IN VIVO AND IN VITRO STUDIES OF ORAL CANDIDAL COLONISATION AND ADHESION IN DIABETIC SUBJECTS

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This thesis is the original work of the author.

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SUMMARY

Diabetes mellitus is the most common endocrine disorder affecting about one per cent of the population although about the same number are still undiagnosed clinically. It is believed that diabetic patients are more predisposed to infections than non-diabetic individuals and oral candidosis is more common in diabetic patients.

Many local and systemic factors have been claimed to predispose an individual to oral candidal infection and the integrity of host defence factors seems to be an important determinant in mucocutaneous candidosis. Therefore, in Chapter One the local host defence factors of known importance, in addition to some general factors thought to increase the risk for oral candidosis have been reviewed, and particular attention has been directed toward those factors that might predispose diabetic patients to oral candidal infection.

Since candidal adhesion to host surfaces is recognised as an essential prerequisite for colonisation and subsequent infection, factors affecting adhesion of candidal species to epithelial cell and denture acrylic surfaces, in addition to the possible mechanisms involved, were reviewed in Chapter Two.

Although oral candidal carriage and infection in diabetic patients has been reported, the various methodologies used have been criticised, and consequently the reasons for increased oral candidal colonisation have never been clearly defined. Chapter Three of this study aimed to study and compare the prevalence of candidal carriage and clinical candidal infection in the oral cavity of a group of diabetic patients and a matched group of control subjects using

current methodology. In addition, the effect if any, of factors such as degree of glycaemic control, type and duration of diabetes as well as the inherited ability to secrete ABO blood group antigens in saliva, on candidal carriage and infection was also evaluated.

Fifty patients with diagnosed diabetes mellitus (19 insulin-dependent and 31 non-insulin dependent) and 50 age, gender and denture status matched non-diabetic individuals underwent routine oral clinical examination where the presence of angular cheilitis or oral candidal infection was recorded. Swabs and smears were collected from clinically infected mucosa and perioral skin and oral candidal carriage was determined using a concentrated oral rinse technique. The samples were inoculated on Sabouraud's dextrose agar plates and incubated aerobically for 48 hours at 37°C.

Unstimulated mixed saliva (2ml) was collected from each subject and assayed for ABO blood group antigen secretor status using an agglutination inhibition technique. Each subject had haematological and biochemical assessment including corrected whole blood folate, ferritin, vitamin B12, haemoglobin and random blood glucose. Diabetic patients were also assayed for glycosylated haemoglobin concentration.

The rate and quantity of oral candidal isolation was higher in diabetic patients compared to non-diabetics although the difference was not significant but, diabetic patients had a significantly increased incidence of clinical oral candidal infection ($p<0.002$). Candida albicans was by far the most commonly isolated species followed by C. glabrata. Age, gender and denture status had no significant relationship to either rate or quantity of candidal isolation but, diabetic patients who wore dentures continuously (day

and night) had a significantly increased rate of candidal isolation ($p<0.05$) and clinical candidal infection ($p<0.0005$) over those who wore dentures during the day only.

Haematinic status had no significant effect on oral candidal isolation. Factors such as blood glucose level, glycosylated haemoglobin, type and duration of diabetes also had no significant relationship to oral candidal isolation in diabetic patients. The ability to secrete ABO blood group antigens in saliva was not different between diabetic patients and control subjects nor between diabetic patients in relation to type of diabetes. However, diabetic patients who were non-secretors had a significantly increased rate of oral candidal isolation compared to secretors ($p<0.05$).

Candidal adhesion to mucosal surfaces was recognised as an essential first step in the process of candidal colonisation and infection. In view of the results obtained and described in Chapter Three, the objectives of the study described in Chapter Four were to investigate and compare adhesion of *C. albicans* to buccal epithelial cells (BEC) in a group of diabetic patients and a closely matched group of non-diabetic individuals to determine whether a relationship existed between the *in vitro* candidal adhesion to BEC and the increased predisposition to clinical oral candidal colonisation and infection.

The *Candida albicans* strain CDS 88 used throughout this study was originally isolated from the oral cavity of an asymptomatic carrier. For candidal adhesion assay *in vitro*, equal volumes (0.5ml) of a standardised suspension of candidal cells and BEC were incubated in an orbital shaker for one hour and then the cells were harvested on 12μm pore-size filter and washed with phosphate buffered saline (PBS).

The BEC and the adherent candidal cells were Gram-stained and the number of C. albicans blastospores adherent to 100 individual BEC in randomly selected fields were counted microscopically.

Mean candidal adhesion (MCA) in the diabetic patients was significantly higher than in the control group ($p<0.001$) and correlated inversely with age ($p<0.05$). In control subjects, a higher MCA was observed in females than males ($p<0.02$) and also in continuous denture wearers compared to those who wore dentures during the day only ($p<0.05$). In both groups, no significant correlation was observed between MCA and smoking or alcohol drinking habits, symptomatic xerostomia, or haematinic status. Nevertheless, a significant correlation was observed between MCA and haemoglobin concentration in control subjects ($p<0.05$). Neither type of diabetes nor blood glucose concentration had a relationship to MCA but, MCA was higher in patients with duration of diabetes less than five years compared with those who had the disease for more than five years ($p<0.05$) and, in patients who had glycosylated haemoglobin higher than 12% compared with those below 12% ($p= 0.05$). The relationship between in vitro candidal adhesion and oral candidal colonisation and infection was not significant in either group and, MCA was not significantly different between secretors and non-secretors.

In the light of the results of Chapter Four and the reports that pretreatment of acrylic strips with chlorhexidine gluconate reduced adherence of Candida species, the objective of Chapter Five was to investigate whether mouth rinsing with 0.2% chlorhexidine would similarly reduce candidal adhesion to BEC comparably in diabetic patients and non-diabetic individuals. Twelve diabetic patients and 12 closely-matched control subjects participated in the study.

