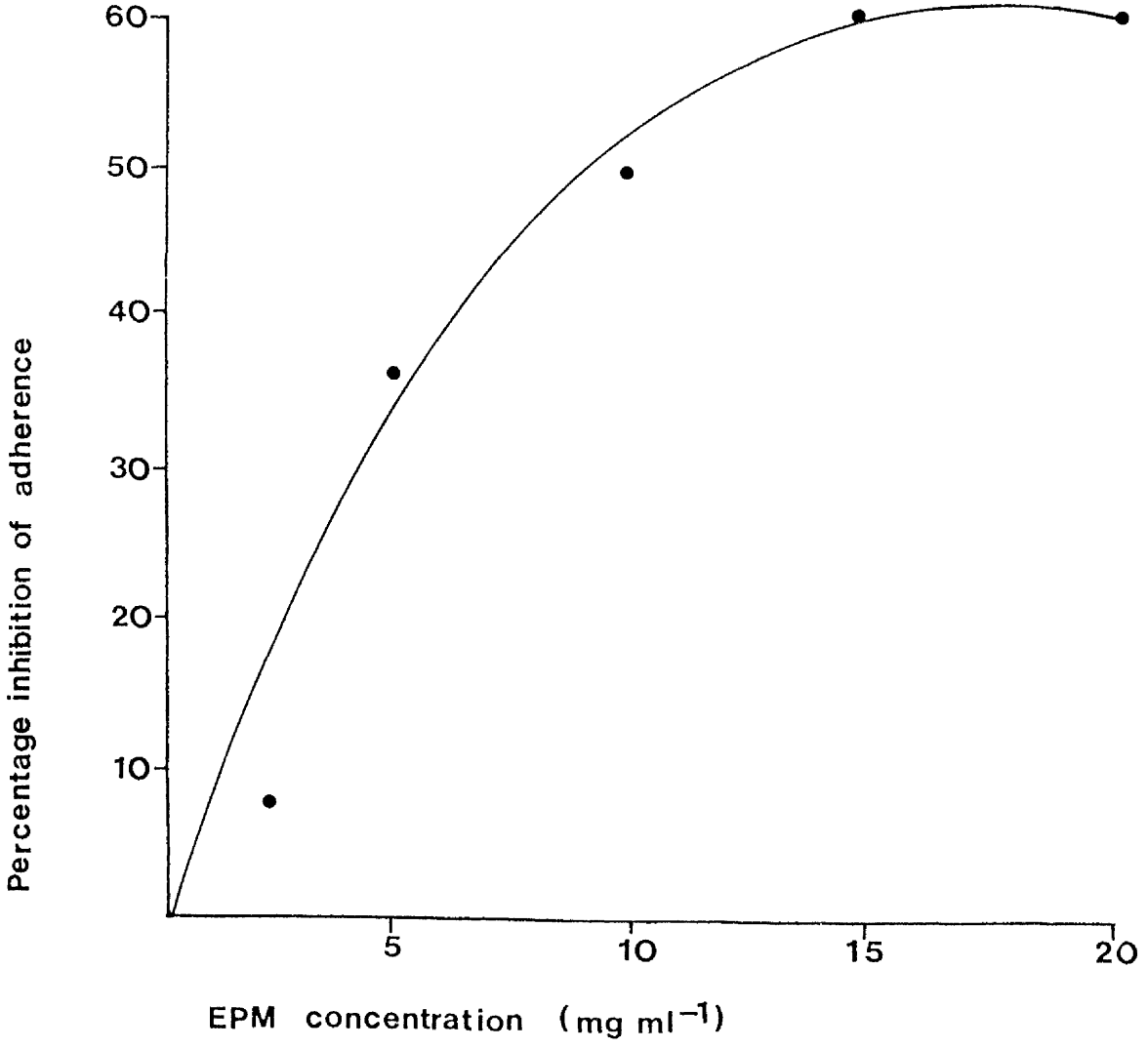

60
50
40
30
20
10
Percentage inhibition of adherence
5
10
15
20
EPM concentration (mg ml^{-1})

Table 12 Effect of EPM on adherence of three strains of *C. albicans* to vaginal epithelial cells from mice

Yeast strain used in assay	Pretreatment of vaginal epithelial cells[a]	Mean no. of adherent yeasts/epithelial cell ± SEM	Percentage inhibition of adherence[b]
GDH 2346	EPM from GDH 2346	3.05 ± 0.23	51.4
	PBS	6.27 ± 0.39	-
GDH 2023	EPM from GDH 2023	3.97 ± 0.19	57.3
	PBS	9.29 ± 0.43	-
GRI 681	EPM from GRI 681	2.33 ± 0.16	25.3
	PBS	3.12 ± 0.18	-

[a]Prior to adhesion assays, vaginal epithelial cells were incubated for 30 min at 37°C in PBS or in a solution (10 $mgml^{-1}$) of EPM produced by *C. albicans* GDH 2346, GDH 2023 or GRI 681

[b]Calculated as the percentage of yeasts not adhering to EPM pretreated epithelial cells when compared with adherence to PBS pretreated epithelial cells

Adherence assays were performed three times in triplicate

before being used in adhesion assays. Results for adherence inhibition assays are shown in Table 12. C. albicans GDH 2346 EPM inhibits adherence of C. albicans GDH 2346 by 51%, this value being similar to that obtained with human buccal epithelial cells and an EPM concentration of 10 $mgml^{-1}$ (Figure 11).

C. albicans GDH 2023 EPM inhibited adherence of C. albicans GDH 2023 and C. albicans GRI 681 inhibited adherence of C. albicans GRI 682 by 57% and 25% respectively.

Vaginal epithelial cells were obtained from six week old mice for these experiments. Attachment of yeasts was found only with cells taken from mice at the oestrous stage of the oestrous cycle. This stage was easily recognised by the appearance of predominantly epithelial cells in vaginal smears.

(iii) Effect of components eluted in column fractions on adherence of C. albicans GDH 2346 to buccal epithelial cells

Attempts were made to purify crude EPM by gel filtration using Sephadex G-200 (Figure 6), ion-exchange chromatography using DE52 (Figure 7) and affinity chromatography using Concanavalin A-Sepharose (Figure 8). Gel filtration and ion-exchange chromatography of C. albicans GDH 2346 EPM both resulted in the elution of five components. Affinity chromatography resulted in the elution of two components before the addition of methyl-α-D-mannoside and several components after the addition of methyl-α-D-mannoside. The components which bound to the Con A-Sepharose column were dialysed and then applied to a DE52 ion-exchange column to see if further purification could be achieved (Figure 9). The components eluted from the various columns were tested for their ability to inhibit adherence of C. albicans GDH 2346 to buccal epithelial cells by preincubating epithelial cells with these components prior to their use in adhesion assays.

(a) Sephadex G-200 column fractions

The five separated components were each tested for their ability to inhibit adherence of C. albicans GDH 2346 (Table 13). Component 5 gave the greatest inhibition of adherence. This component had the highest ratio of carbohydrate to protein and the lowest molecular weight (6×10^3). However, components 1 and 2, which differed considerably from component 5 in both composition and molecular weight, also exhibited significant adherence inhibition. The molecular weights of the five components ranged from 6×10^3 to 7×10^5.

(b) DE52 column fractions

The five components were tested for their ability to inhibit adherence of C. albicans GDH 2346 (Table 14). A major glycoprotein component (component 3, Figure 7) exhibited the greatest degree of adherence inhibition. The extent of adherence inhibition obtained with component 3 was similar to that obtained when epithelial cells were preincubated with crude EPM (10 $mgml^{-1}$). However, the concentrations of carbohydrate and protein in these samples were considerably less than those used in assays with crude EPM (Figure 11). These results indicate that the material which functions as the yeast adhesin has been partially purified by this procedure.

(c) Con A-Sepharose column fractions

The elution profile obtained is shown in Figure 8. All four eluted components were tested in adherence inhibition assays. Adherence inhibition assay results are shown in Table 15. The components eluted after the addition of methyl-α-D-mannoside were the most efficient inhibitors of adherence. This suggests that the yeast adhesin is mannoprotein in nature. Components eluted before the addition of methyl-α-D-mannoside only slightly inhibited adherence (components 1, 2 and 3, Table 15). All

Table 13 Effect of Sephadex G-200 column fractions of EPM isolated from C. albicans GDH 2346 on adherence of C. albicans GDH 2346 to buccal epithelial cells

Component added to assay (Peak no.)[a]	Molecular weight[b]	Composition of component			Mean no. of adherent yeasts/epithelial cell ± SEM	Percentage inhibition of adherence[c]
		Carbohydrate (μg)	Protein (μg)	Carbohydrate: protein ratio		
1	7×10^5	120	75	1.6	20.54 ± 0.45	23.8
2	4×10^5	184	75	2.4	19.42 ± 0.10	27.9
3	6×10^4	0	70	-	22.38 ± 0.24	17.0
4	1.2×10^5	228	80	2.8	25.00 ± 0.13	7.1
5	6×10^3	312	80	3.9	16.53 ± 0.13	39.4
None (PBS)					26.97 ± 0.24	0.0

[a] Peak no. refers to components indicated by bars in Figure 7

[b] Estimated by applying standards of known molecular weight to the column

[c] Calculated as the percentage of yeasts not adhering to EPM pretreated epithelial cells when compared with adherence to PBS pretreated epithelial cells.

Adherence assays were performed three times in triplicate with a yeast cell concentration of 1×10^8 cells ml^{-1}.

Table 14 Effect of ion-exchange chromatography column fractions of EPM isolated from C. albicans GDH 2346 on adherence of C. albicans GDH 2346 to buccal epithelial cells

Component added to assay (Peak no.)[a]	Composition of component			Mean no. of adherent yeasts/epithelial cell ± SEM	Percentage inhibition of adherence[b]
	Carbohydrate (μg)	Protein (μg)	Carbohydrate: protein ratio		
1	208	0	-	14.86 ± 0.48	16.5
2	160	0	-	14.76 ± 0.62	17.1
3	984	80	12.3	8.95 ± 0.52	49.2
4	384	35	10.9	15.40 ± 0.24	13.5
5	32	25	1.3	17.40 ± 0.31	2.2
None (PBS)				17.80 ± 0.38	0.0

[a] Peak no. refers to components indicated by bars in Figure 8

[b] Calculated as the percentage of yeasts not adhering to EPM pretreated epithelial cells when compared with adherence to PBS pretreated epithelial cells

Adherence assays were performed three times in triplicate with a yeast cell concentration of 5×10^7 cells ml^{-1}.

Table 15 Effect of Concanavalin A-Sepharose column fractions of EPM isolated from C. albicans GDH 2346 on adherence of C. albicans GDH 2346 to buccal epithelial cells

Component added to assay (Peak no.)[a]	Composition of component			Mean no. of adherent yeasts/epithelial cell ± SEM	Percentage inhibition of adherence[b]
	Carbohydrate (μg)	Protein (μg)	Carbohydrate: protein ratio		
1	728	70	10.4	17.38 ± 0.25	8.6
2	528	40	13.2	15.72 ± 0.72	17.4
3	0	10	-	17.28 ± 0.54	9.1
4	240	70	3.4	9.77 ± 0.67	48.6
None (PBS)				19.02 ± 0.36	-

[a]Peak no. refers to components indicated by bars in Figure 9

[b]Calculated as the percentage of yeasts not adhering to EPM pretreated epithelial cells when compared with adherence to PBS pretreated epithelial cells.

Adherence assays were performed three times in triplicate with a yeast cell concentration of 5×10^7 cells ml^{-1}.

the material which was eluted after the addition of methyl-α-D-mannoside was pooled. This material was then applied to a DE52 ion-exchange column to see whether further purification of the yeast adhesin could be achieved (Figure 9). The two components eluted were tested for their ability to inhibit adherence of C. albicans GDH 2346 (Table 16). Component 1 inhibited adherence significantly whereas component 2 had little effect. Thus, further purification of the yeast adhesin had been achieved.

To compare the activities of components eluted from the various columns, an adherence inhibition index (AII) was determined for each component (Table 17). The AII is a measure of the relative efficiency with which each component inhibits adherence as compared with crude EPM. At a concentration of 10 $mgml^{-1}$, crude EPM inhibited adherence by 50% (Figure 11). In calculating the AII, the weight of protein and carbohydrate in each purified component required to inhibit adherence by 50% was determined. The respective weights of protein and carbohydrate in 10 mg C. albicans GDH 2346 EPM (ie 1 mg and 7 mg) were then divided by these values. For a worked example of AII calculation see Appendix IV.

The results shown in Table 17 indicate that a two-step separation protocol involving chromatography on Con A-Sepharose and DEAE-cellulose allowed a thirty-fold purification of the yeast adhesin (peak 1 on DE52).

B. Determination of the minimum structure necessary for yeast adherence: dissection of the yeast adhesin

Physical, chemical and enzymic procedures were used to treat EPM from C. albicans GDH 2346 to determine the minimum structure necessary for adherence inhibition. Such treatments yield information on the importance of the carbohydrate or protein portion of the mannoprotein adhesin in mediating adherence. Samples of treated EPM were used to pretreat

Table 16 Effect of DE52 column fractions (Con A-Sepharose bound material) of EPM isolated from *C. albicans* GDH 2346 on adherence of *C. albicans* GDH 2346 to buccal epithelial cells

Component added to assay (Peak no.)[a]	Composition of component			Mean no. of adherent yeasts/epithelial cell ± SEM	Percentage inhibition of adherence[b]
	Carbohydrate (μg)	Protein (μg)	Carbohydrate: protein ratio		
1	190	28	6.7	9.75 ± 0.60	44.0
2	342	44	7.7	16.58 ± 0.65	4.6
None (PBS)				17.39 ± 0.45	

[a]Peak no. refers to components indicated by bars in Figure 10

[b]Calculated as the percentage of yeasts not adhering to EPM pretreated epithelial cells when compared with adherence to PBS pretreated epithelial cells.

Adherence assays were performed three times in triplicate with a yeast cell concentration of 5×10^7 cells ml^{-1}.

Table 17 Purification of the yeast adhesin by combined Con A-Sepharose and DEAE-cellulose chromatography: calculation of the adherence inhibition index (AII)

Fractionation method	Component added to assay (Peak no.)	Percentage inhibition of adherence	AII[a]	
			based on protein content	based on carbohydrate content
Con A-Sepharose	1	8.6	2.50	1.65
	2	17.4	8.68	4.60
	3	9.1	18.30	0
	4	48.6	13.88	28.40
DE52	1	44.0	31.44	32.40
	2	4.6	2.69	1.88

[a]See Appendix IV for worked example of AII calculation

epithelial cells for 30 min at 37°C prior to their use in adhesion assays. The ability of the treated samples to inhibit adherence was used to determine the minimum structure of EPM necessary for adherence inhibition.

(i) Enzyme treatment

EPM samples were treated with the proteolytic enzymes trypsin, chymotrypsin, pronase and papain at a concentration of 0.1 $mgml^{-1}$. All enzymes were then inhibited by the appropriate enzyme inhibitor (trypsin inhibitor, chymotrypsin inhibitor and α_2-macroglobulin for pronase and papain). Results for the effect of enzyme-treated EPM samples on adherence are shown in Table 18. EPM treated with chymotrypsin was the least effective inhibitor of adherence. Trypsin- and pronase-treated EPM samples were also less active inhibitors of adherence than crude EPM. These results suggest that the yeast component which mediates adherence to epithelial cells is protein in nature. However, treatment with papain enhanced the adherence inhibitory ability of EPM.

Further experiments were performed to investigate whether carbohydrate or protein components of the yeast adhesin mediated adherence. As EPM consists essentially of mannoprotein, endoglycosidase H was used to cleave the N-glycosidic bond which links the polysaccharide moiety via a diacetylchitobiose bridge to an asparaginyl residue in the protein part of the molecule. The carbohydrate and protein portions were separated by applying the enzyme-treated sample to an affinity column of Con A-Sepharose. Protein components were eluted with PBS and the bound carbohydrate components were eluted with methyl-α-D-mannoside. The components in the eluted fractions were more efficient inhibitors of adherence than components in the bound fraction, indicating that protein was more important than carbohydrate in mediating adherence (Table 19).

Table 18 Effect of various proteolytic enzyme treatments on the ability of EPM to inhibit adherence of C. albicans GDH 2346 to buccal epithelial cells

Enzyme used to pretreat EPM[a]	Mean no. of adherent yeasts/epithelial cell ± SEM	Percentage inhibition of adherence[b]	Pc	Pd
Trypsin	12.40 ± 0.20	26.6	<0.01	<0.05
Chymotrypsin	15.20 ± 0.26	10.1	<0.05	<0.01
Pronase	12.89 ± 0.25	23.7	<0.01	<0.05
Papain	7.04 ± 0.15	58.3	<0.001	<0.001
None (untreated EPM)	10.14 ± 0.21	40.0	<0.001	-
PBS (Control)	16.90 ± 0.35	0.0	-	<0.001

[a]Prior to adhesion assays, buccal cells were incubated for 30 min at 37°C in PBS or in a solution (10 $mgml^{-1}$) of untreated or enzyme-treated EPM

[b]Calculated as percentage of yeasts not adhering when compared with adherence in the presence of PBS alone

[c]Probability that adherence to epithelial cells pretreated with EPM is significantly different from adherence to cells pretreated with PBS

[d]Probability that adherence to epithelial cells incubated with enzyme-treated EPM is significantly different from adherence to cells pretreated with untreated EPM.

Table 19 Effect of endoglycosidase H treatment on the ability of EPM to inhibit adherence of C. albicans GDH 2346 to buccal epithelial cells

Material used to pretreat epithelial cells[a]	Mean no. of adherent yeasts/epithelial cell ± SEM	Percentage inhibition of adherence[b]
Con A bound material (carbohydrate)	13.56 ± 0.50	17.3
Con A eluted material (protein)	8.78 ± 0.35	46.4
Untreated EPM	10.88 ± 0.35	33.7
PBS	16.40 ± 0.38	0.0

[a] Samples of EPM treated with endoglycosidase H were applied to Concanavalin A-Sepharose mini columns to separate carbohydrate and protein fractions. These fractions were used to pretreat epithelial cells prior to their use in adhesion assays.

[b] Calculated as the percentage of yeasts not adhering to EPM pretreated epithelial cells when compared with adherence to PBS pretreated epithelial cells.

EPM was also treated with α-mannosidase; results are shown in Table 20. This enzyme was found to have little effect on the ability of EPM to inhibit adherence. The activity of the α-mannosidase on EPM was confirmed by running treated samples on paper chromatograms. The chromatograms were treated with silver nitrate reagents for reducing sugars. Results showed that treatment of EPM with α-mannosidase released reducing sugars, as no free reducing sugars were present with untreated EPM samples. The release of these reducing sugars from EPM does not appear to affect its ability to inhibit adherence. The proteolytic enzyme bromelain was found to abolish the ability of EPM to inhibit adherence. In control experiments, α-mannosidase, bromelain and pronase were shown not to have any significant effect on buccal epithelial cells (Table 20).

(ii) Chemical treatment

Mild alkaline treatment of EPM (0.1N NaOH at 25°C for 24 hours) appeared to increase slightly its inhibitory capacity (Table 21). This treatment releases oligosaccharides from O-glycosidic linkages to serine and threonine in protein. Mild acid treatment of EPM (0.01N HCl at 100°C for 30 min) totally abolishes the ability of EPM to inhibit adherence. This is probably the result of protein denaturation either by the acid or by the high temperature required for hydrolysis.

Treatment of EPM with 0.02M and 0.05M periodate had little effect on its ability to inhibit adherence (Table 21). Periodate destroys the carbohydrate component of the glycoprotein, it may affect the protein but this is less likely. Periodate was treated with ethylene glycol to prevent any further activity so that it did not have any effect on epithelial cells.

EPM treated with dithiothreitol (0.012M) at 37°C for 1 hour abolished its ability to inhibit adherence of C. albicans to epithelial cells. This suggests that disulphide bonds may play some role in

Table 20 Effect of various enzyme treatments on the ability of EPM to inhibit adherence of *C. albicans* GDH 2346 to buccal epithelial cells

Sample used to pretreat epithelial cells[a]	Mean no. of adherent yeasts/epithelial cell ± SEM	Relative adherence[b]	Pc	Pd
α-mannosidase-treated EPM	5.26 ± 0.53	0.58	<0.001	NS
bromelain-treated EPM	7.57 ± 0.12	0.84	<0.001	<0.001
α-mannosidase	10.46 ± 1.58	1.16	NS	<0.001
bromelain	9.01 ± 0.98	1.00	NS	<0.001
pronase	8.58 ± 0.53	0.95	NS	<0.01
Untreated EPM	4.49 ± 0.13	0.50	<0.01	-
PBS	8.97 ± 0.38	1.00	-	<0.001

[a]Samples of EPM were treated with various enzymes as indicated in materials and methods. Enzyme treated samples were used to pretreat epithelial cells prior to their use in adhesion assays.

[b]Adherence relative to that of yeasts suspended in PBS.

Pc, probability that adherence to EPM pretreated epithelial cells is significantly different from adherence to PBS pretreated epithelial cells.

Pd, probability that adherence to epithelial cells incubated with enzyme-treated EPM is significantly different from adherence to epithelial cells incubated with untreated EPM.

Table 21 Effect of various chemical treatments on the ability of EPM to inhibit adherence of C. albicans GDH 2346 to buccal epithelial cells

A : Mild acid and mild alkali

Chemical treatment of EPM[a]	Mean no. of adherent yeasts/epithelial cell ± SEM	Relative adherence[b]	Pc	Pd
Mild acid	7.13 ± 0.17	0.92	<0.001	NS
Mild alkali	3.48 ± 0.12	0.44	<0.05	<0.001
None	3.77 ± 0.12	0.48	-	<0.001
PBS control	7.79 ± 1.10	1.00	<0.001	-

B : Other chemical treatments

Chemical treatment of EPM[a]	Mean no. of adherent yeasts/epithelial cell ± SEM	Relative adherence[b]	Pc	Pd
0.02M Periodate	3.50 ± 0.13	0.52	<0.001	NS
0.05M Periodate	3.76 ± 0.17	0.54	<0.001	NS
Periodate and ethylene glycol	7.08 ± 0.09	1.03	NS	<0.01
0.012M Dithiothreitol	6.65 ± 0.21	0.97	NS	<0.01
None	3.47 ± 0.16	0.50	-	<0.001
PBS control	6.85 ± 0.18	1.00	<0.01	-

[a] Samples of EPM were treated with various chemical agents as described in materials and methods. These samples were used to pretreat epithelial cells prior to their use in adhesion assays.

[b] Adherence relative to that of yeasts suspended in PBS

Pc, relative to untreated EPM

Pd, relative to that of yeasts suspended in PBS

maintaining the integrity of the protein adhesin component.

(iii) Physical treatment

EPM samples were heated for 15 min at 50°, 80° and 100°C (Table 22). These samples were used to pretreat buccal epithelial cells prior to their use in adherence assays with C. albicans GDH 2346. Heating EPM to 50°C only slightly affected its ability to inhibit adherence; however, heating to 100°C totally abolished its ability to inhibit adherence. Therefore, protein is either important as an adhesin itself, or is responsible for maintaining the configuration of the adhesin.

(iv) Combined enzyme and chemical treatment

Of the various proteolytic enzymes previously used to treat EPM, papain was the only enzyme which actually increased the ability of EPM to inhibit adherence (Table 18). It is possible that the peptide fragment released with papain treatment still has base labile oligosaccharides bound to serine and threonine in the peptide. Therefore, if EPM is treated with papain, then by mild alkali to remove these oligosaccharides a peptide component may be produced which is capable of binding with the epithelial cell receptor more efficiently. This possibility was tested by the experiment described in Table 23. EPM treated with papain and then with mild alkali was capable of inhibiting adherence of 73% and was considerably more efficient at inhibiting adherence than untreated EPM.

C. Adherence inhibition studies to determine the nature of the epithelial cell receptor

(i) Effect of sugars on adherence to buccal epithelial cells

Adherence assays were performed in the presence of a variety of different sugars at a concentration of 25 $mgml^{-1}$. Results obtained with C. albicans GDH 2346 are shown in Table 24. L-Fucose gave the greatest inhibition of

Table 22 Effect of various physical treatments on the ability of EPM to inhibit adherence of C. albicans GDH 2346 to buccal epithelial cells

Treatment of EPM[a]	Mean no. of adherent yeasts/epithelial cell ± SEM	Percentage inhibition of adherence[b]
50°C, 15 min	14.28 ± 0.81	36.0
80°C, 15 min	18.90 ± 0.95	15.4
100°C, 15 min	22.16 ± 0.34	0.01
None	11.43 ± 0.50	48.8
PBS control	22.34 ± 0.61	0.0

[a]Samples of EPM (10 $mgml^{-1}$) were heat treated as indicated in materials and methods. These samples were used to pretreat epithelial cells prior to their use in adherence assays

[b]Calculated as percentage of yeasts not adhering when compared with adherence of yeasts suspended in PBS

Adherence assays were done with a yeast-cell concentration of 1×10^8 yeasts ml^{-1} (higher than that in the standard assay).

Table 23 Effect of combined papain and mild alkali treatment on the ability of EPM to inhibit adherence of C. albicans GDH 2346 to buccal epithelial cells

Treatment of EPM[a]	Mean no. of adherent yeasts/epithelial cell ± SEM	Percentage inhibition of adherence[b]
Papain followed by mild alkali	2.25 ± 0.08	73.2
None	4.25 ± 0.14	49.5
PBS control	8.42 ± 0.12	0.0

[a]Samples of EPM were treated with papain and then with mild alkali as indicated in materials and methods. This sample was used to pretreat epithelial cells prior to their use in adherence assays

[b]Calculated as percentage of yeasts not adhering when compared with adherence of yeasts suspended in PBS.