Initially, BEC were obtained from each individual and subjected to 10ml of 0.2% chlorhexidine gluconate *in vitro* for one minute, then assayed for candidal adhesion as described previously. In the second part of the experiment, each subject was required to rinse the mouth throughly with 10ml chlorhexidine gluconate mouth rinse for one minute and then BEC were assayed for candidal adhesion. The same subjects participated in both parts of the experiment which was repeated twice with a two week interval between visits. BEC collected from each subject prior to exposure to chlorhexidine were also assayed for candidal adhesion and used as control cells.

Exposure of BEC to 0.2% chlorhexidine gluconate, both *in vitro* and *in vivo*, for one minute resulted in a significant reduction in candidal adhesion to BEC both in diabetic patients and control subjects. The reduction in adhesion was more marked in control subjects than in diabetic patients and, more pronounced *in vitro* than *in vivo* in both groups but not significantly so ($p>0.05$). The reduction in mean candidal adhesion was comparable between secretors and non-secretors in both groups.

The antifungal agent nystatin is widely prescribed for the treatment of oral candidosis and well known for its potent fungicidal activity. Previous reports have shown pretreatment of acrylic strips with nystatin to result in a significant reduction in candidal adhesion. The main aim of Chapter Six was to investigate whether nystatin, in therapeutic doses, would reduce candidal adhesion to buccal epithelial cells and whether any differences were discernable between diabetic patients and non-diabetic individuals.

Twelve diabetic patients and 12 closely-matched control subjects participated in this part of the study. Initially, BEC were

obtained from each subject and exposed in vitro to 1ml of nystatin solution (100,000iu) for ten minutes and then assayed for candidal adhesion. Subsequently, each subject was asked to dissolve a nystatin pastille (100,000iu) in the mouth and then BEC were collected and assayed for candidal adhesion. Each experiment was repeated twice with each subject with a two week interval between experiments. BEC collected from each subject before exposure to nystatin were assayed for candidal adhesion and used as control cells. Pretreatment of BEC for ten minutes with nystatin (100,000iu) in vitro resulted in a significant reduction in candidal adhesion both to BEC from diabetic patients and control subjects ($p<0.002$). However, the effect of dissolution of one nystatin pastille (100,000) did not affect candidal adhesion to BEC significantly in either group. The effect of nystatin pretreatment on candidal adhesion was not sigificantly different between secretors and non-secretors.

Thus this study has shown that patients with diabetes mellitus are more prone than normal to develop oral candidosis. Factors such as type of diabetes, duration or quality of glycaemic control seems to have no significant effect on this susceptibility. However, the significantly increased candidal adhesion to buccal epithelial cells of diabetic subjects compared to controls may partially explain the increased incidence of oral candidosis. Nevertheless, as to why only a limited number of diabetic patients develop oral candidosis while the majority remain free of infection is still far from clear.

The aetiological factors in oral candidosis are often described as multifactorial and are probably increasingly complex in diabetic subjects. Unfortunately, many of the previous studies

undertaken on this topic have been cross-sectional in design and have thus yielded limited informations. What is undoubtedly required are carefully designed longitudinal studies, monitoring oral candidal status in diabetic patients and testing candidal adhesion to oral epithelial cells in different states of glycaemic control. These studies should also take into account other factors that might affect candidal colonisation and adhesion in these patients such as salivary glucose level and the influence of oral commensal bacterial flora. Obviously, standardised oral candidal sampling methods and candidal adhesion assay would be needed to allow comparison of results between different research centres. In that regard, the widespread application of the concentrated oral rinse technique for oral microbial sampling described previously and used in the present study would be beneficial.

Should the present finding of increased candidal adhesion *in vitro* to buccal epithelial cells of diabetic subjects be confirmed in the future, the detailed study of other factors involved in adhesion could be pursued such as analysis of epithelial cell surface lectins.

If one minute oral rinse with 0.2% chlorhexidine gluconate was shown to reduce candidal adhesion to epithelial cells *in vitro*, more studies are required to define the effect of this oral rinsing on reducing the incidence of oral candidosis in population generally and in diabetic subjects in particular. Topical nystatin therapy must continue for at least three to four weeks befor resolution of oral candidal infection. The effect of nystatin pastilles intake over a prolonged period of time on candidal adhesion warrants study.

CHAPTER ONE

PREDISPOSING FACTORS FOR ORAL CANDIDOSIS: REVIEW OF LITERATURE

1.1 INTRODUCTION

Candidal infection has been frequently reported in the oral cavity of compromised patients such as those with diabetes mellitus, haematological malignancy and patients infected with the human immunodeficiency virus. Candida species are commensal inhabitants of the oral cavity of about 40 per cent of normal individuals (Arendorf & Walker, 1980) but, the eventual outcome of the balance between the efficiency of the host defence mechanisms and the pathogenicity of the candidal organisms determines whether clinical disease occurs. This balance between the host defence factors and the virulence of Candida species appears to be surprisingly even so Candida may be able to provoke infection whenever a breakdown of host resistance occurs. Candida albicans is considered the most pathogenic of the medically important Candida species since it is isolated most commonly from cases of oral candidosis. Whilist being potentially capable of causing infection, the virulence of Candida seems to be insufficient to establish clinical infection in an entirely uncompromised host. Therefore, the integrity of local and systemic host defence mechanisms seems to be of prime importance in determining pathogenicity.

The following discussion of the factors predisposing to oral candidosis deliberately emphasises the most important factors rather than being an exhaustive account of all known or possible predisposing factors.

1.2 HOST FACTORS IN ORAL CANDIDAL INFECTION

1.2.1 Epithelial Barrier

The continuous desquamation of the epithelial cells lining the oral cavity constitutes an important factor in limiting candidal colonisation of the oral mucosa. In addition, certain proteins present on the mucosal cells may retard candidal invasion as recent studies have shown that proteins isolated from the rat epidermis exhibit candidacidal activity (Kashima et al., 1989).

Local traumatic factor in denture wearers have been postulated as contributing to chronic atrophic candidosis (Turrell, 1966). A recent animal experimental study showed that inoculation of C. albicans per se did not lead to candidal invasion or tissues changes in the rat palate, except in areas where ulceration of the surface epithelium occurred due to the presence of a prosthesis (Jennings, 1988). In angular cheilitis, the folds at the corners of the mouth may, in some cases, be related to an overclosed bite leading to increased maceration of the mucosa, and the continuous bathing of saliva may facilitate candidal infection (Cawson, 1963).

1.2.2 Salivary Flow

The flushing effect of salivary flow dislodges yeasts from the surface of the oral mucosa, and helps limit oral candidal colonisation. Low salivary output appears to be associated with higher rates of candidal recovery (Parvinen & Larmas, 1981). Oral candidosis has been reported in patients with xerostomia (MacFarlane & Mason, 1974) and animals with reduced salivary flow rate (Olsen & Haanaes, 1977).

1.2.3 Salivary Hydrogen Ion Concentration (pH)

The possible relationship between increased yeast isolation and low pH in the oral cavity has been reiterated by several authors (Young, Resca, Sullivan, 1951; Shipman, 1979; Arendorf & Walker, 1980).

The low pH medium could activate extracellular candidal phospholipase (Samaranayake, Raeside, MacFarlane, 1984) and acid proteinases production (Samaranayake, Hughes, MacFarlane, 1984) which are of potential importance in relation to the virulence of candidal species.

Candidal adhesion to epithelial cells is increased at low pH (Samaranayake & MacFarlane, 1982a). In addition, the aciduric and acidophilic nature of Candida species enables it to thrive in a low pH milieu in contrast to other components of the oral flora which may not survive in such conditions (Odds & Abbott, 1984).

1.2.4 Salivary Glucose Concentration

The study of Knight and Fletcher (1971) showed inhibition of candidal growth when cultured in saliva of normal subjects but this could be reversed by the addition of glucose. In another study, candidal growth was significantly increased in glucose-supplemented saliva when compared with glucose-free saliva (Samaranayake et al., 1986a). In addition, a diet rich in glucose may enhance candidal adhesion to epithelial cells (Samaranayake & MacFarlane, 1982b) and acrylic denture surfaces (Samaranayake & MacFarlane, 1980).

1.2.5 Salivary Lactoferrin

Salivary lactoferrin, the exocrine gland equivalent of transferrin, is an iron-binding glycoprotein synthesised by serous epithelial cells and neutrophils (Arnold, Brewer, Gauthier, 1980). Lactoferrin has been shown to be an active antifungal agent (Kirkpatrick et al., 1971; Arnold et al., 1980). Iron-unsaturated lactoferrin has an iron binding capacity sufficient to inhibit optimal growth of C. albicans but, it loses this capability in an iron-saturated environment (Kirkpatrick et al., 1971).

The immune function of lactoferrin in candidal infection is not clear. Lactoferrin concentration in parotid saliva from patients with chronic mucocutaneous candidosis has been reported to be comparable to that in control subjects (Kirkpatrick et al., 1971).

1.2.6 Salivary Lysozyme

Lysozyme is a low molecular weight protein originating in the oral cavity from saliva, gingival crevicular fluid and polymorphonuclear leucocytes (Fukui, Fukui, Moriyama, 1981). It has a growth inhibitory effect on several Candida species (Tobgi, Samaranayake, MacFarlane, 1988) and has the ability to potentiate the antifungal effect of amphotericin B (Collins & Pappagianis, 1974). Ultrastructural studies have shown lysozyme to alter Candida cell membrane permeability (Marquis et al., 1982).

1.2.7 Salivary Histidine-Rich Polypeptides

The initial findings of an in vitro study showed that human parotid salivary histidine-rich polypeptides had fungistatic and fungicidal activity towards C. albicans at very low concentrations

(Pollock et al., 1984). However, the antifungal activity observed was strain-dependent.

1.2.8 Salivary Immunoglobulin A (IgA)

It has been estimated that about 100mg of salivary IgA is secreted daily in saliva, along with relatively smaller amounts of IgG and IgM (Roitt & Lehner, 1983). As salivary IgA is more resistant to proteolytic degradation by bacterial hydrolases than other immunoglobulins, this renders it capable of continuing function on the oral mucous membranes(Jeganathan et al., 1987).Therefore, salivary IgA is quantitatively and qualitatively the most important immunoglobulin secreted in saliva. Accumulating evidence suggests that dimeric salivary IgA is produced locally in the salivary glands by plasma cells and is different than monomeric IgA produced in the serum (Dolby et al., 1981).

Titers of salivary anticandidal IgA have been shown to correlate with isolation of candidal species from the oral cavity (Jeganathan et al., 1987). Thus titres were low in non-carriers and high in clinically infected subjects (Lehner, 1965), while asymptomatic Candida carriers had anticandidal IgA levels intermediate between those of non-carriers and candidosis patients (Epstein et al., 1982). Two subclasses of IgA (IgA1 and IgA2) exist in approximately equal proportion in oral secretions but, a recent study has shown salivary IgA1 subclass antibodies are produced selectively in response to clinical candidal infection (Jeganathan et al., 1987).

Studies on the significance of salivary IgA defence against candidal infection have yielded conflicting results. Reduced

concentrations of salivary IgA anticandidal antibodies have been reported in patients with chronic mucocutaneous candidosis (Chilgren et al., 1967; Lehner, Wilton, Ivanyi, 1972). Secretory IgA has been shown to inhibit the adhesion of C. albicans to epithelial cells (Vudhichamnong, Walker, Ryley, 1982). Since newborn infants lack salivary IgA, it is believed that the secretory IgA content of human breast milk may be responsible in part for the lower incidence of thrush in breast-fed infants, compared with that in bottle-fed neonates (Ludlam & Henderson, 1942). On the other hand, oral candidosis often persists in spite of a locally detectable salivary IgA response (Epstein et al., 1982), and recurrent candidal infections may develop in the presence of specific IgA in secretions (Gough et al., 1984). These studies suggest that salivary IgA alone lacks the ability to eliminate the yeast, although its presence may limit the development of infection (Lehner et al., 1972). The susceptibility of salivary IgA molecules to degradation by proteinases produced by Candida species may partly explain the inability of salivary IgA to protect against oral candidosis (Reinholdt, Krogh, Holmstrup, 1987).