Table 24 Effect of different sugars on adherence of C. albicans GDH 2346 to buccal epithelial cells

Sugar present in assay mixture	Mean no. of adherent yeasts/epithelial cell ± SEM	Relative adherence[a]	P[c]
D-Mannose	11.68 ± 0.42	0.93	NS[b]
N-Acetyl-D-glucosamine	10.34 ± 0.63	0.83	<0.05
PBS	12.52 ± 0.35	1.00	-
D-Glucose	10.70 ± 0.91	1.19	<0.02
D-Glucosamine	9.89 ± 0.32	1.10	NS
D-Mannosamine	9.22 ± 0.24	1.02	NS
D-Galactose	8.80 ± 0.20	0.98	NS
D-Fucose	7.47 ± 0.46	0.83	<0.01
L-Fucose	6.43 ± 0.36	0.71	<0.001
PBS	8.98 ± 0.12	1.00	-

[a]Adherence relative to that of yeasts suspended in PBS

[b]NS, not significant

[c]Probability values comparing adherence in the presence of sugar with adherence in the presence of PBS only

Adherence assays with D-mannose and N-acetyl-D-glucosamine were performed using 5×10^7 yeasts ml^{-1}.

adherence with a relative adherence value of 0.71. D-Mannose did not significantly affect adherence to buccal cells, while N-acetyl-D-glucosamine had an effect which was only marginally significant. Amino sugars and D-galactose did not have any effect on adherence whereas D-glucose enhanced adherence resulting in a relative adherence value of 1.19.

Different results were obtained when these sugars were examined for their effect on the adherence of C. albicans GDH 2023 (Table 25). N-Acetyl-D-glucosamine and amino sugars had a greater inhibitory effect on adherence of this strain with relative adherence values of 0.66 and 0.68 for D-glucosamine and N-acetyl-D-glucosamine respectively. L-Fucose did not have any inhibitory effect whereas D-mannose did inhibit adherence and D-glucose did not enhance the adherence of this strain.

The results for the effect of sugars on the adherence of C. albicans 'outbreak' strain are shown in Table 26. This strain has an inhibition pattern different from that of C. albicans GDH 2023 in that adherence is sensitive to L-fucose. However, it is also different from C. albicans GDH 2346 as D-glucose did not enhance adherence. These results suggest that there may be several receptors on epithelial cell surfaces and that L-fucose, N-acetyl-D-glucosamine and D-mannose are principal receptor sugars.

(ii) Effect of lectins on adherence to buccal epithelial cells

Lectins were used to study the role of polysaccharide containing moieties on the surface of buccal epithelial cells and their role in mediating C. albicans adhesion. Lectins of known sugar specificity were used to pretreat epithelial cells prior to their use in adherence assays. Such treatments may be used to block epithelial cell receptor sites preventing yeast attachment. Results for the effect of lectins on adherence of C. albicans GDH 2346 are shown in Table 27. Winged pea lectin significantly

Table 25 Effect of different sugars on adherence of C. albicans GDH 2023 to buccal epithelial cells

Sugar present in assay mixture	Mean no. of adherent yeasts/epithelial cell ± SEM	Relative adherence[a]	P[c]
D-Galactose	12.49 ± 0.18	1.07	NS[b]
D-Glucose	12.13 ± 0.27	1.04	NS
L-Fucose	11.98 ± 0.54	1.03	NS
D-Fucose	10.93 ± 0.13	0.94	NS
D-Mannosamine	10.09 ± 0.52	0.86	<0.02
D-Mannose	9.75 ± 0.32	0.83	<0.01
N-Acetyl-D-glucosamine	8.01 ± 0.22	0.68	<0.001
D-Glucosamine	7.70 ± 0.09	0.66	<0.001
PBS	11.63 ± 0.31	1.00	

[a] Adherence relative to that of yeasts suspended in PBS

[b] NS, not significant

[c] Probability values comparing adherence in the presence of sugar with adherence in the presence of PBS only.

Table 26 Effect of different sugars on adherence of C. albicans 'outbreak' strain to buccal epithelial cells

Sugar present in assay mixture	Mean no. of adherent yeasts/epithelial cell ± SEM	Relative adherence[a]	P[c]
D-Glucose	12.68 ± 0.41	0.97	NS[b]
D-Galactose	12.34 ± 0.39	0.95	NS
D-Mannose	11.75 ± 0.25	0.90	<0.02
D-Mannosamine	11.72 ± 0.33	0.90	<0.01
N-Acetyl-D-glucosamine	10.75 ± 0.42	0.83	<0.01
D-Glucosamine	10.65 ± 0.43	0.82	<0.001
L-Fucose	9.13 ± 0.41	0.70	<0.001
PBS	12.94 ± 0.42	1.00	

[a] Adherence relative to that of yeasts suspended in PBS

[b] NS, not significant

[c] Probability values comparing adherence in the presence of sugar with adherence in the presence of PBS only.

Table 27 Effect of different lectins on the adherence of C. albicans GDH 2346 to buccal epithelial cells

Lectin used to pretreat epithelial cells[a]	Sugar specificity of lectin	Mean no. of adherent yeasts/epithelial cell ± SEM	Relative adherence[b]	P
Canavalia ensiformis (Jack bean)	D-mannose, D-glucose	15.08 ± 0.71	1..39	<0.001
Lens culinaris (Lentil)	D-mannose, D-glucose	13.67 ± 0.33	1.27	<0.001
Triticum vulgaris (Wheat germ)	N-acetyl-D-glucosamine	10.05 ± 1.24	0.92	NS[c]
Arachis hypogea (Peanut)	D-galactose	9.33 ± 0.94	0.86	NS
Lotus tetragonolobus (Winged pea)	L-fucose	6.23 ± 1.07	0.57	<0.001
PBS (Control)		10.88 ± 0.90	1.00	

[a]Prior to adhesion assays, buccal epithelial cells were incubated for 30 min at 37°C in PBS or in a solution (100 $\mu g ml^{-1}$) of lectin. After this pretreatment, buccal cells were recovered by centrifugation, resuspended in PBS and used in adhesion assays.

[b]Adherence relative to yeasts suspended in PBS.

[c]NS, not significant.

inhibited adherence suggesting that L-fucose, which is present in glycoprotein or glycolipid, may be the receptor involved in yeast attachment. Epithelial cells pretreated with Concanavalin A (Jack bean) and lentil lectin resulted in an increased number of adhering yeasts. This may be due to the assay being performed at pH 7.2 where Con A exists as a pseudotetrahedral molecule with four saccharide binding sites, one in each subunit. These results suggest that Con A bound to mannose containing moieties on both epithelial and yeast cell surfaces. Lentil lectin has two saccharide binding sites and this may explain why enhanced adherence occurred. Pretreatment of epithelial cells with peanut lectin did not have any effect on adherence suggesting that D-galactose is not involved in the attachment of this strain. Wheat germ agglutinin only slightly inhibited yeast adherence to lectin pretreated epithelial cells.

The effect of these lectins on adherence of C. albicans GDH 2023 are shown in Table 28. Wheat germ agglutinin was the most efficient inhibitor of adherence whereas winged pea and peanut lectin did not have any significant effect. Con A and lentil lectin both enhanced adherence. These results suggest that this strain attaches to a receptor containing N-acetyl-D-glucosamine whereas C. albicans GDH 2346 attaches to a receptor containing L-fucose.

The results for the effects of lectins on C. albicans strains GRI 681 and GRI 682 (Tables 29 and 30) were similar to those obtained with C. albicans GDH 2346. Peanut lectin and wheat germ agglutinin had no effect on yeast adherence, whereas Con A and lentil lectin enhanced adherence and winged pea inhibited adherence.

For comparison, the effect of lectins on adherence of a reference laboratory strain C. albicans MRL 3153 was investigated (Table 31). These results were similar to those obtained with C. albicans GDH 2346.

Table 28 Effect of different lectins on the adherence of *C. albicans* GDH 2023 to buccal epithelial cells

Lectin used to pretreat epithelial cells[a]	Sugar specificity of lectin	Mean no. of adherent yeasts/epithelial cell ± SEM	Relative adherence[b]	P
Lens culinaris (Lentil)	D-mannose, D-glucose	16.97 ± 0.27	1.39	<0.001
Canavalia ensiformis (Jack bean)	D-mannose, D-glucose	15.28 ± 0.76	1.25	<0.001
Lotus tetragonolobus (Winged pea)	L-fucose	11.71 ± 0.79	0.96	NS[c]
Arachis hypogea (Peanut)	D-galactose	11.62 ± 0.69	0.95	NS
Triticum vulgaris (Wheat germ)	N-acetyl-D-glucosamine	9.49 ± 0.67	0.78	<0.001
PBS (Control)		12.17 ± 1.0	1.00	

[a]Prior to adhesion assays, buccal epithelial cells were incubated for 30 min at 37°C in PBS or in a solution (100 $\mu g ml^{-1}$) of lectin. After this pretreatment, buccal cells were recovered by centrifugation, resuspended in PBS and used in adhesion assays.

[b]Adherence relative to that of yeasts suspended in PBS.

[c]NS, not significant.

Table 29 Effect of different lectins on the adherence of *C. albicans* GRI 681 to buccal epithelial cells

Lectin used to pretreat epithelial cells[a]	Sugar specificity of lectin	Mean no. of adherent yeasts/epithelial cell ± SEM	Relative adherence[b]	P
Canavalia ensiformis (Jack bean)	D-mannose, D-glucose	10.59 ± 0.37	2.57	<0.001
Lens culinaris (Lentil)	D-mannose, D-glucose	6.25 ± 0.46	1.52	<0.001
Triticum vulgaris (Wheat germ)	N-acetyl-D-glucosamine	4.33 ± 0.55	1.05	NS[c]
Arachis hypogea (Peanut)	D-galactose	4.09 ± 0.29	0.99	NS
Lotus tetragonolobus (Winged pea)	L-fucose	2.64 ± 0.15	0.64	<0.001
PBS (Control)		4.12 ± 0.49	1.00	

[a]Prior to adhesion assays, buccal epithelial cells were incubated for 30 min at 37°C in PBS or in a solution (100 $\mu g ml^{-1}$) of lectin. After this pretreatment, buccal cells were recovered by centrifugation, resuspended in PBS and used in adhesion assays

[b]Adherence relative to that of yeasts suspended in PBS

[c]NS, not significant

Table 30 Effect of different lectins on the adherence of C. albicans GRI 682 to buccal epithelial cells

Lectin used to pretreat epithelial cells[a]	Sugar specificity of lectin	Mean no. of adherent yeasts/epithelial cell ± SEM	Relative adherence[b]	P
Canavalia ensiformis (Jack bean)	D-mannose, D-glucose	9.05 ± 0.46	2.52	<0.001
Lens culinaris (Lentil)	D-mannose, D-glucose	6.66 ± 0.56	1.85	<0.001
Arachis hypogea (Peanut)	D-galactose	3.98 ± 0.41	1.10	NS[c]
Triticum vulgaris (Wheat germ)	N-acetyl-D-glucosamine	3.57 ± 0.35	0.99	NS
Lotus tetragonolobus (Winged pea)	L-fucose	2.28 ± 0.02	0.63	<0.001
PBS (Control)		3.59 ± 0.28	1.00	

[a]Prior to adhesion assays, buccal epithelial cells were incubated for 30 min at 37°C in PBS or in a solution (100 $\mu g ml^{-1}$) of lectin. After this pretreatment, buccal cells were recovered by centrifugation, resuspended in PBS and used in adhesion assays

[b]Adherence relative to that of yeasts suspended in PBS

[c]NS, not significant

Table 31 Effect of different lectins on the adherence of C. albicans MRL 3153 to buccal epithelial cells

Lectin used to pretreat epithelial cells[a]	Sugar specificity of lectin	Mean no. of adherent yeasts/epithelial cell ± SEM	Relative adherence[b]	P
Canavalia ensiformis (Jack bean)	D-mannose, D-glucose	15.46 ± 1.10	1.55	<0.001
Lens culinaris (Lentil)	D-mannose, D-glucose	11.99 ± 0.39	1.20	<0.001
Triticum vulgaris (Wheat germ)	N-acetyl-D-glucosamine	11.62 ± 0.85	1.16	NS[c]
Arachis hypogea (Peanut)	D-galactose	10.92 ± 0.76	1.04	NS
Lotus tetragonolobus (Winged pea)	L-fucose	6.68 ± 0.60	0.66	<0.001
PBS (Control)		9.98 ± 0.26	1.00	

[a]Prior to adhesion assays, buccal epithelial cells were incubated for 30 min at 37°C in PBS or in a solution (100 $\mu g ml^{-1}$) of lectin. After this pretreatment, buccal cells were recovered by centrifugation, resuspended in PBS and used in adhesion assays

[b]Adhesion relative to that of yeasts suspended in PBS

[c]NS, not significant

(iii) Effect of lectins and sugars on adherence to human vaginal epithelial cells

L-Fucose inhibited adherence of C. albicans GDH 2346 to buccal epithelial cells while N-acetyl-D-glucosamine inhibited adherence of C. albicans GDH 2023. Lectins with specificities for these sugars also inhibited adherence of each respective strain to vaginal epithelial cells. The results shown in Table 32 suggest that the mechanism of adherence is similar for both buccal and vaginal epithelial cells. The adherence of C. albicans GDH 2346 was inhibited by preincubating vaginal cells with winged pea lectin or by adding L-fucose to assay mixtures. The adherence of C. albicans GDH 2023 was similarly inhibited by N-acetyl-D-glucosamine and wheat germ agglutinin.

(iv) Effect of fibronectin on adherence

Fibronectin is known to be present on the surface of buccal epithelial cells. It is thought that it might serve as a receptor for microorganisms or mask receptor molecules preventing adherence. Results in Table 33 reveal the effect of fibronectin on adherence after preincubation of buccal epithelial cells with fibronectin prior to their use in adherence assays. The two samples used in this assay were isolated from human plasma by affinity chromatography with gelatin-Sepharose. Samples were used at a concentration of 100 $\mu g ml^{-1}$. Both samples of fibronectin inhibited adherence (Table 33), although sample 7 was more efficient than sample 6. The major differences between the two samples were that sample 7 contained several small molecular weight components (A.J. Lancaster, unpublished results). It is possible that fibronectin binds to epithelial cells blocking attachment by masking the glycoside receptors for the yeast.

D. 'Lectin-like' interactions and their possible role in adherence

Table 32 Effect of principal inhibitory sugars and lectins on adherence of C. albicans strains GDH 2346 and GDH 2023 to human vaginal epithelial cells

Strain	Sugar or lectin used to pretreat vaginal epithelial cells[a]	Mean no. of adherent yeasts/epithelial cell ± SEM	Relative adherence[b]	P
GDH 2346	L-fucose	6.72 ± 0.41	0.70	<0.001
	N-acetyl-D-glucosamine	9.51 ± 0.48	0.99	NS[c]
	Winged pea lectin	7.01 ± 0.28	0.73	<0.001
	Wheat germ agglutinin	8.39 ± 0.59	0.87	NS
	PBS	9.59 ± 0.24	1.00	
GDH 2023	L-fucose	10.82 ± 0.13	0.98	NS
	N-acetyl-D-glucosamine	6.15 ± 0.33	0.56	<0.001
	Winged pea lectin	10.24 ± 0.45	0.93	NS
	Wheat germ agglutinin	7.56 ± 0.37	0.69	<0.001
	PBS	11.01 ± 0.16	1.00	

[a]Prior to adhesion assays, vaginal epithelial cells were incubated for 30 min at 37°C in PBS or in a solution (100 $\mu g ml^{-1}$) of lectin. Sugars (25 $mg ml^{-1}$) were added to adhesion assay mixtures.

[b]Adherence relative to that of yeasts suspended in PBS

[c]NS, not significant

Table 33 Effect of human plasma fibronectin on the adherence of C. albicans GDH 2346 to buccal epithelial cells

Sample used to pretreat epithelial cells	Mean no. of adherent yeasts/epithelial cell ± SEM	Percentage inhibition of adherence[c]	Pa	Pb
Fibronectin (sample 6)	6.02 ± 0.15	28.9	<0.001	<0.01
Fibronectin (sample 7)	4.61 ± 0.11	45.6	<0.001	
PBS	8.47 ± 0.24	0.0		

Pa, both samples compared with PBS

Pb, comparing fibronectin samples

[c]Calculated as the percentage of yeasts not adhering to fibronectin pretreated epithelial cells when compared with adherence to PBS pretreated epithelial cells

(i) Effect of 'lectin-like' components isolated from EPM on adherence

EPM samples from each strain were applied to affinity columns of sugar coupled to Sepharose. The percentage of EPM protein bound to each affinity column for each strain of C. albicans is shown in Figure 11. All bound fractions were tested for their ability to inhibit adherence by preincubating them with buccal epithelial cells before using the cells in adherence assays. The results for the effect of these column fractions on the adherence of C. albicans GDH 2346 to epithelial cells are shown in Table 34. The L-fucose-bound fraction inhibited adherence by 65% and was 53 times more efficient than crude EPM at inhibiting adherence as revealed by AII values (Table 39). EPM components which bound to D-mannose and N-acetyl-D-glucosamine Sepharose columns were also capable of inhibiting yeast adherence but not to the same extent as the L-fucose-bound fraction.

The L-fucose-Sepharose bound fraction from C. albicans MRL 3153 EPM inhibited yeast adherence by 52% (Table 35) and was 33 times more active an inhibitor of adherence than crude EPM (Table 39). D-Mannose and N-acetyl-D-glucosamine bound fractions also inhibited adherence resulting in 20 and 16% adherence inhibition respectively.

The L-fucose-bound fractions of C. albicans GRI 681 and GRI 682 inhibited adherence of these strains by 30% and 29% respectively (Tables 36 and 37). These two strains produced EPM which had similar sugar binding specificities (Figure 10). The fractions from these two strains which bound to columns of sugar coupled to Sepharose inhibited adherence to the same extent and gave similar AII values (Table 39).

The inhibition pattern obtained with bound fractions from C. albicans GDH 2023 were different from those obtained with the other four strains. All bound fractions from C. albicans GDH 2023 EPM inhibited adherence to different extents (Table 38). The most active adherence

Table 34 Effect of 'lectin-like' material isolated from EPM by affinity chromatography on the adherence of C. albicans GDH 2346 to buccal epithelial cells

Sugar coupled to Sepharose gel[a]	Mean no. of adherent yeasts/epithelial cell ± SEM	Percentage inhibition of adherence[b]
L-fucose	2.73 ± 0.18	65.2
D-mannose	5.77 ± 0.14	16.4
N-acetyl-D-glucosamine	6.28 ± 0.27	19.8
D-galactose	7.19 ± 0.13	8.4
N-acetyl-D-galactosamine	7.88 ± 0.45	0.0
sialic acid	8.33 ± 0.39	0.0
PBS control	7.84 ± 0.54	0.0

[a]Samples of EPM (10 $mgml^{-1}$) were applied to mini columns of Epoxy-activated Sepharose with coupled sugar. Material which bound to the column was eluted with the appropriate sugar. This sample was dialysed and used to pretreat epithelial cells prior to their use in adhesion assays.

[b]Calculated as the percentage of yeasts not adhering when compared with adherence of yeasts suspended in PBS.

EPM was isolated from C. albicans GDH 2346 as described in materials and methods.

Table 35 Effect of 'lectin-like' material isolated from EPM by affinity chromatography on the adherence of C. albicans MRL 3153 to buccal epithelial cells

Sugar coupled to Sepharose gel[a]	Mean no. of adherent yeasts/epithelial cell ± SEM	Percentage inhibition of adherence[b]
L-fucose	4.27 ± 0.19	52.6
D-mannose	7.23 ± 0.13	19.6
N-acetyl-D-glucosamine	7.58 ± 0.16	15.7
N-acetyl-D-galactosamine	8.39 ± 0.14	6.8
sialic acid	8.52 ± 0.22	5.4
D-galactose	8.58 ± 0.18	4.7
PBS control	9.00 ± 0.19	0.0

[a]Samples of EPM (10 $mgml^{-1}$) were applied to mini columns of Epoxy-activated Sepharose with coupled sugar. Material which bound to the column was eluted with the appropriate sugar. This sample was dialysed and used to pretreat epithelial cells prior to their use in adhesion assays.

[b]Calculated as the percentage of yeasts not adhering when compared with adherence of yeasts suspended in PBS.

EPM was isolated from C. albicans MRL 3153 as described in materials and methods.

Table 36 Effect of 'lectin-like' material isolated from EPM by affinity chromatography on the adherence of C. albicans GRI 681 to buccal epithelial cells

Sugar coupled to Sepharose gel[a]	Mean no. of adherent yeasts/epithelial cell ± SEM	Percentage inhibition of adherence[b]
L-fucose	2.42 ± 0.12	30.2
D-mannose	3.13 ± 0.12	10.0
N-acetyl-D-glucosamine	3.23 ± 0.17	7.2
N-acetyl-D-galactosamine	3.52 ± 0.12	0.0
D-galactose	3.60 ± 0.08	0.0
sialic acid	3.80 ± 0.14	0.0
PBS control	3.48 ± 0.13	0.0

[a]Samples of EPM (10 $mgml^{-1}$) were applied to mini columns of Epoxy-activated Sepharose with coupled sugar. Material which bound to the column was eluted with the appropriate sugar. This sample was dialysed and used to pretreat epithelial cells prior to their use in adhesion assays.

[b]Calculated on the percentage of yeasts not adhering when compared with adherence of yeasts suspended in PBS.

EPM was isolated from C. albicans GRI 681 as described in materials and methods.

Table 37 Effect of 'lectin-like' material isolated from EPM by affinity chromatography on the adherence of C. albicans GRI 682 to buccal epithelial cells

Sugar coupled to Sepharose gel[a]	Mean no. of adherent yeasts/epithelial cell ± SEM	Percentage inhibition of adherence[b]
L-fucose	3.36 ± 0.08	24.5
D-mannose	4.03 ± 0.06	9.4
N-acetyl-D-glucosamine	4.19 ± 0.07	5.8
N-acetyl-D-galactosamine	4.38 ± 0.11	1.6
D-galactose	4.49 ± 0.12	0.0
sialic acid	4.64 ± 0.12	0.0
PBS control	4.45 ± 0.12	0.0

[a]Samples of EPM (10 $mgml^{-1}$) were applied to mini columns of Epoxy-activated Sepharose with coupled sugar. Material which bound to the column was eluted with the appropriate sugar. This sample was dialysed and used to pretreat epithelial cells prior to their use in adhesion assays

[b]Calculated as the percentage of yeasts not adhering when compared with adherence of yeasts suspended in PBS

EPM was isolated from C. albicans GRI 682 as described in materials and methods.

Table 38 Effect of 'lectin-like' material isolated from EPM by affinity chromatography on the adherence of C. albicans GDH 2023 to buccal epithelial cells

Sugar coupled to Sepharose gel[a]	Mean no. of adherent yeasts/epithelial cell ± SEM	Percentage inhibition of adherence[b]
L-fucose	6.43 ± 0.16	41.3
D-mannose	9.63 ± 0.31	12.1
N-acetyl-D-glucosamine	9.82 ± 0.38	10.4
N-acetyl-D-galactosamine	10.16 ± 0.31	7.3
D-galactose	10.60 ± 0.13	3.3
sialic acid	10.90 ± 0.19	0.5
PBS control	10.96 ± 0.21	0.0

[a]Samples of EPM (10 $mgml^{-1}$) were applied to mini columns of Epoxy-activated Sepharose with coupled sugar. Material which bound to the column was eluted with the appropriate sugar. This sample was dialysed and used to pretreat epithelial cells prior to their use in adhesion assays.

[b]Calculated as the percentage of yeasts not adhering when compared with adherence of yeasts suspended in PBS.

EPM was isolated from C. albicans GDH 2023 as described in materials and methods.

Table 39 'Lectin-like' material isolated from EPM by affinity chromatography: AII values for different *C. albicans* strains

Sugar coupled to Sepharose gel[a]	*C. albicans* strain				
	GDH 2346	MRL 3153	GRI 681	GRI 682	GDH 2023
D-mannose	25.63	16.23	66.6	62.9	5.71
L-fucose	53.40	33.50	74.15	73.17	33.40
N-acetyl-D-glucosamine	17.70	21.33	48.00	37.80	22.90
D-galactose	-	-	-	-	5.70
sialic acid	-	-	-	-	4.15
N-acetyl-D-galactosamine	-	-	-	-	-

[a]Samples of EPM (10 $mgml^{-1}$) from each strain of *C. albicans* were applied to columns of Epoxy-activated Sepharose with coupled sugar. Material which bound to the column was eluted with the appropriate sugar. This sample was dialysed and used to pretreat epithelial cells prior to their use in adhesion assays. These samples were analysed for protein so that AII values could be calculated (see Appendix IV for worked example).

inhibiting fractions were those which bound to the L-fucose and N-acetyl-D-glucosamine Sepharose columns with AII values of 33.4 and 22.9 respectively (Table 39). This strain had much lower AII values with D-mannose-bound fractions than all the other strains, yet D-mannose bound the largest percentage of protein from EPM (Figure 10). D-Galactose- and sialic acid-bound fractions also had low AII values.

(ii) Effect of cations on the ability of EPM to inhibit adherence

The effect of cations on the inhibitory effect of EPM was determined by dissolving EPM in 50 mM Tris-HCl, pH 7.2 containing Ca^{2+}, Mn^{2+} and Mg^{2+} at a 2 mM concentration. As cations are normally required for the binding activities of most lectins any enhanced adherence inhibition by EPM in the presence of cations may be the result of 'lectin-like' binding. EPM dissolved in buffer containing cations was slightly more efficient at inhibiting adherence than EPM dissolved in PBS. This effect was seen with all three strains used here, the degree of enhancement being similar with each strain (Table 40). These results provide further evidence that 'lectin-like' interactions play a role in mediating adherence of C. albicans to epithelial cells.