1.2.9 Salivary Blood Group Antigens

It has been realised that ABO blood group antigens are not confined to the red blood cells but are secreted in body fluids and other secretions particularly saliva (Ferguson, 1975). The inherited inability to secrete blood group substances has been linked with susceptibility to several infections including candidosis (Blackwell, 1989). Buccal epithelial cells of secretors have been shown to bind Candida blastospores in lesser numbers than non-secretors (May, Blackwell, Weir, 1986). An increased incidence of non-secretors has

been reported in asymptomatic oral Candida carriers (Burford-Mason, Weber, Willoughby, 1988) and in patients with candidal infection (May et al., 1986).

It has been shown that the ability to secrete blood group substances is inherited in a Mendelian fashion controlled by a pair of allelomorphic genes, Se and se with Se dominant over se (Ferguson, 1975). As a consequence, individuals who are either homozygous (SeSe) or heterozygous (Sese) are secretors, while homozygous (sese) individuals are non-secretors.

1.2.10 Phagocytosis

Polymorphonuclear leucocytes and monocytes can phagocytose and kill Candida even in the absence of the specific opsonising antibodies and the mechanism of killing is mediated principally by myeloperoxidase and hydrogen peroxide (Lehrer, 1978). Therefore, defective polymorphs or monocytes predispose to candidosis. This is apparent in patients with primary immunodeficiency who usually have a qualitative rather than a quantitative defect in polymorph or macrophage function. Secondary immunodeficiency, such as that resulting from some advanced malignancies or their treatment may also affect phagocytosis as will be discussed later. However, current evidence points to phagocytic cells as major components of defence against deep-seated rather than superficial candidosis (Hurtrel, Lagrange, Michel, 1980).

1.2.11 Specific Immunity

Most of the specific antibodies to Candida in the serum are of the IgG class (Roitt & Lehner, 1983). Antibody and complement

cannot kill candidal organisms directly but can enhance phagocytosis by polymorphs and macrophages (Lehrer, 1978).

Impairment of T-cell mediated delayed hypersensitivity in response to Candida is probably the basic defect in patients with mucocutaneous candidosis (Kirkpatrick, 1984). In AIDS patients, the T-cell mediated immunity is deficient due to selective reduction in the number of T-helper cells which are infected with the Human Immunodeficiency Virus (HIV). In these patients oral candidosis is a common manifestation and may affect up to 50 per cent of such patients (Klein et al., 1984). This may be the only specific oral finding in HIV infected patients (Chandrasekar & Molinari, 1985). The frequency of candidal isolation and oral candidosis has been shown to correlate with a reversed T-helper / T-suppresssor cell ratio (Korting et al., 1988).

1.2.12 Interaction with Other Oral Commensal Organisms

In vitro, lactobacilli have been claimed both to enhance (Isenberg, Berkman, Carito, 1960) and to inhibit (Young, Krasner, Yudofsky, 1956) candidal growth. In vivo there was a reported correlation between salivary lactobacilli levels and candidal count (Parvinen & Larmas, 1981) but this relationship was not found in preschool young children (Glass, 1951).

C. albicans can colonise the mucous membranes of gnotobiotic animals in higher numbers than conventional animals (Phillips & Balish, 1966). Moreover, bacterial constituents of the human oral flora can suppress colonisation of C. albicans in gnotobiotic mice (Liljemark & Gibbons, 1973).

In theory, some organisms may compete with Candida species for available nutrients (Knight & Fletcher, 1971) or binding sites on the host surface (Liljemark & Gibbons, 1973). The latter mechanism may explain in part why Streptococcus salivarius and mitior, which have been shown to adhere well to mucosal surfaces, were able to suppress oral candidal growth, whereas Strep. mutans which is known to adhere poorly to oral epithelium had little inhibitory effect (Gibbons & van Houte, 1975; Samaranayake & MacFarlane, 1982a). Other studies have suggested that some bacterial constituents of the oral microbial flora may secrete substances which are toxic or inhibitory to Candida species (Kennedy, 1981).

1.3 RISK FACTORS IN ORAL CANDIDAL INFECTION

1.3.1 Iron Deficiency

Cawson (1963) was the first to draw attention to the relationship between iron deficiency and oral candidosis when he reported two cases of chronic atrophic candidosis associated with iron deficiency anaemia which resolved after iron replacement therapy alone. Thereafter many investigators reported an association between iron deficiency (although not necessarily anaemia) and chronic mucocutaneous candidosis (Higgs & Wells, 1972), atrophic glossitis and angular cheilitis (Fletcher et al., 1975) and chronic atrophic candidosis (Rose, 1968). Challacombe (1986) reported sideropenia as being detected in 30 per cent of patients with oral candidosis. However, this has not been the opinion of other investigators (Jenkins et al., 1977; Samaranayake & MacFarlane, 1981a). Experimental animal model studies have indicated that iron deficient animals were less

able to eliminate Candida from their oral tissues compared to haematologically normal animals (Rennie et al., 1983).

Iron plays a modulating role in the activity of both lysozyme and lactoferrin (Kirkpatrick et al., 1971), so iron deficiency may compromise these local host defence factors. Iron deficiency may produce an impairment of iron dependent enzyme systems which in turn may affect the metabolism and hence the kinetics of rapidly dividing oral epithelium (Rennie, MacDonald, Dagg, 1984). The hyperkeratosis and atrophy of the epithelium reported in iron deficient individuals (Rennie et al., 1984) and in experimental animals (Rennie & MacDonald, 1982) may provide a suitable environment for candidal infestation.

Cell-mediated immunity has been reported as being depressed in iron deficiency. Impairment of lymphocyte transformation and migration inhibition factor production in response to stimulation with candidal antigen in iron-deficient individuals has been reported both in vivo and in vitro (Joyson et al., 1972). This immune impairment was restored after iron therapy (Joyson et al., 1972; Fletcher et al., 1975). Decreased peripheral blood lymphocyte count (Fletcher et al., 1975), impaired phagocytosis and antibody production (Samaranayake, 1986) have also been reported in association with iron deficiency but, low peripheral blood lymphocyte count may respond to iron therapy (Fletcher et al., 1975).

1.3.2 Vitamin Deficiency

Jenkins et al. (1977) studied the haematological status of 25 patients with chronic hyperplastic candidosis and found seven patients who were folic acid deficient compared to a single patient in 25

control subjects. Subsequent studies have shown chronic atrophic candidosis to occur in a significant number of patients with folate deficiency (Samaranayake & MacFarlane, 1981a; Challacombe, 1986). However, in one study folic acid replacement alone was not able to resolve the candidal infection (Samaranayake & MacFarlane, 1981a).

Hypovitaminosis A was detected in seven of 12 patients with mucocutaneous candidosis (Montes, Krumdieck, Cornwell, 1973). Animal experiments revealed that mice treated with vitamin A (Cohen & Elin, 1974) or vitamin C (Rogers & Balish, 1980) showed increased resistance to candidal infection. However, these studies have not been substantiated as yet.

1.3.3 Carbohydrate-Rich Diet

Chronic atrophic candidosis and angular cheilitis were reported in four patients consuming a high carbohydrate diet (Shuttleworth & Gibbs, 1960). Repeated oral sucrose rinsing can initiate chronic atrophic candidosis in healthy denture wearers and aggravate preexisting lesions in patients with chronic atrophic candidosis (Olsen & Birkeland, 1976). The *in vitro* study of Knight and Fletcher (1971) demonstrated that the growth of *C. albicans* in saliva was accelerated when supplemented with either glucose or sucrose. Sucrose and glucose may facilitate candidal adhesion to acrylic denture surfaces (Samaranayake, McCourtie, MacFarlane, 1980) and oral epithelium generally (Samaranayake & MacFarlane, 1982b). Recently, it has been shown that high concentrations of glucose can augment the iC3b receptors on *C. albicans* and increase resistance of the organism to phagocytosis (Gilmore et al., 1988).

1.3.4 Denture-Related Factors

Denture wearers have been reported to have a qualitative and quantitative increase in oral candidal isolation compared with dentate individuals (Parvinen, 1984). Candidal species had a propensity to proliferate on the fitting surface of the upper denture rather than the adjacent mucosa (Davenport, 1970). In edentulous subjects, Candida species were recovered in higher rates from the oral cavity of denture wearers than from edentulous non-denture wearers (Vandenbussche & Swinne, 1984). The importance of denture wearing in chronic atrophic candidosis was proven indirectly by Turrell (1966) who demonstrated that non-wearing of the dentures for a two week period was associated with resolution of the infection. The prevalence of denture stomatitis in selected poulations has been shown to vary from 27 per cent (Nyquist, 1952) to 67 per cent (Budtz-Jorgensen, 1981).

Traumatised soft tissues of denture bearing areas resulting from ill-fitting dentures may reduce tissue resistance against infection, and increase the permeability of the palatal epithelium to soluble candidal antigens and toxins (Budtz-Jorgensen, 1978). Moreover, continuous wearing of the denture may keep Candida species in contact with the palatal mucosa for a long enough period to permit microbial metabolic products to cause inflammation (Bartels, 1965). Also, a close fitting denture may restrict the cleansing effect of the tongue and the free flow of saliva to the palatal mucosa, in addition to depriving the tissues of anticandidal salivary constituents (Budtz-Jorgensen, 1974). Thus, continuous wearing of the dentures has been reported to increase the prevalence of chronic atrophic candidosis (Budtz-Jorgensen, 1974).

1.3.5 Antibacterial Antibiotics

Antibiotic therapy has been reported to increase oral candidal carriage in terms of frequency of isolation and density of growth (Johnston et al., 1967; Barlow & Chattaway, 1969; Odds & Evans, 1980).

Early observations (Tomaszewski, 1951) reported cases of oral candidosis following therapeutic use of systemic penicillin, aureomycin and chloramphenicol. Repeated topical application of tetracycline has also been reported as capable of inducing oral candidosis in up to 67 per cent of patients (Lehner & Ward, 1970). Achromycin or terramycin, but not bacitracin, incorporated in periodontal packs have also been associated with the development of oral candidosis (Romanow, 1964). However, the latter antibiotic was only found to increase oral candidal isolation and not clinical infection.

In contrast, McKendrick, Wilson and Main (1967) investigated the oral flora of two groups of subjects with chronic bronchitis. The subjects of one group were treated with systemic tetracycline, while the other group received placebo for a period of six months and there were no significant differences in oral candidal isolation between the two groups. As a consequence, McKendrick (1968) concluded the lack of a relationship between the administration of tetracycline and occurrence of oral candidosis. However, the control group subjects also recieved tetracycline for acute exacerbations of their bronchitis. Nevertheless, it is worth mentioning that apart from the studies of McKendrick et al. (1967) and McKendrick (1968), the effect of antibiotic treatment on oral candidal colonisation has been

compared with healthy control group subjects, who were medically different from the treated subjects. Therefore, such treated subjects may have been debilitated by illness prior to commencing antibiotic therapy.

Theoretically, when oral microbial flora has been exposed to an antibacterial antibiotic, sensitive organisms are suppressed or eliminated, whereas resistant organisms like yeasts may grow in abundance (Seelig, 1966). Therefore, candidal growth may be enhanced due to suppression of host bacteria competing with yeasts for nutrients (Knight & Fletcher, 1971) and attachment sites on the epithelial cells (Samaranayake & MacFarlane, 1982a).

Early studies proposed a direct stimulatory effect of antibiotics on candidal growth but this hypothesis is no longer accepted (Knight & Fletcher, 1971). Another possible mechanism is that some antibiotics may depress the host resistance to Candida. Aminoglycoside antibiotics have been reported to inhibit anticandidal activity of human neutrophil leucocytes in vitro (Ferrari et al., 1980). On the other hand, the capacity of human serum to inhibit the growth of C. albicans was not affected by the systemic administration of therapeutic doses of tetracycline (Roth & Goldstein, 1961). The experimental study of Domer and Hector (1987) demonstrated that therapeutic doses of some antibiotics had no adverse effect on the systemic immune response to Candida. However, patients receiving prolonged broad spectrum antibiotics may already have depressed antimicrobial defenses due to their illness.