IV STRAIN-RELATED DIFFERENCES IN ADHERENCE OF C. ALBICANS

A. Effect of EPM from different strains on adherence of the same strain and other strains to buccal epithelial cells

C. albicans GDH 2346 and other I strains are more adherent to epithelial cells than the C strains GRI 681 and GRI 682 after growth in YNB medium containing 500 mM galactose. It is possible that differences in ability of various strains to adhere reflects differences in the proportion of adhesin material present on the yeast surface and hence EPM.

Table 40 Effect of cations on the ability of EPM to inhibit adherence of C. albicans GDH 2346 to buccal epithelial cells

Pretreatment of epithelial cells[a]	Strain used in assay	Mean no. of adherent yeasts/epithelial cell ± SEM	Relative adherence[b]	P[c]
GDH 2346 EPM + cations		4.94 ± 0.33	0.51	<0.05
GDH 2346 EPM - cations	GDH 2346	5.61 ± 0.33	0.57	
PBS		9.79 ± 0.89	1.00	
GDH 2023 EPM + cations		6.48 ± 0.46	0.48	<0.01
GDH 2023 EPM - cations	GDH 2023	7.68 ± 0.68	0.57	
PBS		13.32 ± 0.40	1.00	
MRL 3153 EPM + cations		4.90 ± 0.27	0.53	<0.01
MRL 3153 EPM - cations	MRL 3153	5.56 ± 0.17	0.60	
PBS		9.26 ± 0.49	1.00	

[a]Prior to adhesion assays, buccal epithelial cells were incubated for 30 min at 37°C in PBS or in a solution (10 $mgml^{-1}$) of EPM produced by C. albicans GDH 2346, GDH 2023 and GRI 681. EPM was dissolved either in PBS or in 0.05M Tris HCl (pH 7.2) containing 0.002M calcium, magnesium and manganese ions.

[b]Adherence relative to that of yeasts suspended in PBS.

[c]Probability values comparing adherence to EPM plus cations treated epithelial cells to EPM treated cells in the absence of cations.

EPM isolated from C strains only significantly inhibited adherence of homologous strains (Table 41) and had little effect on adherence of the I strains (GDH 2346, MRL 3153 and GDH 2023) to epithelial cells. On the other hand EPM isolated from C. albicans strains GDH 2346 and MRL 3153 were efficient inhibitors of adherence of all strains, except C. albicans GDH 2023 (Table 41). Therefore, C. albicans strains GDH 2346 and MRL 3153 produced EPM which was similar in structure but differed slightly in the proportion of adhesin material present in EPM. C. albicans GDH 2023 EPM was only capable of significantly inhibiting adherence of C. albicans GDH 2023 to epithelial cells (Table 41). This result indicated that there were differences in the nature of the EPM produced by C. albicans GDH 2023 and other I strains and that these strains may have different adherence mechanisms.

B. Immunological studies

(i) Agglutination reactions

Previous work by McCourtie and Douglas (1985) found that there were antigenic differences between C. albicans strains GDH 2346 and GDH 2023. They prepared antisera to C. albicans GDH 2346 EPM which was used in cross-agglutination assays with yeast suspensions of homologous yeast cells and GDH 2023 yeast cells. Incubation of sera with suspensions of C. albicans GDH 2023 gave considerably lower titres than homologous cells suggesting that the EPM produced by these strains was different.

In this study antiserum against EPM from C. albicans GDH 2023 grown in medium containing 500 mM galactose was raised in a rabbit using Freund's complete adjuvant. Double diffusion tests, using the Ouchterlony slide method, gave a single precipitin line with homologous antigen. Slide agglutination tests with the antiserum and whole yeast cells also

Table 41 Effect of EPM preparations on the adherence of C. albicans strains to human buccal epithelial cells: summary of results

Source of EPM preparation used in assay[a]	Percentage inhibition of adhesion of C. albicans strain[b]				
	GRI 681	GRI 682	MRL 3153	GDH 2346	GDH 2023
GRI 681	25.0	5.6	0.0	2.1	3.3
GRI 682	0.0	29.0	2.7	3.5	14.8
MRL 3153	46.1	46.3	56.8	30.5	13.7
GDH 2346	60.1	59.2	50.8	50.0	30.8
GDH 2023	12.2	6.9	0.0	0.0	55.5

[a]Epithelial cells were pretreated with EPM from each C. albicans strain at 37°C for 30 min. After this treatment, epithelial cells were recovered by centrifugation, resuspended in PBS and used in adhesion assays with the five strains of C. albicans.

[b]Calculated as the percentage of yeasts not adhering to EPM-treated epithelial cells when compared with adherence of yeasts to PBS-treated cells.

proved positive. Agglutination titres were performed by adding doubling dilutions of antisera in saline to a standardized yeast suspension. The endpoint of the titration was recorded as the highest dilution of antiserum which gave no visible agglutination of yeast suspension. The antiserum was titrated with C. albicans GDH 2023 suspension (homologous cells) and C. albicans GDH 2346 suspension. The mean reciprocal titre with C. albicans GDH 2023 was 768 and with C. albicans GDH 2346 was 32 (Table 42). These results suggest that the antigenic determinants of the EPM from these two strains are different.

(ii) Effect of antiserum on adherence

Antisera raised against C. albicans GDH 2023 EPM was examined for its effect on the adherence of C. albicans GDH 2023, C. albicans GDH 2346 and C. albicans GRI 681 to epithelial cells. The concentration of antiserum used did not agglutinate any of the yeast cells. Higher concentrations of antibody were required to inhibit adherence of C. albicans strains GDH 2346 and GRI 681 than were required to inhibit adherence of C. albicans GDH 2023 (Table 43). Therefore, antiserum is more efficient at inhibiting adherence of the strain from which the EPM was isolated. These results also indicated that there are immunological differences between the cell surfaces of C. albicans GDH 2346 and C. albicans GDH 2023.

V ADHERENCE OF DIFFERENT SPECIES OF CANDIDA

C. albicans has been widely reported to be the most pathogenic and most adherent of the Candida species. In this study the adherence of several Candida species to epithelial and acrylic surfaces was measured after growth in YNB containing 50 mM glucose or 500 mM galactose.

Table 42 Agglutination titres of serum raised against EPM obtained from the culture supernatant of C. albicans GDH 2023

Strains used in agglutination assay[a]	Mean reciprocal titre of antiserum raised against EPM from C. albicans GDH 2023[b]
GDH 2023	768
GDH 2346	32

[a] Yeasts were grown in YNB medium containing 500 mM galactose

[b] Antiserum was raised as described in materials and methods. EPM was isolated from C. albicans GDH 2023 after growth in YNB containing 500 mM galactose.

Agglutination assays were performed twice in duplicate.

Table 43 Effect of antisera raised against EPM from C. albicans GDH 2023 on the adherence of several strains of C. albicans to buccal epithelial cells

Strain used in assay	Dilution of antiserum[a]	Mean no. of adherent yeasts/epithelial cell ± SEM	Relative adherence[b]	P[c]
GDH 2346	1/64	5.04 ± 0.43	0.50	<0.001
	1/1024	10.34 ± 0.37	1.03	NS
	PBS	9.99 ± 0.38	1.00	
GDH 2023	1/1024	4.25 ± 0.54	0.37	<0.001
	1/2048	8.39 ± 0.42	0.74	<0.001
	PBS	11.26 ± 0.29	1.00	
GRI 681	1/64	2.73 ± 0.14	0.74	<0.01
	1/1024	4.10 ± 0.39	1.10	NS
	PBS	3.67 ± 0.19	1.00	

[a]The concentration of antiserum used did not agglutinate yeast cells and was confirmed by agglutination assays and slide agglutination tests. Yeast cells were pretreated with the appropriate concentration of antiserum at 37°C for 30 min. Cells were recovered by centrifugation and used in adhesion assays as described in materials and methods.

[b]Adherence relative to that of yeasts suspended in PBS.

[c]Probability values comparing adherence of yeasts treated with antiserum and yeasts treated with PBS to epithelial cells.

A. Adherence to buccal epithelial cells

The 'outbreak' strain of C. albicans had the highest relative adherence value of all the C. albicans strains. Yeasts were up to 8 times more adherent after growth in medium containing galactose than after growth in medium containing glucose (Table 44). This suggests that the 'outbreak' strain has a potential for cell-surface modification when grown in high galactose-containing media. C. albicans (serotype B) and C. albicans NCTC 3156, also serotype B, showed enhanced adherence after growth in medium containing galactose, although enhancement was not as great as with the 'outbreak' strain. Of the other Candida species tested, only one strain (the Glasgow strain) of C. tropicalis showed an increase in adherence of more than three-fold. C. tropicalis (London strain), C. stellatoidea and C. parapsilosis gave smaller increases in adherence. C. guilliermondii and C. pseudotropicalis were not very adherent and showed no enhancement of adherence after growth in medium containing 500 mM galactose. These results indicate that the less or non-pathogenic yeast species have little or no potential for cell-surface modification. No significant differences in adherence of the non-pathogenic yeast Saccharomyces cerevisiae X2180 was observed after growth in medium containing either carbon source.

B. Adherence to acrylic surfaces

C. albicans 'outbreak' strain was more than 13 times more adherent to acrylic surfaces after growth in medium containing 500 mM galactose than after growth in medium containing 50 mM glucose (Table 45). This strain was by far the most adherent strain of C. albicans. The adherence of C. albicans GDH 2346 to acrylic was enhanced by 9-fold. By contrast, the other yeast species tested showed little or no increase in adherence.

Table 44 Adherence of different yeast species to buccal epithelial cells after growth in medium containing 50 mM glucose or 500 mM galactose

Organism	Carbon source	Mean no. of adherent yeasts/epithelial cell ± SEM	Relative adherence[a]	P[b]
C. albicans 'outbreak' strain	Glucose	1.48 ± 0.04	1.00	<0.001
	Galactose	12.27 ± 0.25	8.29	
C. albicans GDH 2346	Glucose	1.51 ± 0.04	1.00	<0.001
	Galactose	10.88 ± 0.16	7.21	
C. albicans serotype B	Glucose	2.10 ± 0.09	1.00	<0.001
	Galactose	12.67 ± 0.20	6.03	
C. albicans NCTC 3156	Glucose	1.87 ± 0.10	1.00	<0.001
	Galactose	8.20 ± 0.07	4.38	
C. tropicalis (Glasgow strain)	Glucose	1,54 ± 0.11	1.00	<0.001
	Galactose	5.25 ± 0.13	3.41	
C. tropicalis (London strain)	Glucose	2.07 ± 0.05	1.00	<0.001
	Galactose	2.72 ± 0.07	1.31	
C. stellatoidea	Glucose	1.28 ± 0.08	1.00	<0.001
	Galactose	2.37 ± 0.08	1.85	
C. parapsilosis	Glucose	1.06 ± 0.02	1.00	<0.001
	Galactose	1.84 ± 0.07	1.74	
C. guilliermondii	Glucose	1.05 ± 0.07	1.00	NS[c]
	Galactose	1.03 ± 0.06	1.00	
C. pseudotropicalis	Glucose	0.85 ± 0.08	1.00	NS
	Galactose	0.92 ± 0.07	1.10	
S. cerevisiae X-2180	Glucose	0.15 ± 0.01	1.00	NS
	Galactose	0.15 ± 0.01	1.00	

[a] Adherence relative to that of glucose-grown yeasts of the same strain

[b] Probability values comparing adherence of glucose- and galactose-grown yeasts

[c] NS, not significant

Table 45 Adherence of different yeast species to acrylic after growth in medium containing 50 mM glucose or 500 mM galactose

Organism	Carbon source	Mean no. of adherent yeasts/mm^2 ± SEM	Relative adherence[a]	P[b]
C. albicans 'outbreak' strain	Glucose	70 ± 56	1.00	<0.001
	Galactose	960 ± 21	13.80	
C. albicans GDH 2346	Glucose	84 ± 5	1.00	<0.001
	Galactose	792 ± 67	9.43	
C. albicans serotype B	Glucose	71 ± 6	1.00	<0.001
	Galactose	638 ± 52	8.99	
C. albicans NCTC 3156	Glucose	56 ± 5	1.00	<0.001
	Galactose	417 ± 14	7.45	
C. tropicalis (Glasgow strain)	Glucose	414 ± 26	1.00	NS[c]
	Galactose	373 ± 20	0.90	
C. tropicalis (London strain)	Glucose	318 ± 16	1.00	NS
	Galactose	342 ± 17	1.10	
C. parapsilosis	Glucose	383 ± 17	1.00	NS
	Galactose	264 ± 26	0.69	
C. stellatoidea	Glucose	38 ± 3	1.00	<0.001
	Galactose	57 ± 4	1.50	
C. guilliermondii	Glucose	20 ± 1	1.00	<0.001
	Galactose	35 ± 3	1.75	
C. pseudotropicalis	Glucose	39 ± 4	1.00	NS
	Galactose	37 ± 4	0.95	
S. cerevisiae X-2180	Glucose	7 ± 1	1.00	NS
	Galactose	10 ± 2	1.43	

[a]Adherence relative to that of glucose-grown yeasts of the same strain

[b]Probability values comparing adherence of glucose- and galactose-grown yeasts

[c]NS, not significant.

Even the Glasgow strain of C. tropicalis which was more adherent to buccal cells after growth in galactose medium (Table 44) adhered to acrylic in similar numbers following growth on either carbon source. However, glucose-grown cells of both strains of C. tropicalis and C. parapsilosis showed significantly greater adherence to acrylic than glucose-grown yeasts of any other species, including C. albicans (Table 45). These results are different from those with buccal epithelial cells as C. tropicalis was more adherent to acrylic than C. albicans after growth in glucose-containing media. Saccharomyces cerevisiae X2180, a non-pathogenic yeast, was not very adherent to acrylic after growth in medium containing either carbon source.

VI IN VIVO STUDIES ON ADHERENCE OF CANDIDA ALBICANS

A. Vaginal and systemic infections

For most of the animal experiments yeasts were grown in YNB containing 50 mM glucose as a carbon source. C. albicans B2630 was a strain which had similar in vitro adherence capabilities as C. albicans GDH 2346 (Table 5). Three strains of C. albicans were compared for their ability to cause vaginal and systemic infections in mice (Table 46). C. albicans strains GDH 2346, GDH 2023 and B2630 were all capable of infecting the vaginas of mice, as revealed by total vaginal counts which were performed 7 days after inoculation. Total kidney counts were used to assess the extent of systemic infections. C. albicans GDH 2023 gave much lower kidney counts than strains GDH 2346 and B2630.

Three strains of C. albicans which are resistant to the antifungal agent ketoconazole were examined for their ability to infect mouse vaginas after growth in defined medium containing 50 mM glucose or 500 mM galactose.

Table 46 Systemic and vaginal infections produced in mice by three strains of *C. albicans*

Strain of *C. albicans*	Total vaginal count (c.f.u. x 10^{-3}) ± SEM	Total kidney count[a] (c.f.u. x 10^{-3})	Total kidney count[b] (c.f.u. x 10^{-3})	
		Day 7	Day 7	Day 21
GDH 2346	65 ± 20	215 ± 107	17.5 ± 6	459 ± 201
GDH 2023	32.5 ± 11	9.3 ± 3.0	5.1 ± 2	178 ± 51
B2630	141 ± 61	360 ± 92	33 ± 14	240 ± 62

[a]Kidney count performed on mice inoculated with 3×10^6 yeasts

[b]Kidney count performed on mice inoculated with 10^5 yeasts

Details of procedures are given in materials and methods

All strains were capable of adhering to buccal epithelial cells and adherence was enhanced by growing in medium containing galactose (Table 47). Growth of the yeasts in 500 mM galactose-containing medium also enhanced their ability to infect mouse vaginas, although the extent of enhancement was not as high as that observed in in vitro adherence assays (Table 5).

Systemic infections were also assessed by following mortality patterns in mice after intravenous challenge with yeast suspensions. In the first set of experiments (Figure 12) yeasts were grown in YNB medium containing 50 mM glucose. C. albicans GDH 2346 was the most virulent strain followed by C. albicans B2630. C. albicans GDH 2023 was not very virulent; only 30% of the mice inoculated with 3×10^6 yeasts died after 30 days (Figure 12c). When the experiment was repeated with a wider range of yeast concentrations (Figure 13), C. albicans strains GDH 2346 and B2630 were more virulent at the higher concentrations resulting in more rapid mortality. However, C. albicans GDH 2023 was still not very virulent even at higher yeast concentrations (Figure 13b).

The effect of growth medium, age and sex of mice was investigated in a separate experiment. A single strain, C. albicans B2630 was used in this experiment. Yeasts were either grown on Sabouraud agar or in YNB medium containing 50 mM glucose. The mortality curve shown in Figure 14a represents the mortality pattern obtained with yeasts grown on Sabouraud agar using young (4-5 week old) female mice. This curve was similar to that obtained with old ($\geqslant$ 7 weeks) female mice (Figure 14b); thus, the age of the mice did not appear to be a variable factor in mortality tests. The mortality curve shown in Figure 15a was obtained with yeasts grown in YNB containing 50 mM glucose. Yeasts grown in this medium were less virulent and took much longer to give 100% mortality. The mortality pattern obtained with yeasts grown on Sabouraud agar using young male mice

Table 47 Vaginal infections produced in mice by ketoconazole-resistant strains of C. albicans grown in different media

Strain of C. albicans	Growth medium	Total vaginal count[a] (c.f.u. x 10^{-3})	Relative infection[b]
AD	YNB + 50 mM glucose	5.21	1.00
	YNB + 500 mM galactose	13.9	2.67
	SDA	5.04	0.96
KB	YNB + 50 mM glucose	3.5	1.00
	YNB + 500 mM galactose	14.5	4.14
	SDA	4.88	1.39
Darlington	YNB + 50 mM glucose	2.06	1.00
	YNB + 500 mM galactose	5.46	2.65
	SDA	0.87	0.43

[a]Results represent the mean from two independent experiments

[b]Extent of infection relative to that caused by yeasts of the same strain grown on YNB containing 50 mM glucose

Details of procedures are given in materials and methods

Figure 12 Mortality patterns in mice obtained with three different strains of C. albicans

Yeasts were grown in YNB medium containing 50 mM glucose.

Mortality patterns obtained in mice with:

(a) C. albicans B2630

(b) C. albicans GDH 2346

(c) C. albicans GDH 2023

Mice, in groups of 20, were inoculated intravenously with:

A 3×10^6 yeasts

B 1×10^6 yeasts

C 3×10^5 yeasts

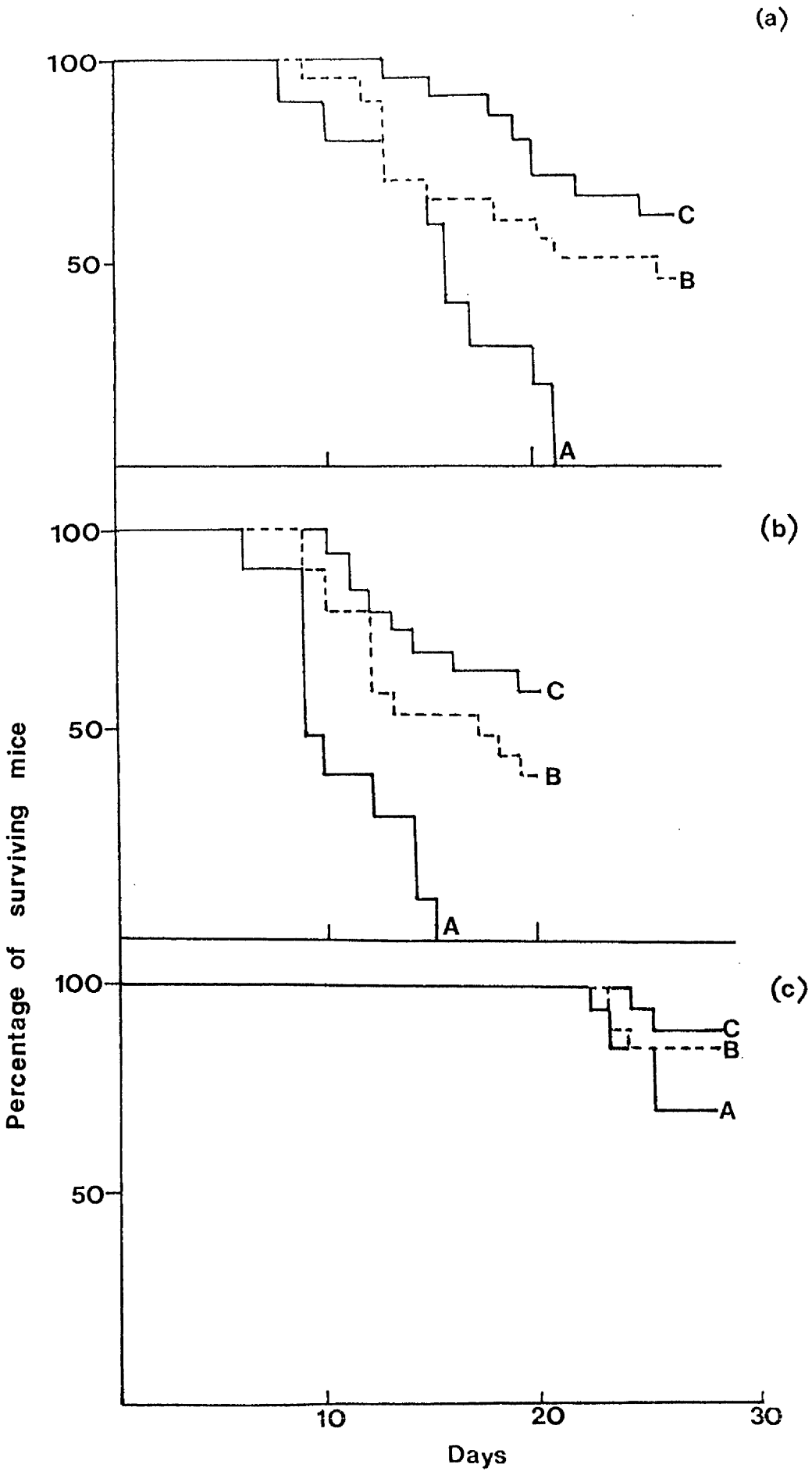

(a)
100
50
C
B
A
(b)
100
50
C
B
A
(c)
100
50
C
B
A
Percentage of surviving mice
10
20
30
Days

Figure 13 Mortality patterns in mice obtained with three different strains of C. albicans: inoculation of mice with five different yeast concentrations.

Yeasts were grown in YNB medium containing 50 mM glucose.

Mortality patterns obtained in mice with:

(a) C. albicans GDH 2346

(b) C. albicans GDH 2023

(c) C. albicans B2630

Mice, in groups of 20, were inoculated intravenously with:

A 1×10^7 yeasts

B 6×10^6 yeasts

C 3×10^6 yeasts

D 6×10^5 yeasts

E 3×10^5 yeasts

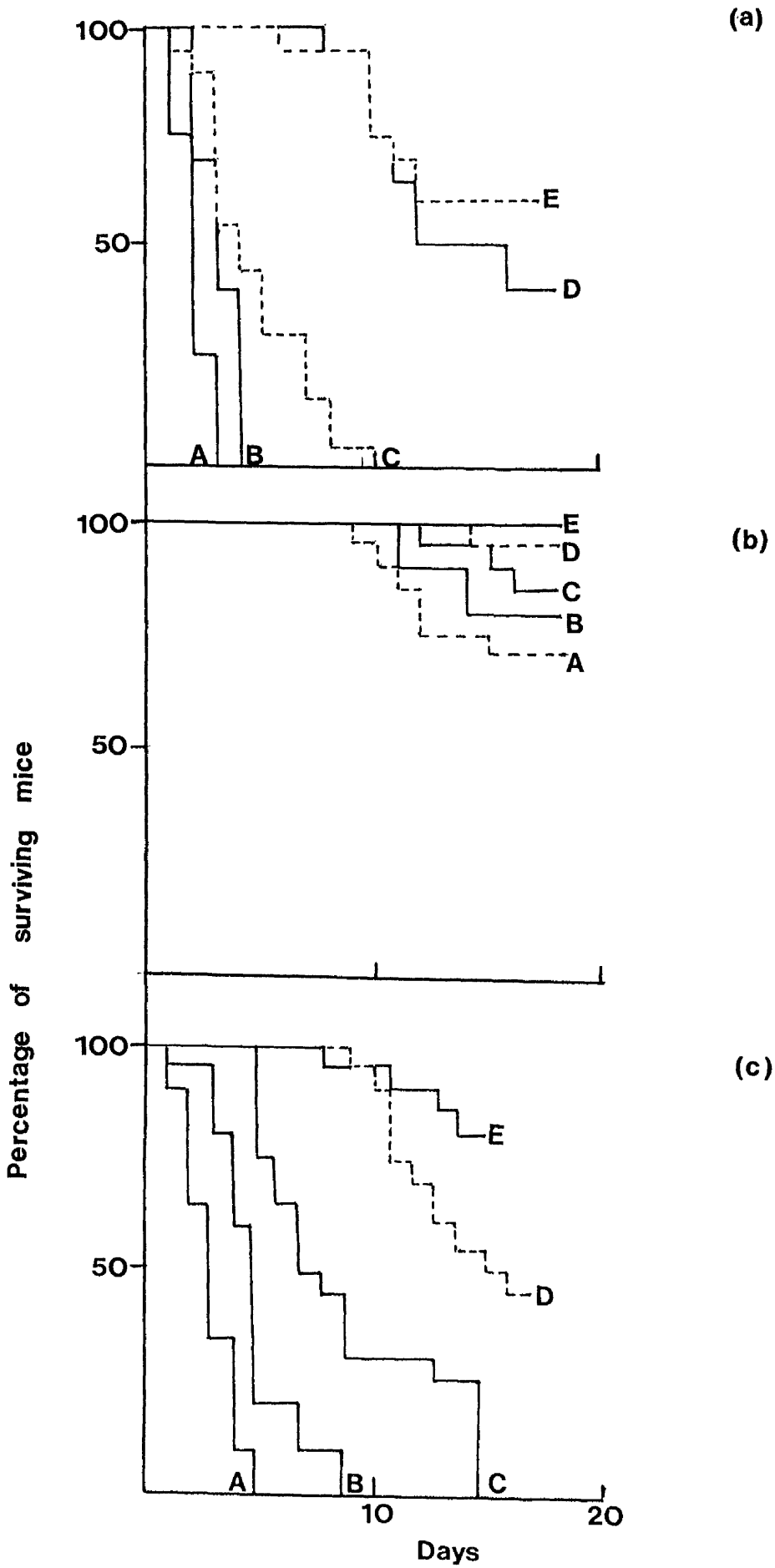

(a)
100
50
E
D
A
B
C
100
(b)
E
D
C
B
A
50
100
(c)
E
D
50
A
B
C
10
20
Days
Percentage of surviving mice

Figure 14 Effect of age of mice on the mortality patterns obtained with C. albicans B2630

(a) Mortality pattern obtained with C. albicans B2630 grown on Sabouraud agar, using young (4 to 5 week) mice (female)

(b) Mortality pattern obtained with C. albicans B2630 grown on Sabouraud agar, using old ($\geqslant$ 7 weeks) mice (female).