For a time it was suggested that alterations in vitamin biosynthesis and vitamin B deficiency in patients receiving antibiotic therapy may be factors in lowering host resistance, and predisposing

to oral candidosis (Harris, 1950). However, the beneficial effect of vitamin replacement therapy in these cases was not consistent and appeared to have varying success (Tomaszewski, 1951; Romanow, 1964). In conclusion, more than one mechanism may operate in individuals receiving antibiotic therapy and predispose them to higher levels of oral candidal growth and infection. In addition, not all antibiotics have the same potential to predispose to secondary oral candidosis (Odds, 1988).

1.3.6 Corticosteroid Therapy

Patients receiving systemic corticosteroid therapy have been reported as having an increased prevalence of oral candidal carriage (Johnston et al., 1967; Odds & Evans, 1980; Thompson et al., 1986), and an increased predisposition to local and systemic candidosis (Folb & Trounce, 1970). Topical steroid application has also been implicated in the development of oral candidosis in man (Lehner & Ward, 1970; Epstein, Komiyama, Duncan, 1986) and in experimental animals (Budtz-Jorgensen, 1975). The use of inhaled steroids has also been reported to increase the incidence of oral candidosis (Milne & Crompton, 1974; Sahay, Chatterjee, Stanbridge, 1979). However, other studies have reported no effect of inhaled steroid therapy on oral candidal carriage in asthmatic children (Godfrey, Hambleton, Konig, 1974).

Not all steroids appear to have the same potential for predisposing to the development of secondary candidosis. Long acting, but not short acting, steroids were associated with an increased incidence of candidal infection (Hurley, Balow, Fauci, 1975). Oral

thrush developed after topical application of betamethasone valerate and triamcinolone acetonide, while hydrocortisone hemisuccinate or betamethasone sodium phosphate did not produce this side effect (Lehner & Ward, 1970; Sahay et al., 1979). It seems that the rate of absorption of the various topical steroids has an effect on the ability to develop secondary candidosis (Lehner & Ward, 1970) and the local effect to be dose-related (Sahay et al., 1979). Thus the incidence of oral candidosis secondary to topical oral use of steroid medications may be expected to be greater than that associated with the use of inhaled aerosols due to longer contact time in the oral cavity (Epstein et al., 1986). However, a simple mouth wash procedure after aerosol inhalation had no prophylactic benefit in reducing the incidence of oral candidosis (Sahay et al., 1979).

The mechanisms responsible for the development of secondary candidosis in association with the use of topical steroid medications are uncertain. This predisposition towards candidal infection appears not to be due to enhancement of candidal growth, as the steroids *per se* had no effect on the growth of *C. albicans in vitro* (Milne & Crompton, 1974). Knight and Fletcher (1971) have shown that patients who received corticosteroid therapy had a higher level of salivary glucose which may promote candidal growth. Steroid therapy can impair cell-mediated immunity (Budtz-Jorgensen, 1975). Lymphocyte transformation in response to antigenic stimulation by *Candida* was depressed in a group of patients treated with high doses of corticosteroids (Folb & Trounce, 1970). Chemotaxis of polymorphs (Ward, 1966) and phagocytosis of *C. albicans* (Pallister & Warnock, 1989) were also adversely affected. Nevertheless, antibody production was not impaired by steroid therapy (Folb & Trounce, 1970; Budtz-

Jorgensen, 1975). A recent in vitro study has shown dexamethasone and triamcinolone acetonide to promote candidal adhesion to epithelial cells (Ghannoum & Abu Elteen, 1987).

Topical antifungal agents have proved effective in controlling secondary oral candidosis in steroid-treated patients, and may be prescribed prophylactically in conjunction with steroids until completion of therapy (Lehner & Ward, 1970; Epstein et al., 1986).

1.3.7 Antineoplastic Chemotherapy and Irradiation

The oral carriage rate and quantity of candidal growth are increased in patients receiving antineoplstic chemotherapy compared to a healthy population (Samaranayake et al., 1984). Fungi such as Saccharomyces cerevisiae, which are generally considered non-pathogenic, may cause disease in such compromised patients (Kostiala, 1986). Trauma and drug-induced epithelial desquamation and ulceration in these patients may lead to haematogenous dissemination of Candida species (Dreizen et al., 1982). In contrast to these reports, Shipman (1979) found no relationship between oral candidal isolation and the administration of antineoplastic chemotherapy. The discrepancy between results of various studies may be partly due to differences in type, dose and duration of chemotherapy administered. In addition, not all the studies used similar microbial isolation techniques.

It is not clear whether this increased candidal infection was due to the underlying disease itself or was a result of the chemotherapy. Corticosteroids and antibiotics are frequently administered in addition to antineoplastic chemotherapy which adds to the confusion. However, a recent study has shown the use of

antibiotics in various combinations with antineoplastic and corticosteroid drugs had no additional impairing effect on phagocytic cell function against C. albicans over that resulting from the antineoplastic chemotherapy alone (Pallister & Warnock, 1989). In haematological malignancies and lymphomas, neutropenia resulting from cytotoxic chemotherapy, bone marrow infiltration by lymphomatous cells and impaired B and T-cell function may be factors predisposing to candidosis (Dreizen et al., 1982). Neutrophils from leukaemic patients exhibit impaired phagocytosis and intracellular killing of C. albicans (Rosner et al., 1970).

In patients receiving radiotherapy, Candida species are recovered in higher numbers from the oral cavity, during and after completion of treatment than prior to commencement of therapy (Martin, Al-Tikriti, Bramley, 1981; Rossie et al., 1987). Patients who were originally free of Candida may acquire these organisms postirradiation and the density of growth seems to be dose-dependent (Rossie et al., 1987). Therefore, some researchers considered that radiotherapy treatment is associated with a higher incidence of oral fungal infection (Schumacher, Ginns, Warren, 1964) whilst others were not of this opinion (Shipman, 1979).

The precise reasons for this increased candidal growth and infection have never been identified. Alterations in major and minor salivary gland function, structural changes in the oral mucosa, with increased keratinisation and atrophy as a result of irradiation have all been reported as being of significance (Robinson, 1976).