Mice, in groups of 20, were inoculated intravenously with: (A) 1×10^7 yeasts; (B) 6×10^6 yeasts; (C) 3×10^6 yeasts; (D) 1×10^6 yeasts; (E) 3×10^5 yeasts and (F) 1×10^5 yeasts.

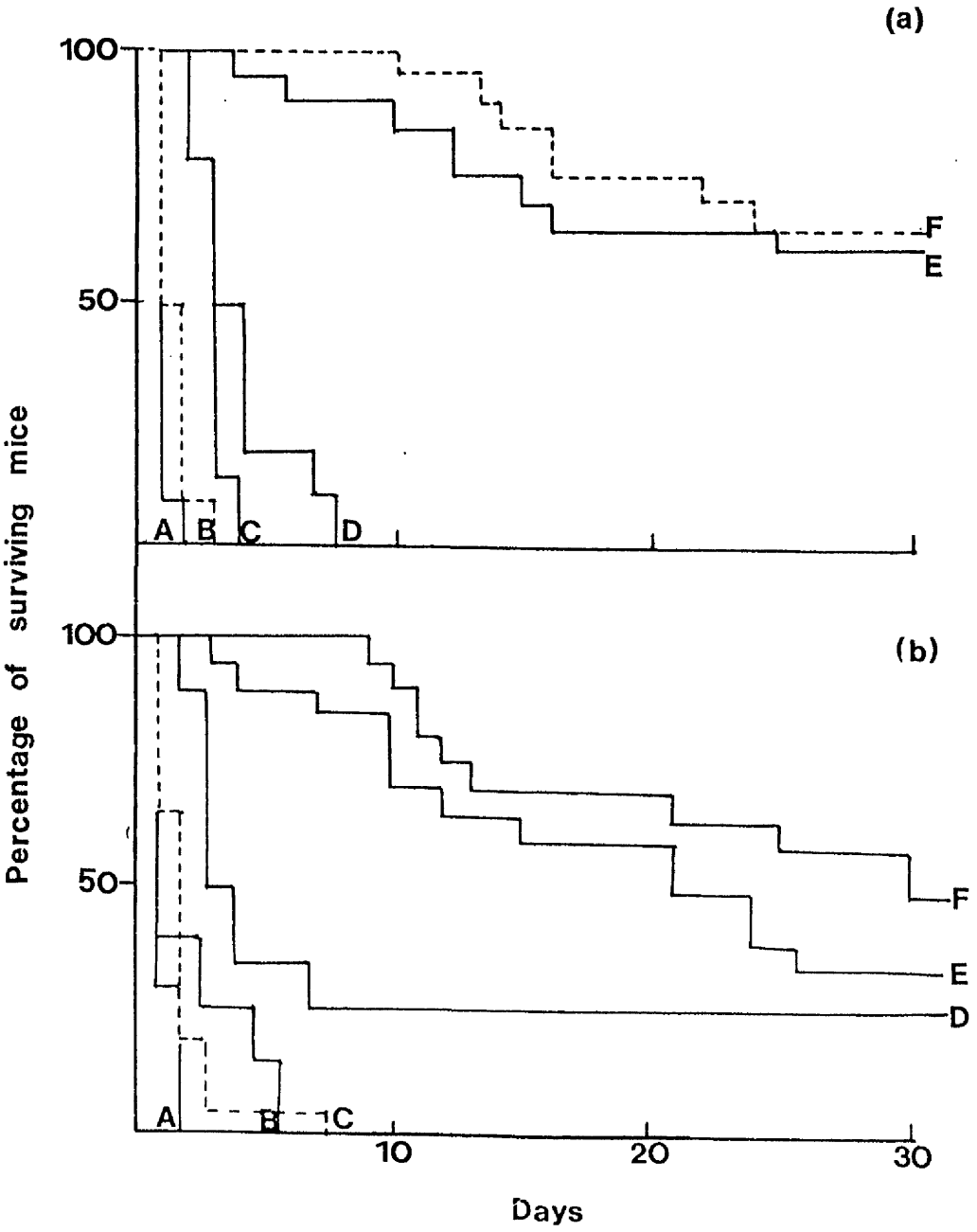
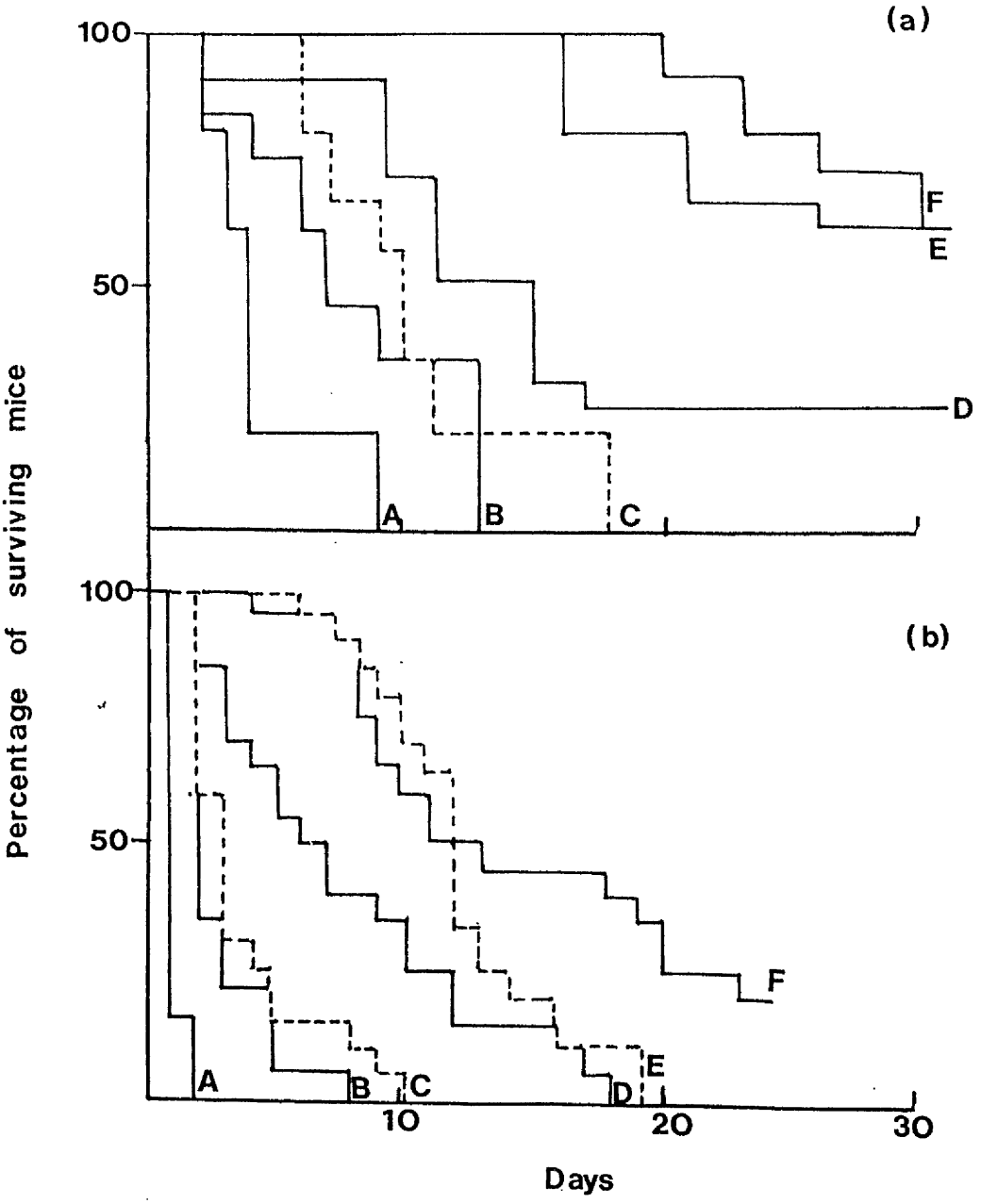

(a)
100
50
A
B
C
D
F
E
(b)
100
50
A
B
C
F
E
D
10
20
30
Days
Percentage of surviving mice

Figure 15 Effect of sex of mice and yeast growth medium on mortality patterns obtained with C. albicans B2630

(a) Mortality pattern obtained with C. albicans B2630 grown on YNB containing 50 mM glucose, using young (4 to 5 weeks) mice (female)

(b) Mortality pattern obtained with C. albicans B2630 grown on Sabouraud agar, using young (4 to 5 weeks) mice (male)

Mice, in groups of 20, were inoculated intravenously with: (A) 1×10^7 yeasts; (B) 6×10^6 yeasts; (C) 3×10^6 yeasts; (D) 1×10^6 yeasts; (E) 3×10^5 yeasts and (F) 1×10^5 yeasts.


(a)
100
50
F
E
D
A
B
C
100
(b)
50
F
E
A
B
C
D
10
20
30
Days
Percentage of surviving mice

is shown in Figure 15b. This mortality pattern is similar to that obtained with agar-grown yeasts using old and young female mice. Therefore, age and sex of mice do not affect the virulence of yeasts; however, the medium used to grow the yeasts appeared to affect their virulence.

B. Use of EPM to block vaginal infections in mice

EPM isolated from the culture supernatant of C. albicans GDH 2346 has been shown to inhibit the adherence of this strain to buccal cells in in vitro assays (Table 48 and Figure 11). The results in Table 49 reveal the effect of pretreating mouse vaginas with EPM (10 $mgml^{-1}$ for 30 min) prior to inoculating them with yeasts. The extent of vaginal infection was assessed from total vaginal counts. Mice pretreated with EPM gave a lower vaginal count than mice pretreated with saline. However, vaginal infection was not totally prevented suggesting that not all receptor sites were blocked.

Table 48 Effect of EPM isolated from C. albicans GDH 2346 on the adherence of C. albicans strains GDH 2346 and B2630 to buccal epithelial cells

Strain[a]	Pretreatment of epithelial cells[b]	Mean no. of adherent yeasts/epithelial cell ± SEM	Percentage inhibition of adherence[c]
GDH 2346	EPM	0.85 ± 0.05	50.6
	PBS	1.72 ± 0.05	
B2630	EPM	0.94 ± 0.04	49.5
	PBS	1.86 ± 0.06	

[a]Yeasts were grown in YNB medium containing 50 mM glucose

[b]Epithelial cells were pretreated in PBS or in a solution of EPM (10 $mgml^{-1}$) for 30 min at 37°C

[c]Calculated as the percentage of yeasts not adhering to EPM pretreated epithelial cells when compared with adherence to PBS pretreated epithelial cells

Table 49 Effect of EPM isolated from C. albicans GDH 2346 on vaginal infections in mice with C. albicans strains GDH 2346 and B2630

Strain of C. albicans	No. of organisms used to infect vagina (yeasts ml^{-1})	Pretreatment of vagina[a]	Total vaginal count (cfu x 10^{-3}) ± SEM Day 4	Day 7	Day 14	Day 21
GDH 2346	10^3	Saline	2.0 ± 0.8	6.5 ± 2.2	4.5 ± 0.7	2.1 ± 0.1
	10^5	Saline	5.0 ± 1.3	22.0 ± 5.6	18.0 ± 4.8	9.2 ± 1.2
	10^7	Saline	11.0 ± 2.3	34.0 ± 5.6	20.0 ± 3.9	13.0 ± 2.4
	10^3	EPM	0.6 ± 0.5	1.8 ± 0.9	2.9 ± 0.3	1.8 ± 0.1
	10^5	EPM	2.7 ± 0.5	13.0 ± 5.7	11.0 ± 2.6	7.6 ± 0.8
	10^7	EPM	4.5 ± 0.9	22.0 ± 4.5	15.0 ± 2.7	10.0 ± 0.6
B2630	10^3	Saline	3.9 ± 1.3	6.5 ± 4.1	3.3 ± 0.8	1.5 ± 0.5
	10^5	Saline	17.0 ± 1.5	32.0 ± 6.3	18.0 ± 2.9	9.6 ± 1.2
	10^7	Saline	32.0 ± 11.7	63.0 ± 35.8	21.0 ± 4.5	13.0 ± 1.8
	10^3	EPM	1.3 ± 0.6	2.6 ± 0.9	2.1 ± 0.5	1.3 ± 0.3
	10^5	EPM	8.2 ± 3.1	22.0 ± 3.8	12.0 ± 2.5	7.2 ± 1.2
	10^7	EPM	18.0 ± 1.6	33.0 ± 5.5	17.0 ± 3.3	9.5 ± 0.9

[a]Mouse vaginas were pretreated with EPM (20 $mgml^{-1}$) for 30 min. Mice were inoculated intravaginally with yeast suspensions containing 10^3, 10^5 or 10^7 yeasts ml^{-1}.

Table 50 Pretreatment of mouse vaginas with EPM: relative infection values

Strain of *C. albicans*	No. of organisms used to infect vagina (yeasts ml^{-1})	Relative infection values[a]			
		Day 4	Day 7	Day 14	Day 21
GDH 2346	10^3	0.33	0.27	0.64	0.86
	10^5	0.54	0.59	0.61	0.83
	10^7	0.41	0.64	0.75	0.76
B2630	10^3	0.33	0.40	0.64	0.86
	10^5	0.48	0.68	0.66	0.75
	10^7	0.56	0.52	0.81	0.73

[a]Values represent extent of infection relative to that of mice pretreated with saline (see Table 49).

DISCUSSION

I ADHERENCE OF CANDIDA ALBICANS TO BUCCAL AND VAGINAL EPITHELIAL CELLS

Various methods have been used to measure adherence of C. albicans to buccal and vaginal epithelial cells. The method used here to study the adherence of C. albicans to epithelial cells was that of Kimura and Pearsall (1978) as modified by Douglas et al (1981). This involved mixing standardised epithelial and yeast cell suspensions under controlled conditions, filtering the assay mixtures on polycarbonate filters and counting the numbers of yeasts adhering to epithelial cells by light microscopy. Yeasts grown to stationary phase in defined medium containing high sugar concentrations were used in adherence assays as stationary-phase yeasts are more adherent than exponential yeasts (King et al, 1980; Douglas and McCourtie, 1981). Contrary to the results of King et al (1980) and Douglas et al (1981), Segal et al (1982) found that yeasts harvested from the exponential phase of growth had a higher rate of adherence than yeasts harvested from the stationary phase of growth. These differences obtained by different investigators remain unexplained.

King et al (1980) devised a radiometric adherence assay in which yeasts were grown on labelled glucose. After incubating, the adherence assay mixture was filtered and washed in PBS. The filters were then immersed in solubilizer and scintillant was added. For the purpose of adherence assays used in this investigation it would not be feasible to grow yeasts on labelled galactose due to the very high concentration (500 mM) which is required.

Most adherence assays in this investigation were done with 1×10^7 instead of 1×10^8 yeasts ml^{-1} used by Douglas et al (1981) as adherence values were very high and counting therefore difficult. Buccal epithelial cells (1×10^5 ml^{-1}) were obtained from a single donor throughout these studies

Adherence assays were also performed using a range of different yeast concentrations and the results obtained were similar to those of Kimura and Pearsall (1978). The fact that variation in adherence occurs with epithelial cells from different donors has been widely documented (King et al, 1980 and Kearns et al, 1983). Buccal epithelial cells obtained from the healthy adult donor in this investigation showed no significant daily changes in C. albicans adherence. The cells were always collected at the same time of day.

In assessing adherence by light microscopy it is possible to observe the distribution of yeasts over the epithelial cell surface. Certain epithelial cells had larger numbers of adherent yeasts than others. This is probably due to the non-uniformity in the population of epithelial cells. Co-adherence of yeast cells was observed in certain assays; however, the problem was not as severe as observed by King et al (1980), and for the purpose of adherence assays only yeasts directly attached to the epithelial cell were counted.

The use of exfoliated buccal and vaginal epithelial cells in in vitro adhesion assays does not permit the study of hormonal and nutritional factors which may act upon the epithelial cell receptor sites (Sobel et al, 1982) and such cells are likely to be contaminated with bacteria and salivary components. Samaranayake and MacFarlane (1981) devised an in vitro technique to quantitate the adhesion of C. albicans to monolayers of human epithelial cell preparations resulting in a more uniform population of epithelial cells.

Initially in this study, the adherence of different strains of C. albicans to buccal epithelial cells was determined after growth in defined medium containing different carbon sources. The results confirmed those of Douglas et al (1981) and McCourtie and Douglas (1984) which

indicated that strains isolated from active infections (I strains) show enhanced adherence after growth in medium with a high sugar content. Strains of C. albicans isolated from symptomless carriers (C strains) show much smaller increases in adherence after growth in 500 mM galactose medium. I strains are capable of synthesizing surface components in response to high sugar concentrations which enhance adhesion to buccal epithelial cells whereas C strains have a diminished capacity for cell surface modification. Kearns et al (1983) did not observe any differences in adherence between yeasts isolated from active infections and yeasts isolated from symptomless carriers. This is probably because they used yeasts grown on malt agar or Lee's medium rather than yeasts grown in galactose-containing liquid medium, so the yeasts did not synthesize the cell surface components which are responsible for enhanced adherence.

McCourtie and Douglas (1981) found that cell surface modification by I strains in response to high sugar concentrations rendered these strains resistant to spheroplast formation by Zymolyase. The C strain of C. albicans remained relatively sensitive to spheroplast formation with Zymolyase after growth in medium containing high sugar concentrations. It was suggested that the cell surface modification responsible for enhanced adhesion after growth in high sugar concentrations involves the production of an outer fibrillar layer (McCourtie and Douglas, 1981). These fibrils may be similar to bacterial fimbriae which have been widely shown to play a role in adhesion (Beachey, 1980). These cell surface changes may play an important role in vivo, particularly in the oral cavity where high concentrations of dietary sugars may be found. Ritchie et al (1969) found that a carbohydrate-rich diet was often a contributing factor in the development and persistance of oral candidosis.

The cell surfaces of several clinical isolates of C. albicans have been examined by electron microscopy after growth of the yeasts in defined media containing different carbon sources (Kulkarni et al, 1980). The mode of budding, bud scar morphology, surface topography and intercellular matrix all varied according to the carbon source. Yeasts grown in defined medium with galactose produced a matrix of dense granular strands whereas yeasts grown on glucose produced a matrix with thin granular strands.

Adherence of I strains of C. albicans to vaginal epithelial cells was similarly enhanced after growth in medium containing high sugar concentrations. The 'outbreak' strain of C. albicans (Burnie et al, 1985), was very highly adherent to vaginal cells after growth in high-galactose medium. Samaranayake and MacFarlane (1982a) suggested that the carbon source may affect oral and vaginal carriage of C. albicans by modifying the adhesive properties of the yeast. The fact that adhesion of C. albicans is enhanced by sugars may also play a role in the pathogenesis of vaginal candidosis as the condition is frequently found during the last trimester of pregnancy and in diabetes. All these conditions are associated with occurrence in the vaginal mucosa of glycogen which may be converted to glucose by tissue enzymes or those present in the commensal flora (Samaranayake and MacFarlane, 1985).

Other factors, apart from available carbon source, may affect the adhesion of C. albicans to epithelial cells. The effect of different cations on the adherence of C. albicans GDH 2346 to buccal epithelial cells after growth in defined medium containing 500 mM galactose was investigated. The results were similar to those reported for the effect of cations on adherence to acrylic surfaces by McCourtie and Douglas (1981). Divalent cations increased adherence slightly, whereas monovalent cations decreased adhesion to buccal cells; calcium ions enhanced adherence to the greatest

extent. The effect of divalent cations may be caused by their ability to reduce the electrostatic repulsion between the yeast and the negatively charged epithelial cell surface. However, the divalent cation effect may not be great enough to be considered significant *in vivo*. Sugarman (1980) found that the adherence of enteric bacteria to buccal epithelial cells was increased with Zn^{2+}, Fe^{3+} and Fe^{2+}, and suggested that high concentrations of iron promote infection by favouring growth.

II EXTRACELLULAR POLYMERIC MATERIAL

A. Isolation and purification

McCourtie and Douglas (1981) have shown that growth of *C. albicans* in medium containing high concentrations of galactose promotes the synthesis of an additional fibrillar layer. These fibrils which enhance yeast adhesion to epithelial cells are released into the surrounding medium. In the present study, cultures of *C. albicans* GDH 2346 were grown for 5 days at 37°C to allow maximum yields of extracellular polymeric material (EPM) to be obtained. The quantity of EPM was found to increase with the age of the culture as higher yields of EPM could be isolated from cultures grown for 5 days than from cultures grown for 24 hours. Pugh and Cawson (1978) also observed an extracellular mucus layer covering the cell surface which was composed of glycoprotein. The quantity of this material increased with the age of the culture. EPM was found to be a glycoprotein with a carbohydrate to protein ratio of 7 to 1. These results confirmed those of McCourtie and Douglas (1985a) who found that EPM contains a large amount of mannose and suggested it was a mannoprotein. McCourtie and Douglas (1985a) isolated EPM by precipitating culture supernates with acetone, whereas the EPM used in the present study was isolated by extensively

dialysing culture supernates to remove low molecular-weight components and freeze-drying the retentate. Therefore, the method used to isolate EPM did not affect its chemical composition; however, the dialysis and freeze-drying method is preferred since it does not affect the protein portion of the glycoprotein, whereas acetone precipitation may result in some denaturation or aggregation of the protein. Masler et al (1966) found that C. albicans produced extracellular polysaccharide-protein complexes which are similar in composition to EPM isolated in these studies.

Attempts to fractionate EPM in this study indicated that it contains more than one component. Molecular weight determinations with a Sephadex G-200 column indicated that the fractions had M_R values ranging from 6×10^3 to 7×10^5. Diedrich et al (1984) isolated low molecular-weight extracellular material from three strains of C. albicans. This material was produced by cells in the exponential phase of growth and was thought to represent the carbohydrate portion of cell surface glycoproteins. They isolated their extracellular material by ethanol precipitation and applied it to a Sephadex G-50 column. Two fractions were eluted, one of which was further fractionated into two components. Diedrich et al (1984) suggested that the extracellular material represented a turnover of protein attached to the cell wall. Fractionation of EPM by ion-exchange chromatography in the present study gave 5 components also with varying carbohydrate and protein contents.

Attempts to resolve EPM by SDS-polyacrylamide gel electrophoresis proved unsuccessful. No bands were detected when gels were stained with Coomassie blue R250 even with high concentrations of EPM (5 $mgml^{-1}$). This may be due to the fact that since EPM consists of glycoprotein containing only 10% protein there was insufficient protein in the sample to stain. Gels were also stained using the periodic acid-Schiff reagent and this, too,

failed to reveal any bands. Samples of EPM did not migrate very far into the gel which may have been due to its high molecular weight. To allow migration of samples into the gel the percentage of acrylamide in the separating gel was reduced from 11% to 7.5% (w/v). These gels were stained with the silver stain procedure which is more sensitive than Coomassie blue. A thick diffuse band was observed for crude EPM preparations and the intensity of the staining was greater towards the sides of the lane. The thick band was observed for all the concentrations of crude EPM used (2, 3.5 and 5 $mgml^{-1}$). This diffuse band may be similar to that observed by Odds and Hierholzer (1973), who applied a glycoprotein acid phosphatase from C. albicans to SDS-polyacrylamide gels. This glycoprotein had a carbohydrate-to-protein ratio similar to that of crude EPM.

There have been few reports of SDS-polyacrylamide gel electrophoresis of glycoproteins. No report of the silver stain being specifically used to stain glycoproteins could be found in the literature. The silver stain was used in the present study because of its ability to detect low concentrations of protein which would not be identified by the Coomassie blue method (Oakley et al, 1980). However, it is not known whether the silver stain reacts with the carbohydrate components of EPM; there may be reducing sugars in the carbohydrate backbone which would be expected to react.

Several investigators have reported successful SDS-polyacrylamide gel electrophoresis of cell wall mannoproteins from S. cerevisiae. Valentin et al (1984) treated purified walls from S. cerevisiae with various chemicals and found that boiling in 2% SDS released mannoproteins. These were resolved by SDS-polyacrylamide gel electrophoresis by the method of Herrero et al (1982), and were rather heterogeneous, as about 60 bands were detected from walls treated with urea or SDS. Pastor et al (1984)

solubilized mannoproteins from radioactively-labelled walls of S. cerevisiae by Zymolyase treatment. The released mannoproteins were precipitated with ethanol and successfully analysed by SDS-polyacrylamide gel electrophoresis and fluorography. Frevert and Ballou (1985) also reported SDS-polyacrylamide gel electrophoresis of cell-wall mannoproteins of S. cerevisiae. They found that their cell-wall mannoprotein was not revealed by protein stains, but could be stained by the periodic acid-Schiff reagent. These observations are consistent with those described here for the analysis of EPM by SDS-polyacrylamide gel electrophoresis. Frevert and Ballou (1985) compared mannoproteins from mutant strains of S. cerevisiae and wild-type cells and found that mannoproteins from the latter were too large to penetrate 7% polyacrylamide gels. This may explain why EPM does not migrate very far into the gel. It may be possible to overcome some of these staining problems by labelling the protein portions of mannoproteins with ^{125}I and subjecting samples to SDS-polyacrylamide gel electrophoresis and autoradiography in a method similar to that described by Zlotnik et al (1984). The successful application of Coomassie blue to mannoprotein staining described by Valentin et al (1984) and Pastor et al (1984) is probably because their samples contained a larger proportion of protein than does EPM from C. albicans.

B. Immunological properties of EPM

EPM produced by C. albicans GDH 2346 is immunologically active. Serological studies by McCourtie and Douglas (1985a) indicated that EPM from 500 mM galactose-grown yeasts contains a larger proportion of antigenic determinants than EPM from yeasts grown on 500 mM sucrose, while EPM from 50 mM glucose-grown yeasts have the fewest determinants. EPM isolated from C. albicans by Masler et al (1966) was also immunologically active.

McCourtie and Douglas (1985a) performed cross-agglutination assays with C. albicans strains MRL 3153 and GDH 2023. Suspensions of C. albicans MRL 3153 were incubated with antisera raised against EPM from strain GDH 2346 and found that the titres were similar to those obtained with the homologous yeast cells. Incubating suspensions of C. albicans GDH 2023 with the same antiserum resulted in much lower titres. McCourtie and Douglas (1985a) concluded that EPM produced by C. albicans strains GDH 2346 and MRL 3153 were similar and that EPM produced by C. albicans GDH 2023 was somewhat different.

In this study antiserum was prepared against EPM isolated from C. albicans GDH 2023 after growth on 500 mM galactose. Cross-agglutination assays were performed with suspensions of C. albicans GDH 2346 and the antiserum raised against EPM from C. albicans GDH 2023. The low titre obtained confirmed the observations of McCourtie and Douglas (1985a) that there are differences in EPM produced by these two strains. It has been suggested that these differences may represent different serotypes; strains GDH 2346 and GDH 2023 may be serotypes A and B respectively, as strain GDH 2346 is similar to strain MRL 3153 which is known to be serotype A (McCourtie and Douglas, 1985a).

C. Role of EPM in adhesion

Evidence that EPM contains the yeast adhesin has come from experiments where it has been used to block adherence by pretreating epithelial cells prior to use in adherence assays (McCourtie and Douglas, 1985a; Tronchin et al, 1984; results of this study). Here, EPM was isolated from five strains of C. albicans (strains GRI 681, GRI 682, MRL 3153, GDH 2346 and GDH 2023), after growth in defined medium containing 500 mM galactose. The EPM was examined for its ability to inhibit adherence to buccal cells, both of the

strains from which it was isolated and the other strains of C. albicans. C. albicans GDH 2023 produced EPM which was capable of inhibiting the adherence of strain GDH 2023 to buccal cells; however, it had no effect on the adherence of strains GDH 2346 and MRL 3153 when used to pretreat buccal cells. This also suggests that there may be differences in EPM produced by strains GDH 2346 and GDH 2023, and confirms the results of cross-agglutination assays (McCourtie and Douglas, 1985a). C. albicans strains GRI 681 and GRI 682 produce EPM which is not as efficient as that from strain GDH 2346 at inhibiting adherence. This may indicate that the EPM produced by these strains does not contain as high a proportion of adhesin components as EPM from C. albicans GDH 2346. Serological analysis of EPM from C. albicans GRI 681 and GRI 682 would be expected to contain fewer antigenic determinants than EPM from C. albicans GDH 2346. This, in turn, suggests that C strains produce EPM with fewer adhesin component(s) than I strains. Environmental conditions in the debilitated host may enable I strains to produce a higher proportion of adhesin components.

Preliminary experiments were done to investigate whether EPM from C. albicans has 'lectin-like' binding properties. The protein portion of yeast mannoprotein appears to have properties similar to those of lectin proteins in that both types of proteins are rich in the amino acids serine and threonine. Most of the plant lectins which have been purified contain covalently bound sugars and are classified as glycoproteins. The carbohydrates found in most lectins include D-mannose, D-glucosamine and D-galactose; other sugars such as D-xylose and D-arabinose are sometimes present, but in smaller amounts (Sharon and Lis, 1972). Most lectins are relatively rich in aspartic acid, serine and threonine which may comprise as much as 30% of their amino acid content; however, they are low in sulphur-containing amino acids (Sharon and Lis, 1972). Lectins may contain

Mn^{2+} and Ca^{2+} which are required for the carbohydrate-binding and agglutinating properties of these lectins (Lis and Sharon, 1973). In this study divalent cations slightly enhanced the binding of C. albicans to buccal epithelial cells. Divalent cations have also been found to enhance slightly adherence of yeasts to denture acrylic (McCourtie and Douglas, 1981). In a separate experiment EPM (10 $mgml^{-1}$) was dissolved in 0.05 M Tris HCl buffer containing 0.002M Ca^{2+}, Mg^{2+} and Mn^{2+} and used to pretreat epithelial cells. Samples of EPM dissolved in buffer containing divalent cations were slightly more efficient inhibitors of adherence than samples dissolved in PBS.

In an attempt to provide further evidence for the 'lectin-like' binding of EPM, fractions with sugar-binding specificities were isolated by applying crude EPM preparations to Sepharose affinity columns containing a coupled sugar. The sugars coupled to the Sepharose gels were chosen because they are known to be present on the surfaces of host cells. It is therefore possible that the protein component of EPM binds in a 'lectin-like' fashion to glycoside receptors on the host cell surface. EPM from strains GDH 2346, MRL 3153, GRI 681 and GRI 682 had similar sugar specificities and bound to L-fucose, D-mannose and N-acetyl-D-glucosamine. EPM from C. albicans GDH 2023 showed a different order of sugar specificities and bound to D-galactose and sialic acid as well as L-fucose, D-mannose and N-acetyl-D-glucosamine. The reason for the different sugar-binding specificities of EPM from this strain remains to be explained.

III MECHANISM OF ADHERENCE

The adherence of certain bacteria to mucosal surfaces is thought to occur via specific adhesin molecules which interact with complementary receptors

on the host cell surface. Adherence mechanisms have been elucidated by attempting to block adherence with an excess of adhesin or receptor components or adhesin/receptor analogues (Figure 4). The adherence of C. albicans appears to involve a specific interaction of cell surface adhesins with receptors on the epithelial cell surface.

A. The yeast adhesin

The adhesin of C. albicans is a component of EPM produced by the yeast after growth in medium containing high concentrations of certain sugars (McCourtie and Douglas, 1985a). Several investigators have shown that yeast adhesins may be released into the culture medium (McCourtie and Douglas, 1985a; Tronchin et al, 1984; Diamond et al, 1980). In the present study EPM was isolated from culture supernatant fluids of C. albicans after growth for 5 days in medium containing 500 mM galactose. Pretreatment of epithelial cells with EPM samples did not have any visible effects on the epithelial cells and there was no aggregation as reported by Diamond et al (1980) for neutrophils in the presence of extracellular material. If buccal epithelial cells are pretreated with EPM (10 $mgml^{-1}$) prior to their use in adherence assays the adhesion of C. albicans GDH 2346 is blocked by 50%. This suggests that adhesin components have bound to epithelial cell receptors leaving fewer receptors for yeasts (Figure 4b). EPM from C. albicans GDH 2346 also blocked the adhesion of this strain to mouse vaginal epithelial cells. The adherence of other I strains was also inhibited when epithelial cells were pretreated with EPM isolated from these strains; however, the extent of inhibition was not as great as that obtained with EPM from C. albicans GDH 2346. This strain produces EPM which may be a rather strong inhibitor of adherence and probably contains a greater proportion of adhesin components than EPM produced by C strains.

On the basis of this assumption, EPM from C. albicans GDH 2023 would also be expected to be an efficient adherence inhibitor of the C strains. This was found not to be the case, and results from experiments investigating effects of sugars and lectins on adherence, and serological studies, suggest that this strain has a different mechanism of adherence.

Attempts to purify the adhesin components by column chromatography proved partially successful. Gel filtration and ion-exchange chromatography fractionated EPM into five components; however, all components exhibited some adherence-inhibiting properties when used to pretreat epithelial cells. A two-step separation protocol involving chromatography on Con A-Sepharose and DEAE-cellulose allowed a thirty-fold purification of the yeast adhesin. This protocol involved eluting the material which bound to the affinity column with methyl-α-D-mannoside and applying it to a DEAE-cellulose column. The mannoprotein components which bound to the Con A-Sepharose column were fractionated into two components. The first fraction which was eluted exhibited an adherence inhibition index value (AII) of 30 (Table 17). The AII was devised to compare the adherence inhibiting activities of components eluted from the various columns. It is defined as a measure of the relative efficiency with which each component inhibits adherence as compared with crude EPM. This means that the fraction eluted in the two-step separation protocol was more than 30 times more efficient than crude EPM (10 $mgml^{-1}$) at inhibiting adherence.

Diamond et al (1980) purified an extracellular product of C. albicans hyphae which inhibited in vitro attachment of yeasts to neutrophils. They applied supernatants to a column of Biogel P-100 and isolated a fraction which was 10,000 times more active at inhibiting attachment to neutrophils than 1 $mgml^{-1}$ of crude mannoprotein.

B. Nature of the epithelial cell receptor

Evidence which suggests that epithelial cell-surface sugars may be attachment sites for certain bacteria has come from experiments in which adherence was inhibited with monosaccharides, lectins, sugar oxidants and glycosides. The binding of bacteria to immobilized sugars and isolation of bacterial lectins (Sharon et al, 1981) also provides evidence for sugars as attachment sites for bacteria. Sugars which may be analogous to epithelial cell receptors were used to elucidate the mechanism of yeast adherence by setting up adherence assays in the presence of different sugars. The sugars used to block adhesion here are thought to be present on host cell surfaces in glycoproteins or glycolipids (Sharon et al, 1981).

C. albicans GDH 2346 was principally inhibited by L-fucose; this sugar has not been detected on the cell surface of C. albicans which rules out the possibility of it acting as an adhesin analogue (Table 24). None of the other sugars investigated had a significant effect on the adherence of this strain to buccal cells. Jones and Freter (1976) recognised that L-fucose and other fucosides, and to a lesser extent D-mannose, may function as receptors for Vibrio cholerae in isolated brush-border membranes. Adherence of Campylobacter species to epithelial cells is thought to be mediated by L-fucose (Cinco et al, 1984). The inhibition of Campylobacter adherence by L-fucose was concentration dependent up to 50 $mgml^{-1}$ with no further inhibition above this concentration. This suggests that another receptor may be present in addition to L-fucose. C. albicans may be similar to Campylobacter as EPM isolated from strain GDH 2346 has also been shown to have several sugar-binding specificities.

C. albicans GDH 2023 gave a different pattern of results when sugars were added to adherence assay mixtures. L-Fucose did not have any effect and the principal inhibitory sugars were N-acetyl-D-glucosamine and

Author: CRITCHLEY, Ian A.

Title: Mechanism of adherence of Candida albicans to epithelial cells

Date of deposit: 3/7/86

If you remove this volume from the shelf, enter on this card the reason for removal, the date, and your initials, and leave the card on the shelf in place of the volume. Before re-shelving the volume, score through the record of removal and replace the card in the volume.

Reason for removal	Date	Initials

Process	Date	Initials
Accessioned	3 7 86	CB
Catalogued		
Classified		
Cataloguing checked		
Processed		
Processing checked	8/7/86	CT
Shelved		

D-glucosamine; D-mannose had a less significant effect (Table 25). Therefore, this strain appears to bind to a different receptor than that of C. albicans GDH 2346. On the other hand, the 'outbreak' strain of C. albicans appears to have a similar adherence mechanism to C. albicans GDH 2346 as L-fucose was the principal monosaccharide inhibitor (Table 26). Other investigators have obtained conflicting results for the effect of sugars on yeast adherence. Sobel et al (1981), found that L-fucose inhibited the adherence of a clinical isolate of C. albicans to vaginal epithelial cells by adding the sugar to the incubation mixture. Sandin et al (1982) found that methyl-α-D-mannoside inhibited adherence, whereas Segal et al (1982) found that amino sugars inhibited yeast adhesion to epithelial cells.

In E. coli, nine adhesins have been described which are proteins and interact in a lectin-like fashion with glycoside receptors on the host cell surface (Jones and Isaacson, 1983). Different C. albicans strains may have different protein adhesins which are specific for different sugars.

An alternative explanation for the different results obtained by different research groups for the effect of sugars on C. albicans adhesion may also be due to differences in receptors on epithelial cells from different individuals. McEachran and Irvin (1985) found differences in sugar inhibition patterns with the adherence of P. aeruginosa to trypsin-treated and untreated epithelial cells. Sialic acid and D-arabinose inhibited adhesion to trypsin-treated buccal cells and enhanced adhesion to non-trypsin-treated cells when these sugars were preincubated with bacteria. N-Acetyl-D-glucosamine enhanced adhesion to both cell types and D-fucose only inhibited adhesion to trypsin-untreated buccal cells. There may be a difference in the receptors on the two cell types and it is possible that salivary proteinases in the oral cavity have an effect similar to that of

trypsin (Woods et al, 1983). Epithelial cells obtained from various individuals may have been subjected to different proteinase levels in their natural environments. This could explain why different research groups obtained different results for the effect of sugars on the adhesion of C. albicans to epithelial cells, as different receptors may have been exposed.

Segal et al (1982) claimed that adherence inhibition obtained with N-acetyl-D-glucosamine was due to its role as an adhesin analogue rather than a receptor analogue. They found that chitin, its hydrolysate derivative and N-acetyl-D-glucosamine inhibited adherence when they were present in adherence assay mixtures. Chitin, which was suggested to mediate adherence is located primarily in the bud scars of yeasts. According to the scheme of Poulain et al (1978) for the cell wall architecture of C. albicans, chitin is not present on the cell surface, but is found several layers beneath the surface where it forms an alkali-insoluble matrix with glucan. Therefore, it would appear that the effect of N-acetyl-D-glucosamine on the adherence of C. albicans observed by Segal et al (1982), is due to the sugar acting as a receptor analogue rather than as an adhesin analogue, as chitin is not readily available for interacting with host receptors.

Differences in adherence mechanism between strains GDH 2346 and GDH 2023 of C. albicans were confirmed by studies with lectins. Lectins were used to pretreat buccal epithelial cells prior to their use in adherence assays. C. albicans GDH 2346 was principally inhibited by winged pea lectin which is specific for L-fucose (Table 27). This lectin binds to L-fucose-containing moieties on the buccal cell surface and prevents the yeast binding to the epithelial cell receptor. The fact that yeast adherence was not totally inhibited suggests that there may be other

receptors available which do not bind winged pea lectin. Wheat-germ agglutinin and peanut lectin, which are specific for N-acetyl-D-glucosamine and D-galactose respectively, did not significantly affect the adhesion of C. albicans GDH 2346. These results confirm the results obtained in the sugar inhibition experiments and suggest that D-galactose and N-acetyl-D-glucosamine do not play any role as receptors for this strain. Concanavalin A and lentil lectin both significantly enhanced the adherence of strain GDH 2346. This is probably due to the pH value (7.2) used in the assay, since Con A has been shown to exist as a pseudo-tetrahedral molecule at this pH, with four saccharide binding sites, one in each subunit (Bittiger and Schnebli, 1976). Therefore, it is possible that Con A can bind to mannose-containing moieties on both the epithelial and yeast cell surfaces and thus function as a bridge. Yeast cell envelopes are abundant in manno-protein which is composed of α-D-mannopyranosyl residues. The backbone consists of α(1 - 6) linkages with side chains varying in length from 1 to 3 monomers joined by α(1 - 2) and α(1 - 3) linkages which are recognised by Con A (Goldstein et al, 1965). This increase in adherence may be similar to the situation encountered by Bar-Shavit and Goldman (1976), who found that incubation of macrophages with 80-160 μg Con A resulted in about 90% saturation of Con A binding sites on the macrophage. They stated that binding of Con A to surface receptors does not involve occupation of all four binding sites of the lectin. Therefore, Con A-coated cells were able to bind to non Con A-coated cells, resulting in Con A-mediated attachment and ingestion. The increase in adherence of C. albicans by pretreating buccal epithelial cells with lentil lectin may also be due to the lectin having more than one sugar-binding site. No aggregation of buccal epithelial cells occurred when they were pretreated with Con A and lentil lectin.

The adherence of C. albicans GDH 2023 was principally inhibited by pretreating buccal epithelial cells with wheat-germ agglutinin (Table 28). This result implies that N-acetyl-D-glucosamine may be involved as an epithelial cell receptor and confirms the results from sugar inhibition tests. As with C. albicans GDH 2346 adherence was not totally inhibited by pretreating epithelial cells with this lectin which suggests that other receptors may be involved. Lentil lectin and Con A both enhanced adherence of this strain suggesting that the same bridging mechanism as that postulated for C. albicans GDH 2346 may occur. Winged pea lectin which was the principal adherence inhibitor of C. albicans GDH 2346 had no effect on the adherence of this strain, indicating that L-fucose does not play any role as a receptor for this strain. Peanut lectin did not have any effect on adherence indicating that D-galactose does not function as a receptor.

The effect of different lectins on the adherence of C. albicans to human buccal epithelial cells was also investigated by Sandin et al (1982). They performed different adherence assays in which either the yeasts or the epithelial cells were pretreated with lectins. They found that Con A inhibited the adherence of pretreated yeasts to buccal cells and also inhibited adherence of yeasts to pretreated buccal cells. Adherence was inhibited by up to 80% but could be restored by preincubating the Con A with a mannose derivative; preincubating with other sugars had no such effect. These results are in contrast to those reported here for the effect of Con A on C. albicans GDH 2346 adhesion. In the light of results obtained in the present study, pretreatment of yeasts as described by Sandin et al (1982) would not be expected to result in adherence inhibition due to the fact that Con A-mediated adherence would have been expected to occur. Makrides and MacFarlane (1983) found that the adherence

of C. albicans to HeLa cell monolayers was enhanced when the cells were incubated in the presence of Con A. They found that enhanced adhesion was also produced when E. coli was present in the assay; this bacterium binds to mannose-containing moieties on both HeLa and yeast cell surfaces. Diamond and Krzesicki (1978) added Con A to incubation mixtures of pseudohyphae and neutrophils and found that attachment was not inhibited by the lectin. In fact, they found that 10 $\mu g ml^{-1}$ of Con A stimulated the attachment of C. albicans to neutrophils by 29.3% as revealed by the ^{14}C cytosine assay. This enhanced adherence may be due to Con A binding to both the neutrophil and yeast surfaces.

C. albicans GRI 681 and GRI 682 both showed similar results when lectins were investigated for their effect on the adherence of these strains (Tables 29 and 30). Winged pea lectin, which is specific for L-fucose, was the principal adherence inhibitor of both strains suggesting that these organisms may bind to L-fucose-containing moieties on the epithelial cell surface. Of the five strains investigated in this study C. albicans GDH 2023 was the only strain which showed different results for the effect of lectins on adherence. The results of the sugar-inhibition tests and lectin experiments suggest that glycosides on epithelial cell surfaces play some role as receptors for C. albicans. The nature of the sugar residue appears to depend on the strain of C. albicans.

Only recently has attention been paid to the possible role of sugar-mediated attachment in the colonization of mucosal surfaces by microorganisms. Evidence has been obtained which suggests that sugar-binding proteins, similar to lectins and present on bacterial surfaces, may serve to attach certain pathogens to sugar residues on a variety of animal cells (Sharon et al, 1981). It was suggested that cell-surface carbohydrates may be acting as determinants of intercellular recognition. Ofek et al

(1977) reported that the adherence of E. coli to epithelial cells was mediated by a lectin-like substance which was specific for mannose residues on epithelial cell surfaces. This interaction with mannose residues was similar to that of lectins with sugars (Sharon and Lis, 1972) in that it is reversible and can be inhibited in the presence of sugars. A similar situation may occur with C. albicans GDH 2346 in that L-fucose binds to the adhesin, when added to adhesion mixtures, making it less accessible to the epithelial cell receptor. It would be interesting to investigate whether interaction of the yeast adhesin with L-fucose is reversible. If L-fucose was added to a solution of EPM then the polymer would not be expected to bind to the epithelial cell receptors and block adherence to the same extent. A similar finding would be expected for C. albicans GDH 2023 and N-acetyl-D-glucosamine.

Two D-mannose-specific lectin proteins have been isolated from two different pathogenic strains of E. coli (Eshdat and Sharon, 1982). Both these lectin-like proteins were found to be located at the outer surface of the bacteria and to mediate their adherence to host cells. The lectin of one strain was isolated as flagella and was composed of identical protein subunits. The lectin of the other strain was organized as hair-like appendages and termed Type I pili; this lectin could not be dissociated into subunits. The adhesin of C. albicans may be similar as fibrils have been observed to mediate adherence of the yeast to epithelial cell surfaces (Marrie and Costerton, 1981) and serological studies by Gardiner et al (1982) have demonstrated the presence of fimbriae on C. albicans.

The adherence of the protozoan organism Entamoeba histolytica to Chinese hamster ovary cells has been reported to be mediated by an amoebic lectin which is inhibited by N-acetyl-D-galactosamine (Ravdin et al, 1985). This observation suggests that the presence of adhesins with lectin-like

properties is quite widespread amongst different types of microorganisms. In most cases bacterial adhesins appear to be proteinaceous in nature and their receptors appear to be carbohydrate. Jones and Isaacson (1983) provide several examples of proteinaceous bacterial adhesins and their carbohydrate receptors on host cell surfaces.

C. Characterization of the yeast adhesin component

The adhesin of C. albicans is thought to be mannoprotein in nature. Surface mannan is important in mediating adherence of C. albicans to fibrin-platelet clots formed in vitro (Maisch and Calderone, 1981). Sandin et al (1982) suggested that mannose-containing moieties on the yeast cell surface could mediate in vitro adherence to epithelial cells. Ray et al (1984) concluded that cell wall mannan may participate in the adhesin complex. Ray et al (1984) found that only minor adherence inhibition was obtained after performing assays in the presence of graded concentrations of mannan. This minor inhibition may be due to the fact that the mannan was derived from S. cerevisiae and not C. albicans. EPM isolated from C. albicans GDH 2346 in this study, which was mannoprotein in nature, was capable of inhibiting adherence of C. albicans to epithelial cells.

Further evidence for the role of a mannoprotein adhesin component in mediating adherence was obtained by studying the effect of the antibiotic tunicamycin on adherence. Tunicamycin only affects the synthesis of mannoprotein and has no effect on the synthesis of glucan or chitin (Douglas and McCourtie, 1983). Yeasts which had been treated with tunicamycin were significantly less adherent than untreated yeasts.

Contrary to these results Lehrer et al (1983) claimed that cell-wall chitin was the adhesin component mediating attachment to vaginal epithelial cells. They pretreated epithelial cells with chitin, chitin

soluble-extract and N-acetyl-D-glucosamine and obtained significant adherence inhibition; pretreatment of yeasts had no such effect. The chitin soluble-extract was prepared by shaking a 20% suspension of chitin in PBS at room temperature for 5 h followed by dialysis and freeze-drying the supernatant. The nature of this soluble extract is rather obscure since chitin is thought to be a highly insoluble polymer (Phaff, 1971). Chitin soluble-extract was concentration-dependent in its inhibitory activity as 1 $mgml^{-1}$ had only a slight effect and concentrations of 50 $mgml^{-1}$ were required to obtain significant inhibition of adhesion. Tronchin et al (1984) suggested that loss of material from the yeast cell surface could unmask other receptors such as N-acetyl-D-glucosamine which are present under the external cell wall coat. This could explain the observations of Lehrer et al (1983); however, details of the ultrastructural components of the yeast cell wall would need to be investigated further.

Results from the experiments of several investigators have suggested that the yeast adhesin component is protein in nature and is the protein portion of mannoprotein. Lee and King (1983a) found that several proteolytic enzymes reduced adherence to vaginal cells after yeasts were pretreated with these enzymes. Treatment of yeasts with various proteolytic enzymes did not have any effect on yeast viability (Lee and King, 1983a; Sobel et al, 1981). However, one of the major disadvantages of treating intact yeast cells with proteolytic enzymes is that such treatments may affect components other than the adhesin. Therefore, proteolytic treatment of EPM, which contains the adhesin component, yields more reliable information on the mechanism of adherence than that obtained from direct treatment of yeast cells, and was the approach used in this study.

Evidence for the role of a proteinaceous adhesin mediating the adherence of C. albicans to epithelial cells was provided from attempts to

dissect the crude adhesin material by chemical, enzymic and physical treatments to determine the minimum structure necessary for adherence inhibition. Untreated EPM (10 $mgml^{-1}$) inhibits the adherence of C. albicans GDH 2346 to buccal epithelial cells by 50% (Figure 11). However, EPM samples which had been heated to 100°C for 15 minutes were no longer able to inhibit adherence (Table 22). This observation suggests that a protein adhesin component has been denatured by such an extreme treatment; therefore EPM is no longer capable of binding to epithelial cell receptors and blocking yeast attachment.