1.4 FACTORS PREDISPOSING DIABETIC PATIENTS TO INFECTIONS

1.4.1 Introduction

It is generally believed that there is an increased susceptibility to infections in patients with diabetes mellitus. Although the question of the prevalence of infections in diabetic patients is controversial, some infections are believed to occur with increased frequency in diabetic patients such as urinary tract infection, tuberculosis and superficial staphylococcal skin infections (Wheat, 1980). However, the reasons for this increased frequency of a variety of infections are likely to be complex and are not completely understood.

It is well known that severe infection can disturb carbohydrate, mineral and other aspects of metabolism in man (Rayfield et al., 1982). In diabetic patients particularly, this may render the control of diabetes more difficult and in some circumstances can be life threatening. Acute infections can adversely affect carbohydrate metabolism principally in two main ways; either directly by destruction of pancreatic islet cells by an infectious agent, or indirectly through antagonism of insulin action by certain hormones secreted in excess during infection such as growth hormone, glucagon or cortisol (Baird, 1987). Therefore, minor infections which may pass virtually unnoticed clinically in non-diabetics, may lead to serious sequalae in diabetic patients (Larkin, Frier, Ireland, 1985).

Although a significant correlation between the overall prevalence of infections and high plasma glucose level in diabetic patients has been described (Rayfield et al., 1982; Larkin et al., 1985), this does not alone identify the reason of the claimed lower

resistance to infection associated with poor glycaemic control, although several mechanisms are possible.

Since diabetics usually have normal serum levels of antibodies (Rayfield et al., 1982), much interest has been directed toward the function of polymorphonuclear leucocytes and cell-mediated immunity in diabetic patients. Impairment of various aspects of neutrophil function in diabetic patients have been described (Wilson, 1986) but their significance remain questionable.

1.4.2 Impaired Granulocyte Adherence

Granulocyte adherence to vascular endothelium is an important early event in the normal inflammatory response. Studies have shown this adherence to be defective in diabetic patients (Mowat & Baum, 1971). This functional abnormality has been reported to correlate inversely with the fasting plasma glucose level and to be corrected by insulin treatment (Bagdade, Stewart, Walters, 1978). However, the role of impaired granulocyte adherence in candidal infection has not been determined.

1.4.3 Abnormal Leucocyte Biochemistry

Although glucose transport across the neutrophil cell membrane is independent of insulin (Wilson, 1986), neutrophils from diabetic patients have reduced glucose uptake, and low levels of lactate production (lactate is considered important for neutrophil function). This glucose uptake defect could be corrected by insulin in certain concentrations (Esmann, 1963). Polymorphs from diabetic patients exhibit a significant decrease in stimulated glucose

oxidation and activity of the myeloperoxidase and hydrogen peroxide system (Qvist & Larkins, 1981) which may further affect the intracellular killing ability of these cells.

1.4.4 Impaired Chemotaxis

Impaired chemotaxis of polymorphs has been reported in patients with diabetes mellitus (Mowat & Baum, 1971; Miller & Baker, 1972) both in insulin-dependent and non-insulin dependent patients (Molenaar et al., 1976), as well as in rats rendered diabetic by alloxan therapy (Pereira, Sannomiya, Leme, 1987). Serum from diabetic patients may contain substances which could inhibit neutrophil chemotaxis, and insulin administration appears crucial for correction of this defect (Mowat & Baum, 1971; Pereira et al., 1987).

Impaired chemotaxis in diabetic patients may be an inherited defect in leucocyte function (Molenaar et al., 1976). However, this defective chemotaxis could also be the result of impaired granulocyte adherence reported in other studies (Bagdade et al., 1978).

1.4.5 Impaired Phagocytosis

Impaired polymorph phagocytic activity has been reported in diabetic patients with ketoacidosis (Bybee & Rogers, 1964), in poorly controlled diabetics without ketoacidosis (Bagdade, Root, Bulger, 1974; Tan et al., 1975) and in mice with experimentally-induced diabetes (Saiki et al., 1980). In some of these studies the defect was restored after the correction of the metabolic state of the patients (Bagdade et al., 1974; Tan et al., 1975).

Experimental studies have shown that mouse macrophages recognise and bind microorganisms by means of "lectin-like" surface

receptors that interact with microbial cell wall sugars. In diabetes mellitus, the raised level of glucose in the tissue fluids may block the sugar specific "lectin-like" phagocyte receptors, leading to reduced binding of organisms to phagocytes with consequent reduced elimination of the potential pathogenic organisms (Weir, Blackwell, McLean, 1981).

1.4.6 Impaired Intracellular Killing

In well controlled diabetic patients, the intracellular killing capacity of neutrophils to Staphylococcus aureus was comparable to that from control subjects (Repine, Clawson, Goetz, 1980). Neutrophils from poorly controlled diabetics showed reduced intracellular killing capacity towards that organism (Tan et al., 1975; Nolan, Beaty, Bagdade, 1978; Repine et al., 1980). This functional impairment has also been observed towards Escherichia coli (Rayfield et al., 1982).

In acute bacterial infections, neutrophils from diabetic patients, particularly those with poor diabetic control, fail to stimulate their bactericidal activity, although this does happen in neutrophils from non-diabetics (Repine et al., 1980). Although this functional neutrophil impairment was claimed to be corrected after institution of better glycaemic control and insulin therapy (Nolan et al., 1978; Repine et al., 1980), this finding does not have universal acceptance (Tan et al., 1975).

Recent studies have shown the intracellular killing capacity of neutrophils from diabetic patients to C. albicans to be impaired under conditions of hyperglycaemia and ketoacidosis (Wilson & Reeves,

1986; Wilson, Tomlinson, Reeves, 1987). The proposed explanation was that, in diabetics, conversion of glucose to sorbitol reduced the available concentration of NADPH leading to inhibition of the oxidative killing mechanisms of neutrophils; this was normal in well controlled diabetic patients (Davies & Denning, 1972).