Sobel et al (1981) found that yeasts heated at 63°C for 90 to 120 min were significantly less adherent to vaginal epithelial cells than untreated yeasts; similar results were obtained by Kimura and Pearsall (1978). The ability of non-viable yeasts to adhere is probably dependent upon the severity of the treatment used to kill the yeast cells. If killing treatments were less vigorous, such as 65°C for 5 min, or a 10 min exposure to UV light, as used by Lee and King (1983a) then the yeasts were still capable of adhering to epithelial cells. However, if very harsh treatments were used, such as prolonged exposure to heat or a high concentration of formalin, then yeasts were no longer capable of adhering (Lee and King, 1983a).

Further evidence for the role of a protein yeast adhesin was derived from experiments examining the effect of EPM samples treated with various proteolytic enzymes on the adherence of C. albicans to buccal epithelial cells. Trypsin, chymotrypsin and pronase all reduced the ability of EPM to inhibit adherence (Table 18). After treating EPM samples with these enzymes an equimolar concentration of the appropriate enzyme inhibitor was added. These results suggest that enzyme treatments have either degraded the adhesin component or altered its configuration in such

a way that it is no longer capable of binding to the epithelial cell receptor and blocking adherence as efficiently as untreated EPM. On the other hand, treating EPM with papain enhances its ability to inhibit adherence. This suggests that papain treatment releases an intact fragment which is capable of binding to epithelial cell receptors more efficiently than untreated EPM. This peptide fragment may be similar to the fragment described by Lee and King (1982b) which was released after treating yeast cells with papain. They used immunofluorescence techniques to show that their peptide fragment was capable of binding to vaginal epithelial cells and was capable of inhibiting adherence of C. albicans to vaginal epithelial cells. Diedrich et al (1984) found that a low molecular weight glycoprotein could be isolated from the culture medium of exponential cultures of C. albicans and could also be generated artificially by treating yeasts with papain. Diamond et al (1980) isolated peptides from material released from C. albicans hyphae after treatment with UV light. This material inhibited the attachment of live C. albicans hyphae to neutrophils and was capable of impairing neutrophil functions.

Treatment of EPM with dithiothreitol totally abolished its ability to inhibit adhesion. Therefore, disulphide bonds appear to play some role in maintaining the configuration of the yeast adhesin. Reducing agents such as dithiothreitol and β-mercaptoethanol, have been found to inhibit significantly the adherence of C. albicans to vaginal cells when yeasts were pretreated with these agents (Lee and King, 1983a). Significant adherence inhibition was also obtained in the adherence assays described by Tronchin et al (1984) after yeasts were pretreated with 2-mercaptoethanol.

As EPM from C. albicans is largely composed of mannoprotein, it was treated with the enzyme endoglycosidase H. This enzyme was used to cleave the N-glycosidic bond which links the polysaccharide moiety of the

glycoprotein via a di-N-acetylchitobiose linkage to an asparaginyl residue in the protein part of the molecule. Endoglycosidase H has been widely used for structural analysis in cell surface research (Tarentino et al, 1974). Such treatment of EPM should cleave it into its respective protein and carbohydrate components. The carbohydrate and protein components were separated by applying the enzyme-treated sample to an affinity column of Con A-Sepharose. The protein components were eluted with PBS and carbohydrate components eluted with methyl-α-D-mannoside. The bound (carbohydrate) material was dialysed against cold PBS to remove methyl-α-D-mannoside. These fractions were tested separately for their ability to inhibit the adherence of C. albicans to buccal epithelial cells (Table 19). The eluted fraction which contains protein was a more efficient adherence inhibitor than the bound fraction. This provides further evidence that the protein portion of C. albicans may mediate adhesion to epithelial cells. The slight inhibitory activity of the carbohydrate fraction may be due to the fact that it contains some protein material. It is possible that endoglycosidase H cleaves a peptide fragment which still has base-labile oligosaccharides attached to serine and threonine. Such oligosaccharides may also bind to the Con A-Sepharose column and this would explain why the carbohydrate fraction had some inhibitory activity.

Treating EPM with α-mannosidase had little or no effect on its ability to inhibit C. albicans adhesion to buccal cells (Table 20). This suggests that mannose residues of the mannoprotein are not important in binding to epithelial cell receptors and blocking adhesion. The effect of α-mannosidase on EPM from C. albicans GDH 2346 was confirmed by applying enzyme-treated samples to paper chromatograms. Treatment of EPM with α-mannosidase was found to result in the release of reducing sugars, which do not appear to have any role in the adherence process. Lee and King (1983a)

treated yeast cells with α-mannosidase and endoglycosidase H and found that neither treatment significantly affected the ability of yeasts to adhere to vaginal epithelial cells. However, they found that α-mannosidase reduced the ability of isolated cell wall fragments to adhere to vaginal cells. The mannoprotein preparations used by Lee and King (1983a) gave only slight inhibition of adherence to vaginal cells. This lack of adherence inhibition may be due to the severity of the extraction procedures which could have destroyed or denatured the protein component.

Destroying the sugar residues of the carbohydrate portion of EPM does not affect its ability to inhibit adherence, since treated samples gave relative adherence values similar to those of untreated samples (Table 21). Periodate oxidizes and cleaves carbon-carbon bonds which contain free adjacent hydroxyl groups or a hydroxyl group and an aldehyde or ketone. This treatment might affect the protein portion of the glycoprotein, but this is unlikely, which is why EPM retains its inhibitory capabilities. Activity of periodate was inhibited by treating samples with ethylene glycol before pretreatment of epithelial cells.

Mild acid and mild alkali treatments have been used successfully in the structural analysis of yeast mannoproteins (Ballou, 1976) and such treatments may be used to characterize the adhesin component of EPM. Phaff (1971) found that phosphodiester bonds were the most acid-labile linkages in yeast mannans. Mild acid treatment should break the α-D-mannopyranosyl phosphodiester linkage resulting in a quantitative release of free mannose. Samples of EPM which were subjected to mild acid treatment lost their ability to inhibit adherence (Table 21). This is probably due to the protein being denatured either by the acid or the high temperature required for hydrolysis. Mild alkali treatment slightly increased the ability of EPM to inhibit adherence. The samples treated

with mild alkali gave a relative adherence value of 0.44, which is less than the untreated sample which gave a value of 0.5 when these samples were compared with the PBS control (Table 21). This treatment releases oligosaccharides from O-glycosidic linkages to serine and threonine (Ballou, 1976). Thus, removal of base-labile oligosaccharides from the protein may make the protein adhesin more accessible to the epithelial cell receptor.

Similar techniques were used by Wright et al (1984) to determine the structural features of mannan required to mediate the formation of myeloperoxidase-mannan-neutrophil complexes. These treated samples of mannan from S. cerevisiae were tested for their ability to bind isolated myeloperoxidase. The binding of mannan to neutrophils appeared to involve mannose oligosaccharides bound to the peptide portion of the molecule. Mannoproteins which had been subjected to β-elimination with mild alkali interacted with meyloperoxidase but were incapable of forming myeloperoxidase-mannan-neutrophil complexes. The interaction of mannan with myeloperoxidase was found to be mediated by phosphate groups of the mannan outer chain. The binding of human neutrophil myeloperoxidase to cell wall mannan is important for the candidacidal activity of the enzyme (Wright et al, 1983).

Maisch and Calderone (1981) conjugated an alkali-soluble extract from the cell wall of C. albicans to sheep erythrocytes; such treatment rendered the erythrocytes adherent to a fibrin-platelet matrix. To determine the active component which mediated attachment, the alkali-soluble extract was treated with α-mannosidase, pronase, or glusulase, or chemically degraded by acetolysis. Extracts treated with α-mannosidase or degraded by acetolysis failed to promote the adherence of erythrocytes. The results from these experiments indicated that cell surface mannan was important in mediating adherence to fibrin-platelet matrices.

If all the chemical and enzymic treatments of EPM are compared,

the two treatments which separately enhanced the ability of EPM to inhibit adherence were papain (Table 18) and mild alkali (Table 21). Therefore, in a separate experiment EPM from C. albicans GDH 2346 was first treated with papain and then with mild alkali. Such treatment resulted in the release of an adherence-inhibiting component which was considerably more active than any component produced by other treatments (Table 23). Papain treatment may have released an active peptide fragment which still has base-labile oligosaccharides attached to it. Mild alkali treatment has possibly removed these oligosaccharides leaving a peptide component which can bind to epithelial cell receptors more efficiently.

These results suggest that the yeast adhesin is protein in nature. Therefore, an attempt was made to provide further evidence that the yeast adhesin interacts with glycosidase receptors on the epithelial cell surface. Fractions of EPM from different strains of C. albicans which bound to affinity columns containing coupled sugars were tested for their effect on adherence. Components of EPM isolated from C. albicans GDH 2346 bound to affinity columns with L-fucose, D-mannose and N-acetyl-D-glucosamine. The column containing L-fucose bound the greatest proportion of protein (Figure 10) and this fraction was the most active adherence inhibitor (Table 34). EPM from C. albicans MRL 3153 was similar to EPM from GDH 2346, as here, too, the L-fucose affinity column bound the greatest proportion of protein and this fraction was also the most efficient adherence inhibitor (Table 35). The C strains of C. albicans were also examined for lectin-like interactions. The EPM preparations from these strains showed similar sugar specificities to EPM from strain GDH 2346, except that the percentage of protein which bound to the affinity columns was lower. The bound fractions were investigated for their effect on adherence and, as expected, the L-fucose-bound fractions were the most

efficient inhibitors of adherence. However, the percentage of adherence inhibition was not as great as that obtained with the L-fucose fraction from GDH 2346 EPM. This confirms the earlier observation (Table 41) that EPM samples from C strains do not contain as many adhesin components as EPM samples from I strains of C. albicans.

C. albicans GDH 2023 produces EPM which has a different pattern of sugar specificities (Figure 10). Fractions which bound to affinity columns containing L-fucose, D-mannose, N-acetyl-D-glucosamine and N-acetyl-D-galactosamine exhibited some capacity to inhibit adherence (Table 38). Therefore, C. albicans GDH 2023 EPM is not only different immunologically from that of GDH 2346, but also has different sugar-binding specificities. This may explain why EPM preparations isolated from one strain may not necessarily inhibit adhesion of another. For example, C. albicans GDH 2346 EPM could bind to L-fucose receptors leaving N-acetyl-D-glucosamine receptors available for C. albicans GDH 2023. The binding of protein-containing fractions from EPM to sugars coupled to Sepharose may be analogous to the binding of yeasts to glycoside receptors on epithelial cell surfaces. The fact that bound fractions are capable of inhibiting adherence suggests that the adhesin component is contained in these fractions, thus providing further evidence that 'lectin-like' interactions may be involved in yeast adhesion.

D. Relationship between yeast adhesion and fibronectin

The data obtained so far suggest that C. albicans attaches to a glycoside receptor on the host cell surface. These glycosides are probably present in cell surface glycoproteins or glycolipids. Skerl et al (1984) claimed that human plasma fibronectin was the receptor on buccal and vaginal cells for C. albicans. They found that the adherence of C. albicans to fibro-

nectin was similar to the binding of the yeasts to epithelial cells as yeasts treated with proteolytic enzymes were less adherent and mannan blocked adhesion. The source of fibronectin on buccal cells has not yet been established but is thought to originate from saliva (Stanislawski et al, 1985). The relationship between salivary fibronectin and other forms of the molecule remains to be determined. In this study, in contrast to the experiments of Skerl et al (1984), buccal epithelial cells were pretreated with human plasma fibronectin before use in adhesion assays. This pretreatment resulted in fewer yeasts attaching to the epithelial cells (Table 33). This situation may be similar to that described by Simpson et al (1985) in which plasma fibronectin was used to block adherence of Type I fimbriated strains of E. coli. Simpson et al (1985) found that fibronectin bound to buccal epithelial cells at the physiological hydrogen ion concentration which occurs in the oral cavity. Such treatment of buccal cells resulted in an increase in adherence of S. pyogenes (Simpson and Beachey, 1983). Therefore, fibronectin acts as a receptor for some bacteria and as a blocking agent for others.

The inhibition of yeast adhesion to buccal cells obtained after fibronectin pretreatment in this study may be due to the masking of receptors by fibronectin. Adhesion of yeasts to buccal cells increases when the epithelial cells have been briefly treated with proteolytic enzymes such as trypsin (L. McCallum and L.J. Douglas, unpublished results). It is possible that treatment with proteolytic enzymes removes cell-surface fibronectin exposing receptors for the yeast to bind. Woods et al (1983) demonstrated that patients colonized with P. aeruginosa had higher salivary proteinase levels than control patients. Whether or not a similar situation occurs in patients colonized with C. albicans has yet to be determined.

Simpson et al (1985) found that the buccal epithelial cells used in their in vitro adherence assays consisted of a mixture of fibronectin-positive and fibronectin-deficient cells. This observation may explain why C. albicans adheres to certain buccal cells and not to others, as epithelial cells coated in fibronectin would have fewer receptors available. The binding of fibronectin to buccal cells appears to have an optimal pH value of 5.0 and the ability of buccal cells from individual donors to bind fibronectin varies (Simpson et al, 1985). This variation may be due to the different salivary proteinase levels of the various individuals.

IV STRAIN-RELATED DIFFERENCES IN THE ADHESION OF CANDIDA ALBICANS

Different C. albicans strains have been shown to have different adherence capabilities, particularly when grown in defined medium containing 500 mM galactose as a carbon source. McCourtie and Douglas (1984) divided different strains into two categories. Strains in the first category, which are referred to as I strains (strains isolated from active infection), are highly adherent to epithelial cells after growth in galactose which allows extensive cell-surface modification. The adherence of C. albicans strains GDH 2346, GDH 2023 and MRL 3153 is enhanced by growth in galactose. The second category of strains (C strains), usually isolated from asymptomatic carriers, do not show enhanced adhesion after growth in galactose. C. albicans strains GRI 681 and GRI 682 are examples of such strains (Table 5). These strains also showed similar adherence patterns when adherence to acrylic surfaces was measured (McCourtie and Douglas, 1984). One strain of C. albicans - the 'outbreak' strain - was exceptionally adherent to both epithelial (Tables 6 and 44) and acrylic surfaces (Table 45) after growth in galactose medium. It is possible that the ability of this

strain to cause an outbreak of systemic candidosis was due to its enhanced ability to undergo cell-surface modification. This strain is probably capable of synthesizing a greater proportion of adhesin components allowing it to adhere more efficiently than other strains. To observe the different adherence capabilities of the various strains, yeasts must be grown on galactose as these differences are not as apparent when yeasts are grown on glucose. This may explain why Kearns et al (1983) did not observe any differences in adherence between virulent and non-virulent strains, as the growth medium they used did not enable the yeasts to modify their cell surface composition.

McCourtie and Douglas (1984) found that I strains showed enhanced virulence for mice, when injected intravenously, after growth in galactose medium, whereas C strains of C. albicans did not show any enhanced virulence. McCourtie and Douglas (1984) suggested that there is a relationship between adherence, virulence and cell surface composition. On the basis of this relationship the 'outbreak' strain of C. albicans would be expected to be exceptionally virulent for mice. It can be concluded that different strains of C. albicans vary in their ability to adhere to epithelial and acrylic surfaces. The origin of the strain appears to be relevant to its capacity to adhere and cause infection. The strain-related differences in adherence of C. albicans are also reflected in the ability of EPM samples from different strains to inhibit adherence to different extents.

V ADHERENCE OF DIFFERENT CANDIDA SPECIES

It has been widely documented that C. albicans is the most adherent and most virulent of the Candida species. King et al (1980) were the first

group to rank the adherence capabilities of Candida species and several subsequent investigators have confirmed these observations (Macura et al, 1983; Ray et al, 1984). In this study the adherence of several different species of Candida and one strain of S. cerevisiae to epithelial and acrylic surfaces was measured after growth of the yeasts in defined medium containing 50 mM glucose or 500 mM galactose. C. albicans strains were, by far, the most adherent to buccal epithelial cells after growth on galactose. C. tropicalis was the next most adherent species followed by C. stellatoidea (Table 44). The C. tropicalis (Glasgow strain) showed a slight enhancement of adherence to buccal cells after growth in medium containing galactose. C. tropicalis (London strain) gave a smaller increase in adherence along with C. stellatoidea and C. parapsilosis. Therefore, these species are capable of undergoing cell surface modification, but in a much reduced form. C. guilliermondii, C. pseudotropicalis and S. cerevisiae do not appear to be capable of modifying their cell surfaces after growth in galactose as these species showed no enhanced adherence at all.

The adherence of these species to denture acrylic was also measured (Table 45). Growth in medium containing 500 mM galactose also promoted the adhesion of C. albicans strains to acrylic. The 'outbreak' strain of C. albicans was 13 times more adherent after growth in medium containing 500 mM galactose than after growth in medium containing 50 mM glucose. These results confirm those obtained previously for C. albicans by McCourtie and Douglas (1981). The adhesion of C. albicans to acrylic surfaces does not involve a specific interaction between adhesins or receptors but is probably mediated by non-specific polymer bridging.

None of the other species investigated showed any enhanced adhesion to acrylic after growth on galactose. However, both strains of C. tropicalis and the C. parapsilosis strain were more adherent to acrylic

after growth on glucose than was C. albicans. Other investigators have also observed that C. tropicalis is more adherent than C. albicans to various inert surfaces (Rotrosen et al, 1983; Minagi et al, 1985). Hydrophobic interactions are thought to play a role in the attachment of C. tropicalis to inert surfaces (Minagi et al, 1985). The surface-free energies of C. albicans and C. tropicalis have been measured by Minagi et al (1985) and C. albicans was found to have a higher surface free energy than most denture base resin materials while the surface-free energy of C. tropicalis was lower than that of these materials. It seems that the closer the surface-free energies of the substrate surface and organism, the higher is the probability of adherence.

The adherence of Candida species to inert surfaces may be significant in relation to the aetiology of denture stomatitis and catheter-related candidaemia. Rotrosen et al (1983) quantified adherence of Candida species to two catheter materials which are in common clinical use. Catheters made from polyvinyl chloride are usually used for central venous lines and catheters made from Teflon are used for peripheral access. Candida species adhere to both materials, adherence to polyvinylchloride being greater than that to Teflon. Marrie and Costerton (1984) found C. parapsilosis biofilms with large numbers of large coccoid cells in a fibrous matrix resembling fibrin when accretions on vascular catheters were examined by transmission electron microscopy. C. parapsilosis has also been responsible for a recent outbreak of systemic infections which were caused by exogenous infection from contaminated intravenous feeds (Solomon et al, 1984). C. albicans has been found to adhere to Tenckhoff peritoneal catheters when catheters were examined by scanning and transmission electron microscopy (Marrie et al, 1983). C. albicans along with bacterial cells occurred in clumps and demonstrated no flattening when in contact with the

catheter surface. The ability of Candida species to adhere to inert surfaces may also play a role in establishing prosthetic valve endocarditis; it is possible that such conditions could be minimized by using materials to which yeasts are poorly adherent.

VI ROLE OF ADHERENCE IN VIVO

In view of the data collected on the adhesion of C. albicans to epithelial cells in vitro, animal experiments were performed to determine whether there was any correlation between in vitro and in vivo adherence of the yeast. To establish a vaginal infection in mice, the animals must be at a particular stage of the hormonal cycle, usually the oestrous stage. The oestrous stage is usually indicated by the presence of fully keratinized, anucleate, squamous epithelia and this horny layer is shed after oestrous has reached its peak (Taschdjian et al, 1960b). Exfoliated vaginal epithelial cells were obtained in this study by swabbing the vaginas of mice in the oestrous stage and the adhesion of C. albicans to these cells was measured (Table 12). The average duration of the oestrous stage in mice is usually between 1 to 2 days. In this study the oestrous stage was induced and maintained by subcutaneously injecting mice with oestradiol benzoate (Ryley et al, 1984). Kita et al (1985) found that injecting mice with oestradiol affected the function of polymorphonuclear leukocytes responsible for eliminating gonococci. It may be that the administration of oestradiol enables C. albicans to infect the vagina as a consequence of impaired leukocyte activity.

A model of chronic vaginal candidosis in rats was devised by Sobel et al (1985). This procedure was similar to that used with mice except that the rats had to be overiectomized before administering

oestradiol valerate. Sobel et al (1985) assessed vaginal infections by performing vaginal lavages and quantitatively culturing the lavage fluid rather than performing total vaginal counts, which was the procedure used in this study. Sobel et al (1985) examined histologically the vaginal mucosa of rats and found that within 48 h of injecting with oestrogen the vaginal mucosa was profoundly altered. The vaginal mucosa in rats is normally composed of non-cornified columnar cells which undergo cyto-proliferation and transformation to a stratified squamous epithelium with extensive keratinization after administration of oestradiol. The role of keratinization in human vaginal candidosis is not certain as the human vaginal epithelium is not keratinized and does not possess a stratum corneum. The human vaginal mucosa also differs from that of rodents in that it has a lower pH value and a different bacterial flora (Sobel et al, 1985).

C. albicans strains GDH 2346, GDH 2023 and B2630 were all capable of causing vaginal infections in mice (Table 46). These strains were also used to establish systemic infections by inoculating mice intravenously with yeast suspensions. Systemic infections were assessed by kidney counts, and higher counts were obtained with C. albicans strains GDH 2346 and B2630 than with C. albicans GDH 2023. This observation confirms that of McCourtie and Douglas (1984) as strain GDH 2023 gave high adherence values in assays with buccal cells and acrylic but was not very virulent for mice. The low virulence of C. albicans GDH 2023 was confirmed by following the mortality pattern obtained with these strains (Figure 12). C. albicans GDH 2023 does not adhere to the kidney as efficiently as GDH 2346 and B2630; this is probably why low kidney counts were obtained and may explain why strain GDH 2023 is not very virulent.

Age and sex of mice did not appear to affect the virulence of these strains of C. albicans (Figure 14). However, the medium used to grow the yeasts did affect virulence. A higher rate of mortality was obtained when C. albicans B2630 was grown on SDA rather than in YNB containing 50 mM glucose. This is probably because SDA contains 4% glucose and YNB has only 0.9% glucose added to it. McCourtie and Douglas (1984) have shown that the virulence of several strains of C. albicans was enhanced by growing yeasts in medium with a higher concentration of sugar. Therefore, yeasts grown on SDA were capable of cell surface modification which makes them more virulent than YNB and glucose-grown yeasts.

The growth of C. albicans in medium containing high concentrations of sugars such as galactose may have useful applications in antifungal screening tests. Three strains of C. albicans which were resistant to the antifungal agent ketoconazole gave low total vaginal counts after yeasts were grown on SDA. As a result it was impossible to use these strains in antifungal screening tests. Growth of these strains in YNB containing 500 mM galactose results in a higher total vaginal count (Table 47) enabling an in vivo drug screening procedure to be used with these strains.

Vaginal infections in mice were reduced when vaginas were pretreated with EPM isolated from the culture supernatant of C. albicans GDH 2346 (Tables 49 and 50). This suggests that the adhesin component of EPM has bound to vaginal epithelial cell receptors leaving fewer receptors available for yeast attachment. The effect of EPM was more pronounced when a smaller inoculum was used to infect the vagina. The blocking effect of EPM became less pronounced as the infection progressed with time. A similar study was conducted by Lehrer et al (1983) except that chitin-soluble-extract was used to reduce the vaginal infection caused by the yeast.

However, Lehrer et al (1983) did not inject their mice with oestradiol so the mice were not permanently in the oestrous phase of the hormonal cycle. This might explain why they did not get a high infection rate in all of their mice. These authors also used a different method to assess the extent of the vaginal infection. They prepared vaginal smears which were Gram-stained. In each smear 100 epithelial cells were counted and epithelial cells with 20 or more attached yeasts were considered as adhering cells. It would be interesting to compare total vaginal counts, vaginal lavages and vaginal smears as procedures for assessing the extent of vaginal infections. The results for the effect of EPM on vaginal infections in mice suggests that adhesin components could be used as prophylactic agents. Adhesin components would bind to epithelial cell receptors and thus block yeast adhesion and prevent infection.

It would be interesting to investigate whether EPM has any effect on systemic infections by blocking kidney receptor sites. The mechanism of adherence to the kidney is thought to be different from that to mucosal surfaces (Lee and King, 1983b). Yeasts treated with proteolytic enzymes colonized the kidney to the same extent as untreated yeasts, whereas enzyme-treated yeasts were less adherent to mucosal surfaces than untreated yeasts (Lee and King, 1983b). Segal et al (1985) protected mice against systemic infection by injecting subcutaneously with ribosomes from C. albicans. They found that there was a significant decrease in yeast colonization of the kidneys of mice injected with ribosomes. The mechanism of protection elicited by ribosome vaccination remains to be established. It is hoped that future studies will clarify whether either vaccination or therapy with surface fibrillar polymers have any practical value in man.

APPENDICES

I BIOCHEMICAL ASSAYS

A. Carbohydrate determination using the phenol-sulphuric acid method (Dubois et al, 1956)

Reagents

1. Concentrated sulphuric acid (Reagent grade)
2. 80% phenol (v/v)

Procedure

Phenol reagent (0.05 ml) was added to sample (2 ml) containing (0-100 $\mu g ml^{-1}$ carbohydrate). Reagent grade concentrated sulphuric acid (5 ml) was rapidly added and samples were left for 30 min at room temperature to allow colour development. Samples were read at 485 nm against a blank of distilled water in a spectrophotometer (Unicam SP6-550). A standard curve was produced using mannose.

B. Protein determination using the Lowry method (Lowry et al, 1951)

Reagents

A. 2% Na_2CO_3 in 0.1N NaOH

B. 0.5% $CuSO_4.5H_2O$ in 1% sodium tartrate (separate double strength solutions mixed 1:1 before use)

C. Folin-Ciocalteau reagent diluted 1:1 with distilled water (to give a total acidity of exactly 1.0N

D. Mix 50 vol of reagent A with 1 vol of reagent B (renewed daily)

Procedure

Reagent D (3 ml) was added to sample 0.6 ml), containing 0-200 $\mu g ml^{-1}$ of protein, mixed well and left to stand at room temperature for 10 min. Reagent C (0.3 ml) was added rapidly with immediate mixing. Samples were left to stand for 30 min at room temperature then read against a reagent

blank in a spectrophotometer at 750 nm with a light path of 1 cm. A standard curve was produced using bovine serum albumin.

C. Phosphorus determination (Chen et al, 1956)

Reagents

1. Digestion mixture

conc H_2SO_4 : 60% $HClO_3$ (3:2, v/v)

2. Colour reagent: 1 vol 6N H_2SO_4

2 vol distilled water

1 vol 2.5% ammonium molybdate

1 vol 10% ascorbic acid

Colour reagent is prepared fresh just before use.

3. Standard KH_2PO_4 solution (10 $\mu g ml^{-1}$).

Procedure

A few carborundum chips were added to samples (0.2 ml), containing 0-10 $\mu g ml^{-1}$, in pyrex tubes. Digestion mixture (0.1 ml) was added to samples which were heated at 140°C for 1 h in an oven. After cooling, water (3.9 ml) was added to samples followed by colour reagent (4.0 ml). Tubes were covered with nescofilm, mixed by inversion and incubated at 37°C for 1.5 to 2 h. Samples were read in a spectrophotometer (Unicam SP6-550) at 820 nm against a reagent blank. A standard curve was produced using KH_2PO_4.

II PREPARATION OF STOCK SOLUTIONS FOR SDS-PAGE (Laemmli method)

Separating and stacking gels contained 11% and 5% (w/v) acrylamide respectively. Gels and electrode buffers contained 0.1% (w/v) SDS. Gels were prepared in glass moulds 8.0 x 80 x 0.3 cm and electrophoresis was done in a Uniscil slab gel electrophoresis unit.

Stock solutions

1. Acrylamide (30.0 g) and NN'methylenebisacrylamide (0.8 g) made up to 100 ml with distilled water. This was then filtered and stored at $4^{o}C$.

2. 1M Tris-HCl buffer (pH 8.8) which was made up with 2M Tris (50 ml), 1N HCl (16.2 ml) and H_2O (33.8 ml).

3. 0.5M Tris-HCl buffer (pH 6.8) which was prepared by making up 1M Tris (50 ml) and adding 1N HCl (45 ml) and H_2O (up to 100 ml). This buffer was stored frozen.

4. Tris-Glycine buffer (pH 8.3) was made up at 10 times the required concentration. This was prepared by adding glycine (144.2 g) and Tris (30.28 g) to 1 l of distilled water.

5. Ammonium persulphate 0.8% (w/v). Made up fresh.

6. SDS made up as 20% (w/v).

7. Bromophenol blue made up as 0.1% (w/v).

8. Solubilizing buffer was prepared containing the following stock solutions: 0.5M Tris HCl pH 6.8 (25 ml), SDS (20 ml), β-mercaptoethanol (10 ml), glycerol (20 ml), bromophenol blue (2 ml) and distilled water (23 ml).

9. Fixing-staining solution was made up containing Coomassie blue R250 (1.25 g), 50% methanol (454 ml) and glacial acetic acid (46 ml).

10. Destaining solution was prepared containing methanol (50 ml), acetic acid (75 ml) and distilled water (875 ml).

11. Staining reagent for the LPS silver stain was prepared by adding 20% silver nitrate (5 ml) to 0.1N NaOH (28 ml) and concentrated NH_4OH (2 ml) while the solution was being stirred. Distilled water (115 ml) was added to give 150 ml of staining reagent.

III PREPARATION OF AGAROSE FOR OUCHTERLONY SLIDE METHOD

A 50:50 solution was prepared with 2% agarose and 0.02M phosphate buffer containing 0.01% sodium azide and kept at 56°C. A microscope slide was placed on a level bench and the above solution was used to cover the slide (2.5 ml) and allowed to set. Wells were cut in the agarose and samples (5 µl) were added to each well. After incubation slides were placed in saline containing sodium azide and kept at 4°C for 48 h. Slides were washed with distilled water and pieces of filter paper, slightly damp, were placed on top of the slide. Layers of paper towels were placed on top of this with a heavy weight on top. After 10 min all paper layers were removed and slides blown dry. Slides were then placed in a petri dish containing Coomassie blue G250 (0.05% w/v) for 2 min. The Coomassie blue was removed and slides were placed in destain.

Staining solution

This was prepared containing 96% ethanol (450 ml), glacial acetic acid (100 ml), distilled water (450 ml) and adding Coomassie brilliant blue G250 to give a concentration of 0.05% (w/v).

Destaining solution

This was prepared by adding acetic acid (75 ml) to methanol (50 ml) and adding distilled water to make up 1 l.

IV CALCULATION OF ADHERENCE INHIBITION INDEX (AII) : WORKED EXAMPLE

Calculation of the AII, based on protein content, for component 4 eluted from the Con A-Sepharose affinity column (Figure 8). The AII is a measure of the relative efficiency with which each component inhibits adherence as compared with crude EPM. At a concentration of 10 $mgml^{-1}$ crude EPM from

C. albicans GDH 2346 inhibits adherence of this strain to buccal cells by 50%. In calculating the AII, the weights of carbohydrate and protein in each component required to inhibit adherence by 50% was determined.

For component 4 eluted from the Con A-Sepharose column (Table 14) 70 μg protein inhibited adherence by 48.6%

$\therefore \frac{70 \times 50}{48.6}$ = 72.01 μg protein are required to inhibit adherence by 50%.

To calculate the AII value the weight of protein in the crude EPM sample is divided by this value

$\therefore$ AII = $\frac{1}{72.01} \times 1000 = 13.88$.

Therefore, the component eluted in component 4 is more than 13 times more efficient than crude EPM from C. albicans GDH 2346 at inhibiting adherence of this strain to epithelial cells.

REFERENCES

Adams, F. (1939). Hippocrates, Circa 460-470 B.C., Epidemics, book 3, Baltimore, Williams and Wilkins.

Arnold, W.N. (1983). Yeast cell envelopes, biochemistry, biophysics and ultrastructure. Volumes I and II, CRC Press.

Auger, P.S., Peloquin, S., Joly, J. and Marquis, G. (1984). Relationship between serotype, sensitivity to ketoconazole and inhibition of pseudomycelial growth in Candida albicans. Mykosen 27: 498-505.

Babu, J.P., Simpson, A.W., Courtney, H.S. and Beachey, E.H. (1983). Interaction of human plasma fibronectin with cariogenic and non-cariogenic oral streptococci. Infection and Immunity 41: 162-168.

Baccarini, M., Vecchiarelli, A., Cassone, A. and Bistoni, F. (1985). Killing of yeast, germ-tube and mycelial forms of Candida albicans by murine effectors as measured by a radiolabelled release microassay. Journal of General Microbiology 131: 505-513.

Bacon, J.S.D. and Farmer, V.C. (1968). The presence of a predominantly β(1-6) component in preparations of yeast glucan. Biochemical Journal 110: 34P-35P.

Ballou, C.E. (1976). Structure and biosynthesis of the mannan component of the yeast cell envelope. Advances in Microbial physiology 14: 93-158.

Banno, Y., Yamada, T. and Nozawa, Y. (1985). Secreted phospholipases of the dimorphic fungus, Candida albicans; separation of three enzymes and some biological properties. Sabouraudia 23: 47-54.

Barnes, J.L., Osgood, R.W., Lee, J.C., King, R.D. and Stein, J.H. (1983). Host-parasite interactions in the pathogenesis of experimental renal candidiosis. Laboratory Investigation 49: 460-467.

Barrett-Bee, K., Hayes, Y., Wilson, R.G. and Ryley, J.F. (1985). A comparison of phospholipase activity, cellular adherence and pathogenicity of yeasts. Journal of General Microbiology 131: 1217-1221.

Bar-Shavit, Z. and Goldman, R. (1976). Concanavalin A-mediated attachment and ingestion of yeasts by macrophages. Experimental Cell Research 99: 231-236.

Beachey, E.H. (1980). Bacterial adherence. Receptors and recognition, series B, volume 6. Chapman and Hall, London.

Beachey, E.H. (1981). Bacterial adherence: Adhesin-receptor interaction mediating the attachment of bacteria to mucosal surfaces. Journal of Infectious Diseases 143: 325-345.

Beachey, E.H., Simpson, A.W., Ofek, I., Hasty, D.L. and Dale, J.B. (1982). Role of surface polysaccharides in bacterial adhesion: Pathogenic role of surface polymers of Streptococcus pyogenes. p327-330. In D. Schlessinger (ed), Microbiology - 1982. American Society for Microbiology, Washington, DC.

Beachey, E.H., Simpson, A.W., Ofek, I., Hasty, D.L., Dale, J.B. and Whitnack, E. (1983). Attachment of Streptococcus pyogenes to mammalisn cells. Reviews of Infectious Diseases 5: S670-S677.

Beggs, W.H. (1982). Combined activity of ketoconazole and sulphamethoxy-azole against Candida albicans. Journal of Antimicrobial Chemotherapy 10: 539-543.

Benham, R.W. (1931). Certain monilias parasitic on man: their identification by morphology and agglutination. Journal of Infectious Diseases 49: 183-215.

Bistoni, F., Baccarini, M., Blasi, E., Marconi, P., Pucetti, P. and Garcia, E. (1983). Correlation between in vivo and in vitro studies of modulation of resistance to experimental Candida albicans infection by cyclophosphamide in mice. Infection and Immunity 40: 46-55.

Bistoni, F., Vecchiarelli, A., Cenci, E., Sheraglia, G., Perito, S. and Cassone, A. (1984). A comparison of experimental pathogenicity of

Candida species in cyclophosphamide- immunodepressed mice. Sabouraudia 22: 409-418.

Bittiger, H. and Schnebli, H.P. (1976). Concanavalin A as a tool. H. Bittiger and H.P. Schnebli (eds). Wiley and Sons.

Botta, G.A. (1979). Hormonal and type dependent adhesion of Group B streptococci to human vaginal cells. Infection and Immunity 25: 1084-1086.

Broughton, R.A. and Baker, C.J. (1983). Role of adherence in the pathogenesis of neonatal group B streptococcal infection. Infection and Immunity 39: 837-843.

Burnie, J.P., Odds, F.C., Lee, W., Webster, C. and Williams, J.D. (1985). Outbreak of systemic Candida albicans in an intensive care unit caused by cross infection. British Medical Journal 290: 746-748.

Burnie, J.P. and Williams, J.D. (1985). Evaluation of the Ramco Latex agglutination test in the early diagnosis of systemic candidosis. European Journal of Clinical Microbiology 4: 98-101.

Cassone, A., Simonetti, N. and Strippoli, V. (1973). Ultrastructural changes in the cell wall during germ tube formation in blastospores of Candida albicans. Journal of General Microbiology 77: 417-426.

Centeno, A., Davis, C.P., Cohen, M.S. and Warren, M.M. (1983). Modulation of Candida albicans attachment to human epithelial cells by bacteria and carbohydrates. Infection and Immunity 39: 1354-1360.

Chen, P.S., Toribara, T.Y. and Warner, H. (1956). Microdetermination of phosphorus. Analytical Chemistry 28: 1756-1758.

Cihlar, R.L., Hoberg, K. and Calderone, R.A. (1984). Isolation of cerulenin-resistant mutants of Candida albicans. p148-149. In D. Schlessinger (ed). Microbiology-1984. American Society for Microbiology, Washington, DC.

Cinco, M., Banfi, E., Ruaro, E., Gevatin, D. and Grotti, D. (1984). Evidence for L-fucose (6-deoxy-L-galactopyranose)-mediated adherence of Campylobacter spp. to epithelial cells. FEMS Microbiology Letters 21: 347-351.

Clegg, H., Guenna, N., Langerman, S., Kessler, T.W., Gruerina, V. and Goldman, D. (1984). Pilus-mediated adherence of Escherichia coli K1 to human oral epithelial cells. Infection and Immunity 45: 299-301.

Cockayne, A. and Odds, F.C. (1984). Interaction of Candida albicans yeast cells, germ tubes and hyphae with human polymorphonuclear leukocytes in vitro. Journal of General Microbiology 130: 465-471.

Cohen, P.S., Arnuda, J.C., Williams, T.J. and Laux, D.C. (1985). Adhesion of a human focal Escherichia coli strain to mouse colonic mucus. Infection and Immunity 48: 139-145.

Collins-Lech, C., Kalbfleisch, J.H., Franson, T.R. and Sohnle, P.G. (1984). Inhibition by sugars of Candida albicans adherence to human buccal mucosal cells and corneocytes in vitro. Infection and Immunity 46: 831-834.

Cox, F. (1983). Adherence of Candida albicans to buccal epithelial cells in children and adults. Journal of Laboratory and Clinical Medicine 102: 960-972.

Cruickshank, R. (1934). Conversion of glycogen of the vagina into lactic acid. Journal of Pathological Bacteriology 39: 213-219.

Cutler, J.E., Friedman, L. and Milner, K.C. (1972). Biological and chemical characterization of toxic substances from Candida albicans. Infection and Immunity 6: 616-627.

Davidson, S., Brish, M. and Rubinstein, E. (1984). Adherence of Candida albicans to buccal epithelial cells of neonates. Mycopathologia 85: 171-173.

De Repentigny, L. and Reiss, E. (1984). Current trends in immunodiagnosis of candidiasis and aspergillosis. Reviews of Infectious diseases 6: 301-312.

Diamond, R.D. and Krzesicki, R. (1978). Mechanisms 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. Journal of Clinical Investigation 61: 360-369.

Diamond, R.D., Krzesicki, R. and Wellington, J. (1978). Damage to pseudohyphal forms of Candida albicans by neutrophils in the absence of serum in vitro. Journal of Clinical Investigation 61: 349-359.

Diamond, R.D., Oppenheim, F., Nakagawa, R., Krzesicki, R. and Haudenschild, C.C. (1980). Properties of a product of Candida albicans hyphae and pseudohyphae that inhibits contact between the fungi and human neutrophils in vitro. Journal of Immunology 125: 2797-2804.

Diamond, R.D. and Haudenschild, C.C. (1981). Monocyte-mediated serum independent damage to hyphal and pseudohyphal forms of Candida albicans in vitro. Journal of Clinical Investigation 67: 172-183.

Diedrich, D.L., Mendel, S.M. and Lawrence, R.M. (1984). Apparent extracellular glycoprotein turnover product from Candida albicans. Mycopathologia 86: 65-70.

Dixon, D., Shadomy, S., Shadomy, H.J., Espinel-Ingroff, A. and Kerbering, A.M. (1978). Comparison of the in vitro antifungal activities of miconazole and a new imidazole R.41.400. Journal of Infectious Diseases 138: 245-248.

Djaczenko, W. and Cassone, A. (1972). Vizualization of the new ultrastructural components in the cell wall of Candida albicans with fixative containing TAPO. Journal of Cell Biology 52: 186-190.

Douglas, L.J., Houston, J.G. and McCourtie, J. (1981). Adherence of Candida albicans to human buccal epithelial cells after growth on different carbon sources. FEMS Microbiology Letters 12: 241-243.

Douglas, L.J. and McCourtie, J. (1981). Adherence of Candida albicans to denture acrylic as affected by changes in cell-surface composition. p375-380. In G.G. Stewart and I. Russell (eds). Current developments in yeast research. Pergamon Press, Toronto.

Douglas, L.J. and McCourtie, J. (1983). Effect of tunicamycin treatment on the adherence of Candida albicans to human buccal epithelial cells. FEMS Microbiology Letters 16: 199-202.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28: 350-356.

Epstein, J.B., Pearsall, N.N. and Truelove, E.L. (1980). Quantitative relationships between Candida albicans in saliva and the clinical status of human subjects. Journal of Clinical Microbiology 12: 475-476.

Epstein, J.B., Kimura, L.H., Menard, T.W., Truelove, E.L. and Pearsall, N.N. (1982). Effect of specific antibodies on the interaction between the fungus Candida albicans and human oral mucosa. Archives of Oral Biology 27: 469-474.

Epstein, J.B., Truelove, E.L. and Izutzu, K.T. (1984). Oral candidiasis and host defense. Reviews of Infectious Diseases 6: 96-106.

Eshdat, Y. and Sharon, N. (1982). Escherichia coli surface lectins. Methods in Enzymology 83: 386-391.

Evans, Z.A. and Mardon, D.W. (1977). Organ localization in mice challenged with a typical Candida albicans strain and a pseudohyphal variant (39780). Proceedings of the Society for Experimental Biology and Medicine 155: 234-238.

Evans, Z.A. (1980). Tissue responses to blastospores and hyphae of Candida albicans in the mouse. Journal of Medical Microbiology 14: 307-319.

Fader, R.C., Nuney, D., Undbehagen, J. and Linares, H.A. (1985). Experimental candidiasis after thermal injury. Infection and Immunity 49: 780-784.

Freter, R. and Jones, G.W. (1983). Models for studying the role of bacterial adherence in virulence and pathogenesis. Reviews of Infectious Diseases 5: 647-658.

Frevert, J. and Ballou, C.E. (1985). Saccharomyces cerevisiae structural cell wall mannoproteins. Biochemistry 24: 753-759.

Gale, E.F., Johnson, A.M., Kerridge, D. and Koh, T. (1975). Factors affecting the changes in amphotericin B sensitivity of Candida albicans during growth. Journal of General Microbiology 87: 20-36.

Gardiner, R., Podgorski, C. and Day, A.W. (1982). Serological studies on the fimbriae of yeasts and yeastlike species. Botanical Gazette 143: 534-541.

Gentles, J.C. and La Touche, C.J. (1969). Yeasts as human and animal pathogens. p108-182. In the Yeasts Vol.1. A.H. Rose and J.S. Harrison (eds). Academic Press, London.

Gibbons, R.J. and Van Houte, J. (1971). Selective bacterial adherence to oral epithelial surfaces and its role as an etiological determinant. Infection and Immunity 3: 567-573.

Gibbons, R.J., Van Houte, J. and Liljemark, W.f. (1972). Parameters that affect the adherence of Streptococcus salivarius to oral epithelial surfaces. Journal of Dental Research 51: 424-435.

Goldstein, I.J., Hollerman, C.E. and Merrick, J.M. (1965). Protein-carbohydrate interactions I. The interaction of polysaccharides with concanavalin A. Biochimica et Biophysica Acta 97: 68-76.

Gough, P.M., Warnock, D.W., Richardson, M.D., Mansell, N.J. and King, J.M. (1984). IgA and IgG antibodies to Candida albicans in the genital tract secretions of women with or without vaginal candidosis. Sabouraudia 22: 265-271.

Grappel, S.F. and Calderone, R.A. (1976). Effect of antibodies on the respiration and morphology of Candida albicans. Sabouraudia 14: 51-60.

Handley, P.S., Carter, P.L. and Fielding, J. (1984). Streptococcus salivarius strains carry either fibrils or fimbriae on the cell surface. Journal of Bacteriology 157: 64-72.

Hasenclever, H.F. and Mitchell, W.O. (1961a). Antigenic studies of Candida. I. Observation of two antigenic groups in Candida albicans. Journal of Bacteriology 82: 570-573.

Hasenclever, H.F. and Mitchell, W.O. (1961b). The pathogenicity of Candida albicans and Candida tropicalis. Sabouraudia 1: 16-21.

Hattori, M., Yoshiura, K., Negi, M. and Ogawa, K. (1984). Keratinolytic proteinase produced by Candida albicans. Sabouraudia 22: 175-183.

Hector, R.F., Dorner, J.E. and Carrow, E.W. (1982). Immune responses to Candida albicans in genetically distinct mice. Infection and Immunity 38: 1020-1028.

Helstrom, P.B. and Balish, E. (1979). Effect of oral tetracycline, the microbial flora, and the athymic state on gastrointestinal colonization and infection of BALB/c mice with Candida albicans. Infection and Immunity 23: 764-774.

Herrero, E., Pastor, J. and Sentandreu, R. (1982). Turnover of protein components of the plasma membrane of Saccharomyces cerevisiae. Biochimica et Biophysica Acta 689: 38-44.

Howlett, J.A. and Squier, C.A. (1980). Candida albicans ultrastructure: colonization and invasion of oral epithelium. Infection and Immunity 29: 252-260.

Huang, F.L. and Evans, W.H. (1973). Differences in protein and glycoprotein patterns between mature and immature neutrophil leukocytes. Biochimica et Biophysica Acta 317: 394-402.

Hurley, R. and Winner, H.I. (1962). The pathogenicity of Candida tropicalis. Journal of Pathology and Bacteriology 84: 33-38.

Ishibashi, Y. and Matsumoto, Y. (1984). Oral ketoconazole therapy for experimental Candida albicans keratitis in rabbits. Sabouraudia 22: 323-330.

Johanson, W.G.Jr., Woods, D.E. and Chaudhuni, T. (1979). Association of respiratory tract colonization and adherence of gram-negative bacilli to epithelial cells. Journal of Infectious Diseases 139: 667-673.

Johnson, E.M., Richardson, M.D. and Warnock, D.W. (1984). In vitro resistance to imidazole antifungals in Candida albicans. Journal of Antimicrobial Chemotherapy 13: 547-548.

Jones, G.W. and Freter, R. (1976). Adhesive properties of Vibrio cholerae: nature of the interaction with isolated rabbit brush border membranes and human erythrocytes. Infection and Immunity 14: 240-245.

Jones, G.W. (1977). The attachment of bacteria to the surfaces of animal cells. In Microbial Interactions, Receptors and Recognition, Series B, Vol.3 (ed.) J.L. Reissig. Chapman and Hall, London.

Jones, G.W. and Isaacson, R.E. (1983). Proteinaceous bacterial adhesins and their receptors. CRC Reviews in Microbiology 10: 229-260.

Jorizzo, J.L. (1982). Chronic mucocutaneous candidiasis: An update. Archives of Dermatology 118: 963-965.

Kearns, M.J., Davies, P. and Smith, H. (1983). Variability of the adherence of Candida albicans strains to human buccal epithelial cells; inconsistency of differences between strains related to virulence. Sabouraudia 21: 93-98.

Kessler, G. and Nickerson, W.J. (1959). Glucomannan-protein complexes from the cell walls of yeasts. Journal of Biological Chemistry 234: 2281-2285.

Kimura, L.H. and Pearsall, N.N. (1978). Adherence of Candida albicans to human buccal epithelial cells. Infection and Immunity 21: 64-68.

Kimura, L.H. and Pearsall, N.N. (1980). Relationship between germination of Candida albicans and increased adherence to human buccal epithelial cells. Infection and Immunity 28: 464-468.

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

Kita, E., Takahashi, S., Yasui, K. and Kashiba, S. (1985). Effect of estrogen (17β-estradiol) on the susceptibility of mice to disseminated gonococcal infection. Infection and Immunity 49: 238-243.

Klotz, S.A., Drutz, D.J., Harrison, J.L. and Huppert, M. (1983). Adherence and penetration of vascular endothelium by Candida yeasts. Infection and Immunity 42: 374-384.

Klotz, S.A., Drutz, D.J. and Zajic, J.E. (1985). Factors governing adherence of Candida species to plastic surfaces. Infection and Immunity 50: 97-101.

Kreger van Rij, N.J.W. (1984). The Yeasts:- a taxonomic study. Elsevier Science Publishers BV Amsterdam.

Kulkarni, R.K., Hollingsworth, P.J. and Volz, P.A. (1980). Variation in cell surface features of Candida albicans with respect to carbon sources. Sabouraudia 18: 255-260.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

La Force, F.M., Mills, D.M., Iveson, K., Cousins, R. and Everett, E.D. (1975). Inhibition of leukocyte candidacidal activity by serum from patients with disseminated candidiasis. Journal of Laboratory and Clinical Medicine 86: 657-666.

Lee, J.C. and King, R.D. (1983a). Characterization of Candida albicans adherence to human vaginal epithelial cells in vitro. Infection and Immunity 41: 1024-1030.

Lee, J.C. and King, R.D. (1983b). Mechanism of pathogenesis. Adherence of Candida albicans. p269-272. In D. Schlessinger (ed). Microbiology - 1983. American Society for Microbiology, Washington DC.

Leffler, H. and Svanborg-Eden, C. (1981). Glycolipid receptors for uropathogenic Escherichia coli on human erythrocytes and uroepithelial cells. Infection and Immunity 34: 920-929.

Lehrer, N., Segal, E. and Barr-Nea, L. (1983). In vitro and in vivo adherence of Candida albicans to mucosal surfaces. Annales de Microbiologie (Inst. Pasteur) 134B: 293-306.

Liljemark, W.F. and Gibbons, R.J. (1973). Suppression of Candida albicans by human oral streptococci in gnotobiotic mice. Infection and Immunity 8: 846-849.

Lis, H. and Sharon, N. (1973). The biochemistry of plant lectins (phytohemagglutinins). Annual Review of Biochemistry 42: 541-574.

Lopez-Martinez, R., Ruiz-Sanchez, D. and Vertiz-Chavez, E. (1984). Vaginal candidosis. Mycopathologia 85: 167-170.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193: 265-275.

MacDonald, F. and Odds, F.C. (1980). Inducible proteinase of Candida albicans in diagnostic serology and in the pathogenesis of systemic

candidosis. Journal of Medical Microbiology 13: 423-435.

MacDonald, F. and Odds, F.C. (1983). Virulence for mice of a proteinase secreting strain of Candida albicans and a proteinase deficient mutant. Journal of General Microbiology 129: 431-438.

MacDonald, F. (1984). Secretion of inducible proteinase by pathogenic Candida species. Sabouraudia 22: 79-82.

Macura, A.B., Pawlik, B. and Wita, B. (1983). Candida adherence to mucosal epithelial cells with regard to its pathogenicity. Zentralblatt für Bakteriologie und Hygiene. I. Abt.Orig.A. 254: 561-565.

Maisch, P.A. and Calderone, R.A. (1980). Adherence of Candida albicans to a fibrin-platelet matrix formed in vitro. Infection and Immunity 27: 650-656.

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

Makrides, H.C. and MacFarlane, T.W. (1982). Effect of commensal bacteria on the adherence of Candida albicans to epithelial cells in vitro. Microbios Letters 21: 55-61.

Makrides, H.C. and MacFarlane, T.W. (1983). An investigation of the factors involved in increased adherence of Candida albicans to epithelial cells mediated by Escherichia coli. Microbios 38: 177-185.

Manjeet, S., Jayakamur, A. and Prassad, R. (1979). Lipid composition and polyene antibiotic sensitivity of isolates of Candida albicans. Microbios 24: 7-18.

Mankowski, Z.T. (1968). Production of glycoprotein by Candida albicans in a synthetic medium and its influence on the growth of newborn mice. Mycopathologia et Mycologia Applicata 34: 113-118.

Marrie, T.J. and Costerton, J.W. (1981). The ultrastructure of Candida albicans infections. Canadian Journal of Microbiology 27: 1156-1164.

Marrie, T.J., Noble, M.A. and Costerton, J.W. (1983). Examination of the morphology of bacteria adhering to peritoneal dialysis catheters by scanning and transmission electron microscopy. Journal of Clinical Microbiology 18: 1388-1398.

Marrie, T.J. and Costerton, J.W. (1984). Scanning and transmission electron microscopy of in situ bacterial colonization of intravenous and intraarterial catheters. Journal of Clinical Microbiology 19: 687-693.

Marrie, T.J., Cooper, J.H. and Costerton, J.W. (1984). Ultrastructure of Candida parapsilosis endocarditis. Infection and Immunity 45: 390-398.

Martin, M.V., Craig, G.T. and Lamb, D.J. (1984). An investigation of the role of true hyphae production in the pathogenesis of experimental oral candidosis. Sabouraudia 22: 471-476.

Masler, L., Sikl, D., Bauer, S. and Sandula, J. (1966). Extracellular polysaccharide-protein complexes produced by selected strains of Candida albicans (Robin) Berkhout. Folia Microbiologia. Prague 11: 373-378.

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

McCourtie, J. and Douglas, L.J. (1984). Relationship between cell surface composition, adherence and virulence of Candida albicans. Infection and Immunity 45: 6-12.

McCourtie, J. and Douglas, L.J. (1985a). Extracellular polymer of Candida albicans: Isolation, analysis and role in adhesion. Journal of General Microbiology 131: 495-503 (1985).

McCourtie, J. and Douglas, L.J. (1985b). Unequal distribution of adhesins within populations of Candida albicans. FEMS Microbiology Letters 27: 111-115.

McEachran, D.W. and Irvin, R.T. (1985). Adhesion of Pseudomonas aeruginosa to human buccal epithelial cells: evidence for two classes of receptors. Canadian Journal of Microbiology 31: 563-569.

Meunier-Carpentier, F., Kiehn, T.E. and Armstrong, D. (1981). Fungemia in the immunocompromised host: changing patterns, antigenemia, high mortality. American Journal of Medicine 71: 363-370.

Minagawa, H., Kituara, K. and Kakarmizo (1983). Effects of pH on the activity of ketoconazole against Candida albicans. Antimicrobial Agents and Chemotherapy 23: 105-107.

Minagi, S., Mujake, Y., Inagaki, K., Tsuru, H. and Suginaka, H. (1985). Hydrophobic interactions in Candida albicans and Candida tropicalis adherence to various denture base resin materials. Infection and Immunity 47: 11-14.

Montes, L.F. and Wilborn, W.H. (1968). Ultrastructural features of host-parasite relationship in oral candidiasis. Journal of Bacteriology 96: 1349-1356.

Morgan, M.A., Wilkowske, C.J. and Roberts, G.D. (1984). Candida pseudo-tropicalis fungemia and invasive disease in an immunocompromised patient. Journal of Clinical Microbiology 20: 1006-1007.

Nakajima, T. and Ballou, C.E. (1974). Structure of the linkage region between the polysaccharide and protein parts of Saccharomyces cerevisiae mannan. Journal of Biological Chemistry 249: 7685-7695.

Nealon, T.J. and Mattingly, S.J. (1984). Role of cellular lipoteichoic acids in mediating adherence of serotype III strains of group B streptococci to human embryonic, fetal and adult epithelial cells. Infection and Immunity 43: 523-530.

Negi, M., Tsuboi, A., Matsui, T. and Ogawa, H. (1984). Isolation and characterization of a proteinase from Candida albicans:- substrate specificity. Journal of Investigative Dermatology 83: 32-36.

Nickerson, W.J. (1953). Reduction of inorganic substances by yeasts. I. Extracellular reduction of sulphite by species of Candida. Journal of Infectious Diseases 93: 45-46.

Niederman, M.S., Rafferty, T.D., Sasaki, C.T., Merrill, W.W., Matthay, R.D. and Reynolds, H.Y. (1983). Comparison of bacterial adherence to ciliated and squamous epithelial cells obtained from the human respiratory tract. American Reviews of Respiratory Diseases 127: 85-90.

Niimi, M., Kamujama, A., Tokunaga, M., Tokunaga, J. andNakajama, H. (1985). Germ-tube forming cells of Candida albicans are more susceptible to clotrimazole-induced killing than yeast cells. Sabouraudia 23: 63-68.

Nosalova, V., Trnovec, T., Greguskova, M. and Nosal, R. (1979). The effect of polysaccharide-protein complex isolated from Candida albicans on regional blood flow in rats. Experentia 35: 341-345.

Oakley, B.R., Kirsch, D.R. and Morris, N.R. (1980). A simplified ultra-sensitive silver stain for detecting proteins in polyacrylamide gels. Analytical Biochemistry 105: 361-363.

Odds, F.C. (1979). Candida and candidosis. Leicester University Press, Leicester.

Odds, F.C. (1984). Ecology and epidemiology of Candida species. Zentralblatt für Bakteriologie und Hygiene I. Abt.Orig.A. 257: 207-212.

Odds, F.C. and Abbott, A.B. (1980). A simple method for the presumptive identification of Candida albicans and identification of strains within the species. Sabouraudia 14: 301-317.

Odds, F.C. and Hierholzer, J.C. (1973). Purification and properties of a glycoprotein and phosphatase from Candida albicans. Journal of Bacteriology 114: 257-266.

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

Ofek, I. and Beachey, E.H. (1978). Mannose binding and epithelial cell adherence of Escherichia coli. Infection and Immunity 22: 247-254.

Ofek, I. and Silverblatt, F.J. (1982). Bacterial surface structures involved in adhesion to phagocytic and epithelial cells. p296-300. In D. Schlessinger (ed). Microbiology - 1982. American Society for Microbiology, Washington, DC.

Oriel, D.S., Pertridge, M.B. and Denny, J.M. (1972). Genital yeast infections. British Medical Journal 4: 761-766.

Ouchterlony, O. (1958). Diffusion-in-gel methods for immunological analysis. Progress in Allergy 5: 1-78.

Pastor, F.I.J., Valentin, E., Herrero, E. and Sentandreu, R. (1984). Structure of the Saccharomyces cerevisiae cell wall mannoproteins released by zymolyase and their contribution to wall architecture. Biochimica et Biophysica Acta 802: 292-300.

Peat, S., Whelan, W.J. and Edwards, T.E. (1961). Polysaccharides of baker's yeast. II. Yeast glucan. Journal of the Chemical Society p3862-3868.

Persi, M.A., Burnham, J.C. and Duhring, J.L. (1985). Effects of carbon dioxide and pH on adhesion of Candida albicans to vaginal epithelial cells. Infection and Immunity 50: 82-90.

Phaff, H.J. (1971). Structure and biosynthesis of the yeast cell envelope. p135-210. In A.H. Rose and J.S. Harrison (eds). The Yeasts vol.2. Academic Press, London.

Polak, A. and Grenson, M. (1973). Evidence for a common transport system of cytosine, adenine and hypoxanthine in Saccharomyces cerevisiae and

Candida albicans. European Journal of Biochemistry 32: 276-282.

Pollock, J.J., Denepitya, L., MacKay, B.J. and Iacono, V.J. (1984). Fungistatic and fungicidal activity of human parotid salivary histidine-rich polypeptides on Candida albicans. Infection and Immunity 44: 702-707.

Pope, L.M. and Cole, G.T. (1981). Scanning electron microscope studies of adherence of Candida albicans to the gastrointestinal tract of infant mice. Scanning Electron Microscopy 3: 73-80.

Pope, L.M. and Cole, G.T. (1982). Comparative studies of gastrointestinal colonization and systemic spread by Candida albicans and non-lethal yeast in the infant mouse. Scanning Electron Microscopy 4: 1667-1676.

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

Price, M.F., Wilkinson, I.D. and Gentry, L.O. (1982). Plate method for detection of phospholipase activity in Candida albicans. Sabouraudia 15: 179-185.

Pugh, D. and Cawson, R.A. (1975). The cytochemical localization of phospholipase A and lysophospholipase in Candida albicans. Sabouraudia 13: 110-115.

Pugh, D. and Cawson, R.A. (1977). The cytochemical localization of phospholipase in Candida albicans infecting the chick chorioallantoic membrane. Sabouraudia 15: 29-35.

Pugh, D. and Cawson, R.A. (1978). The surface layer of Candida albicans. Microbios 23: 19-23.

Rajasingham, K.C. and Cawson, R.A. (1982). Ultrastructural identification of extracellular material and appresoria in Candida albicans. Cytobios 35: 77-83.

Ravdin, J.L., Murphy, C.F., Salata, R.A., Guerrant, R.L. and Hewlett, E.L. (1985). N-Acetyl-D-galactosamine-inhibitable adherence lectin of Entamoeba histolytica. I. Partial purification and relation to amoebic virulence in vitro. Journal of Infectious Diseases 151: 804-815.

Ray, T.L., Digre, K.B. and Payne, C.D. (1984). Adherence of Candida species to human epidermal corneocytes and buccal epithelial cells: correlation with cutaneous pathogenicity. Journal of Investigative Dermatology 83: 37-41.

Ritchie, G.M., Fletcher, A.M., Main, D.M.G. and Prophet, A.S. (1969). The etiology, exfoliative cytology and treatment of denture stomatitis. Journal of Prosthetic Dentistry 22: 185-200.

Rogers, T.J. and Balish, E. (1980). Immunity to Candida albicans. Microbiological Reviews 44: 660-682.

Roitt, I.M. and Lehner, T. (1983). Immunology of Oral Diseases. 2nd edition, Blackwell, London.

Rotrosen, D., Gibson, T.R. and Edwards, J.E.Jr. (1983). Adherence of Candida species to intravenous catheters. Journal of Infectious Diseases 147: 594.

Rotrosen, D., Edwards, J.E.Jr., Moore, J.C. and Cohen, A.H. (1985). Adherence of Candida to cultured endothelial cells: mechanisms of attachment and endothelial cell penetration. Abstract, 9th ISHAM, Atlanta, 1985 No. S16-5.

Rüchel, R. (1981). Properties of a purified proteinase from the yeast Candida albicans. Biochimica et Biophysica Acta 659: 99-113.

Rüchel, R. (1984). A variety of Candida proteinases and their possible targets of proteolytic attack in the host. Zentralblatt für Bakteriologie und Hygiene Abt.Orig.A. 257: 266-274.

Russell, C. and Jones, J.H. (1973a). Effect of oral inoculation of Candida albicans in tetracycline-treated rats. Journal of Medical Microbiology 6: 275-279.

Russell, C. and Jones, J.H. (1973b). Effect of oral inoculation of yeast and mycelial phases of Candida albicans in rats fed on normal and carbohydrate diets. Archives of Oral Biology 18: 409-412.

Rutler, P. and Vincent, B. (1980). The adhesion of microorganisms to surfaces: physico-chemical aspects. p79-82. In Microbial adhesion to surfaces. R.C.W. Berkely (ed). Ellis Harwood, Chichester.

Ryley, J.F., Wilson, R.G. and Barrett-Bee, K.J. (1984). Azole resistance in Candida albicans. Sabouraudia 22: 53-63.

Saltarelli, C.G. (1973). Growth stimulation and inhibition of Candida albicans by metabolic bi-products. Mycopathologia Mycologia applicata 51: 53-56.

Saltarelli, C.G., Gentile, K.A. and Mancuso, S.C. (1975). Lethality of Candida strains as influenced by the host. Canadian Journal of Microbiology 21: 648-653.

Saltarelli, C.G. and Coppola, C.P. (1980). Inhibitory effect of Candida albicans extracellular polysaccharide on mouse Sarcoma 180. Journal of Surgical Oncology 15: 99-106.

Samaranayake, L.P. and MacFarlane, T.W. (1980). An in vitro study of adherence of Candida albicans to acrylic surfaces. Archives of Oral Biology 25: 603-609.

Samaranayake, L.P., McCourtie, J. and MacFarlane, T.W. (1980). Factors affecting the in vitro adherence of Candida albicans to acrylic surfaces. Archives of Oral Biology 25: 611-615.

Samaranayake, L.P. and MacFarlane, T.W. (1981). The adhesion of the yeast Candida albicans to epithelial cells of human origin in vitro. Archives of Oral Biology 26: 815-820.

Samaranayake, L.P. and MacFarlane, T.W. (1982a). The effect of dietary carbohydrates on the in vitro adhesion of Candida albicans to epithelial cells. Journal of Medical Microbiology 15: 511-517.

Samaranayake, L.P. and MacFarlane, T.W. (1982b). Factors affecting in vitro adherence of the fungal oral pathogen Candida albicans to epithelial cells of human origin. Archives of Oral Biology 27: 869-873.

Samaranayake, L.P., Raeside, J.M. and MacFarlane, T.W. (1984). Factors affecting the phospholipase activity of Candida species in vitro. Sabouraudia 22: 201-207.

Samaranayake, L.P. and MacFarlane, T.W. (1985). Hypothesis on the role of dietary carbohydrates in the pathogenesis of oral candidosis. FEMS Microbiology Letters 27: 1-5.

Sams, W.M., Jorizzo, J.L., Snyderman, R. et al (1979). Chronic muco-cutaneous candidosis: Immunologic studies of three generations of a single family. American Journal of Medicine 67: 948-958.

Sandin, R.L. and Rogers, A.L. (1982). Inhibition of adherence of Candida albicans to human epithelial cells. Mycopathologia 77: 23-26.

Sandin, R.L., Rogers, A.L., Patterson, R.J. and Beneke, E.S. (1982). Evidence for mannose-mediated adherence of Candida albicans to human buccal cells in vitro. Infection and Immunity 35: 79-85.

Savage, N. and Balish, E. (1971). Morphology, physiology and virulence of some mutants of Candida albicans. Infection and Immunity 3: 141-148.

Scaletsky, I.C.A., Silva, M.L.M. and Trabulsi, L.R. (1984). Distinctive patterns of adherence of enteropathogenic Escherichia coli to HeLa cells. Infection and Immunity 45: 534-536.

Scheld, W.M., Calderone, R.A., Brodeur, J.P. and Sande, M.A. (1983). Influence of preformed antibody on the pathogenesis of experimental Candida albicans endocarditis. Infection and Immunity 40: 950-955.

Segal, E., Lehrer, N. and Ofek, I. (1982). Adherence of Candida albicans to human vaginal epithelial cells: inhibition by amino sugars. Experimental Cell Biology 50: 13-17.

Segal, E., Soroka, A. and Schreichter, A. (1984a). Correlative relationship between adherence of Candida albicans to human vaginal epithelial cells in vitro and candidal vaginitis. Sabouraudia 22: 191-200.

Segal, E., Soroka, A. and Lehrer, N. (1984b). Attachment of Candida to mammalian tissues - clinical and experimental studies. Zentralblatt für Bakteriologie und Hygiene I Abt.Orig.A. 257: 257-265.

Segal, E., Nussbaum, S. and Barr-Nea, L.(1985).Protection against systemic infections with various Candida species elicited by vaccination with Candida albicans ribosomes. Sabouraudia 23: 275-285.

Segrest, J.P. and Jackson, R.L. (1972). Methods in Enzymology, V. Ginsberg (ed). Vol.27. p54-63. Academic Press, New York.

Sentandreu, R., Elorza, M.V. and Villaneuva, J.R. (1975). Synthesis of yeast wall glucan. Journal of General Microbiology 90: 13-20.

Sharon, N., Eshdat, Y., Silverblatt, F.J. and Ofek, I. (1981). Bacterial adherence to cell surface sugars. p119-141. In Adhesion and Microorganism Pathogenicity, Ciba Foundation Symposium 80. Pitman Medical, Tunbridge Wells.

Sharon, N. and Lis, H. (1972). Lectins:- Cell agglutinating and sugar specific proteins. Science 177: 949-959.

Sikl, D., Masler, L. and Bauer, S. (1969). Relationship between extracellular and cell wall polysaccharides of selected yeasts and yeast-like microorganisms. Antonie van Leeuwenhoek 35: A9.

Simpson, A.W., Courtney, H. and Beachey, E.H. (1982). Fibronectin - a modulator of the oropharangeal bacterial flora. p346-347. In D. Schlessinger (ed). Microbiology - 1982. American Society for Microbiology, Washington, DC.

Simpson, A.W. and Beachey, E.H. (1983). Adherence of group A streptococci to fibronectin on oral epithelial cells. Infection and Immunity 39: 275-279.

Simpson, A.W., Hasty, D.L. and Beachey, E.H. (1985). Binding of fibronectin to human buccal epithelial cells inhibits the binding of Type I fimbriated Escherichia coli. Infection and Immunity 48: 318-323.

Skerl, K.G., Calderone, R.A. and Sreevalsan, T. (1981). Platelet interactions with Candida albicans. Infection and Immunity 34: 938-943.

Skerl, K.G., Calderone, R.A., Segal, E., Sreevalsan, T. and Scheld, W.M. (1984). In vitro binding of Candida albicans yeast cells to human fibronectin. Canadian Journal of Microbiology 30: 221-227.

Sklavanou, A. and Germaine, G.R. (1980). Adherence of oral streptococci to keratinized and non-keratinized human oral epithelial cells. Infection and Immunity 27: 686-689.

Smith, C.B. (1985). Candidiasis, host resistance and predisposing factors p53-70. In G.P. Bodey and V. Fainstein (ed). Candidiasis. Raven Press, New York.

Sobel, J.D., Myers, P.G., Kaye, D. and Levison, M.E. (1981). Adherence of Candida albicans to human vaginal and buccal epithelial cells. Journal of Infectious Diseases 143: 76-82.

Sobel, J.D., Myers, P.G., Levison, M.E. and Kaye, D. (1982). Comparison of bacterial and fungal adherence to vaginal exfoliated epithelial cells and human vaginal epithelial tissue culture cells. Infection and Immunity 35: 697-701.

Sobel, J.D. and Obedeanu, N. (1983). Effects of subinhibitory concentrations of ketoconazole on in vitro adherence of Candida albicans to vaginal epithelial cells. European Journal of Clinical Microbiology 2: 445-452.

Sobel, J.D., Muller, G. and Buckley, H. (1984). Critical role of germ tube formation in the pathogenesis of candidal vaginitis. Infection and Immunity 44: 576-580.

Sobel, J.D., Muller, G. and McCormick, J.F. (1985). Experimental chronic vaginal candidosis in rats. Sabouraudia 23: 199-206.

Solomon, J.L., Khabbaz, R.F., Parker, R.H., Anderson, R.L., Geraghty, M.A., Furman, M.A. and Martone, W.J. (1984). An outbreak of Candida parapsilosis bloodstream infections in patients receiving parenteral nutrition. Journal of Infectious Diseases 149: 98-102.

Staib, F. (1965). Serum proteins as nitrogen source for yeastlike fungi. Sabouraudia 4: 187-193.

Stanislawski, L., Simpson, A.W., Hasty, D., Sharon, N., Beachey, E.H. and Ofek, I. (1985). Role of fibronectin in attachment of Streptococcus pyogenes and Escherichia coli to human cell lines and isolated oral epithelial cells. Infection and Immunity 48: 257-259.

Sud, I.J. and Feingold, D.S. (1983). Effect of ketoconazole on the fungicidal action of amphotericin B in Candida albicans. Antimicrobial Agents and Chemotherapy 23: 185-187.

Sugarman, B. (1980). Effect of heavy metals on bacterial adherence. Journal of Medical Microbiology 13: 351-354.

Svanborg-Eden, C., Hagberg, L., Hanson, L.A., Korhonen, T., Leffler, H. and Olling, S. (1981). Adhesion of Escherichia coli in urinary tract infection. p161-187. In Adherence and Microorganism Pathogenicity. Ciba Foundation Symposium 80. Pitman Medical, Tunbridge Wells.

Takamiya, H., Vogt, A., Batsford, S., Kuttin, E.S. and Muller, J. (1984). Further studies on the immunoelectronmicroscopic localization of polysaccharide antigens on ultra thin sections of Candida albicans. Mykosen 28: 17-32.

Tarentino, A.L., Plummer, T.H. and Maley, F. (1974). The release of intact oligosaccharides from specific glycoproteins by endo-β-N-acetylglucosaminidase H. Journal of Biological Chemistry 249: 818-824.

Taschdjian, C.L., Burchall, J.J. and Kozinn, P.J. (1960a). Rapid identification of Candida albicans by filamentation on serum and serum substitutes. American Journal of Diseases of Children 99: 212-215.

Taschdjian, C.L., Reiss, F. and Kozinn, P.J. (1960b). Experimental vaginal candidiasis in mice; its implications for superficial candidiasis in humans. Journal of Investigative Dermatology 34: 89-94.

Tkacz, J.S. and Lampen, J.O. (1972). Wall replication in Saccharomyces species; use of fluorescein-conjugated concanavalin A to reveal the site of mannan insertion. Journal of General Microbiology 72: 243-247.

Tronchin, G., Poulain, D. and Verries, A. (1984). Cytochemical and ultrastructural studies of Candida albicans. Archives of Microbiology 139 : 221-224.

Tsai, C. and Frasch, C.E. (1982). A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Analytical Biochemistry 119: 115-119.

Valentin, E., Herrero, E., Pastor, F.I.J. and Sentandreu, R. (1984). Solubilizotion and analysis of mannoprotein molecules from the cell wall

of Saccharomyces cerevisiae. Journal of General Microbiology 130: 1419-1428.

Vercellotti, G.M., Lussenhop, D., Peterson, P.K., Furcht, L.T., McCarthy, J.B., Jacob, H.S. and Moldow, C.F. (1984). Bacterial adherence to fibronectin and endothelial cells: a possible mechanism for bacterial tissue tropism. Journal of Laboratory and Clinical Medicine 103: 34-43.

Vudhichamnong, K., Walker, D.M. and Ryley, H.C. (1982). The effect of secretory immunoglobulin A on the in vitro adherence of the yeast Candida albicans to human oral epithelial cells. Archives of Oral Biology 27: 617-621.

Warnock, D.W., Speller, D.C.E., Kay, J.K. and Farell (1979). Resistogram method for differentiation of strains of Candida albicans. Journal of Applied Bacteriology 46: 571-578.

Weber, K. and Osborne, M. (1969). The reliability of molecular weight determinations by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Journal of Biological Chemistry 244: 4406-4412.

Wingard, J.R., Merz, W.G. and Seral, R. (1979). Candida tropicalis: A major pathogen in immunocompromised patients. Annals of Internal Medicine 91: 539-543.

Winner, H.I. and Hurley, R. (1964). Candida albicans. Churchill, London.

Woods, D.E., Bass, J.A., Johanson, W.G.Jr. and Strauss, D.C. (1980). Role of adherence in the pathogenesis of Pseudomonas aeruginosa lung infection in cystic fibrosis patients. Infection and Immunity 30: 649-699.

Woods, D.E., Strauss, D.C., Johanson, W.G. and Bass, J.A. (1981a). Role of salivary protease activity in adherence of gram-negative bacilli to mammalian buccal epithelial cells in vivo. Journal of Clinical Investigation 68: 1435-1440.

Woods, D.E., Strauss, D.C., Johanson, W.G.Jr. and Bass, J.A. (1981b). Role of fibronectin in the prevention of adherence of Pseudomonas aeruginosa to buccal cells. Journal of Infectious Diseases 143: 784-790.

Woods, D.E., Strauss, D.C., Johanson, W.G.Jr. and Bass, J.A. (1983). Factors influencing the adherence of Pseudomonas aeruginosa to mammalian buccal epithelial cells. Reviews of Infectious Diseases 5: S846-851.

Wright, C.D., Bowie, J.U., Gray, G.R. and Nelson, R.D. (1983). Candidacidal activity of myeloperoxidase: mechanisms of inhibitory influence of soluble cell wall mannan. Infection and Immunity 42: 76-80.

Wright, C.D., Bowie, J.U. and Nelson, R.D. (1984). Influence of yeast mannan on release of myeloperoxidase by human neutrophils: determination of structural features of mannan required for formation of myeloperoxidase-mannan neutrophil complexes. Infection and Immunity 43: 467-471.

Yu, R.J., Bishop, C.T., Cooper, F.P., Hasenclever, H.F. and Blank, F. (1967). Structural studies of mannans from Candida albicans (serotype A and serotype B), Candida parapsilosis, Candida stellatoidea and Candida guilliermondii. Canadian Journal of Chemistry 45: 2205-2211.

Zlotnik, H., Fernandez, M.P., Powers, B. and Cabib, E. (1984). Saccharomyces cerevisiae mannoproteins from an external cell wall layer that determines wall porosity. Journal of Bacteriology 159: 1018-1026.