1.4.7 Impaired Cell-Mediated Immunity

A reduced cell-mediated immune response has been reported in diabetic patients to staphylococcal antigens (Casey, Heeter, Klyshevich, 1977) and to tubercle bacillus in mice with experimentally-induced diabetes (Saiki et al., 1980). This immune defect probably resides in lymphocytes rather than in serum (Casey et al., 1977). Peripheral blood lymphocyte transformation in response to phytohaemagglutinin was depressed in diabetic patients and directly proportional to the severity of diabetes (Delespesse et al., 1974). The effect of insulin therapy on the lymphocytic response remains controversial (Mahmoud et al., 1975).

In contrast, peripheral blood lymphocyte transformation in well controlled diabetic patients in response to phytohaemagglutinin (Casey et al., 1977) and to C. albicans antigens (Ragab, Hazlett, Cowan, 1972) was comparable to that of non-diabetic subjects and no relationship between serum glucose level and degree of immunological response was noted (Ragab et al., 1972).

1.4.8 Salivary Antimicrobial Factors

Reduced lysozyme activity has been observed in the saliva of diabetic patients (Velikov et al., 1985). In hamsters rendered diabetic with streptozotocin, salivary lysozyme and peroxidase

activity was reduced but, insulin treatment was able to restore these functions to normal (Muratsu & Morioka, 1985).

Some researchers have claimed that there was a significant increase in the proportion of non-secretors of the blood group antigens among insulin-dependent diabetic patients compared to non-insulin dependent diabetics (Blackwell et al., 1987). Inability to secrete these antigens was postulated as being responsible for an increased incidence of candidal infection (Blackwell, 1989).

1.4.9 High Tissue and Salivary Glucose levels

Diabetic patients have a higher level of cutaneous glucose and lower level of lactic acid than non-diabetics (Kandhari et al., 1969). These may be factors predisposing diabetic patients to more candidal skin infection than non-diabetic individuals.

Knight and Fletcher (1971) reported a significant correlation between salivary glucose and candidal growth in vitro in patients with diabetes mellitus. They concluded that the increased incidence of oral candidosis in diabetic patients could be attributed to higher levels of glucose in their saliva.

1.4.10 Miscellaneous Factors

The fourth complement component (C4), which is important in virus neutralisation, has been reported as being quantitatively and qualitatively deficient in insulin-dependent diabetics (Senaldi et al., 1988). Vascular insufficiency of small and medium-sized blood vessels in diabetic patients may compromise the host defences by reducing tissue blood flow and consequently decrease oxygen

concentration of these tissues which in turn may promote the growth of microaerophilic and anaerobic organisms (Rayfield et al., 1982). The increase of the oral capillary basement membrane thickness in diabetic patients (Keene, 1975) may further impede leucocyte migration and prevent adequate diffusion of insulin and glucose to extravascular leucocytes at the site of microbial invasion.

Sensory neuropathy contributes to the development of neuropathic ulcers which may subsequently predispose to infection. Autonomic neuropathy affecting the bladder may result in urine retention and stasis which may predispose to bacteruria (Rayfield et al., 1982). Therefore, both sensory and autonomic neuropathy may play an indirect role in promoting candidal infection in diabetic subjects.

1.5 ORAL CANDIDAL COLONISATION IN DIABETIC PATIENTS

Whether the prevalence of oral candidal carriage in patients with diabetes mellitus is higher than that in non-diabetic individuals has not been clearly defined. Substantial evidence points towards a greater prevalence among diabetics (Weinstein et al., 1960; Bhatt, Doshi, Bilimoria, 1983; Bartholomew, Rodu, Bell, 1987; Lamey et al., 1988) where a rate of oral candidal carriage between 30 per cent (Johnston et al., 1967) and 80 per cent (Weinstein et al., 1960) has been reported. Among Candida carriers, diabetic patients were shown to have a higher density of oral candidal colonisation than non-diabetics (Tapper-Jones et al., 1981; Agarunowa et al., 1986). On the other hand, some early studies did not find a relationship between diabetes mellitus and oral candidal colonisation (Mehnert & Mehnert, 1958; Peters, Bahn, Barens, 1966). Variations in candidal isolation and culturing techniques used by different researchers (Table 1.1), in

addition to variations in clinical status of the subjects studied probably led to conflicting results which make comparison of data difficult and confusing.

Study	Yeast isolation (%)	Sampling technique
Mehnert & Mehnert (1958)	50	Whole saliva
Weinstein et al. (1960)	80	Whole saliva
Peters et al. (1966)	46	Oral swabs
Johnston et al. (1967)	38	Sabouraud mouth rinse
Barlow & Chattaway (1969)	62.5	Oral swabs
Farman and Nutt (1976)	46.2	Oral swabs
Odds et al. (1978)	41	Mouth wash
Tapper-Jones et al. (1981)	60	Imprint culture
Bhatt et al. (1983)	58	Oral swabs
Bartholomew et al. (1987)	75	Cytology
Banoczy et al. (1987)	34.5	Special diagnostic kit
Fisher et al. (1987)	51	Concentrated rinse
Lamey et al. (1988)	57	Concentrated rinse
Pohjamo et al. (1988)	56.8	Special diagnostic kit
Hill et al. (1989)	50	Oral swabs

Table 1.1 Candidal isolation rates from the oral cavity of diabetic patients and isolation techniques used in different studies.

The contention that poor glycaemic control in diabetic patients is associated with increased oral candidal colonisation is controversial. Odds, Evan and Taylor (1978) and Hill et al. (1989) related quantitatively the extent of oral candidal colonisation to the quality of glycaemic control, while other studies showed oral candidal carriage to decrease after improvement of glycaemic control in diabetic patients (Odds & Evans, 1980). However, recent investigations have emphasised the lack of a relationship between oral candidal carriage and quality of diabetic control (Bartholomew et al., 1987; Lamey et al., 1988; Pohjamo et al., 1988). Furthermore, factors such as type of diabetes mellitus or method of glycaemic control (Tapper-Jones et al., 1981; Lamey et al., 1988) as well as the presence of complications, such as diabetic retinopathy (Bartholomew et al., 1987), seem to bear no relationship to oral candidal carriage.

1.6 CANDIDAL INFECTION OF THE ORO-FACIAL REGION IN DIABETIC PATIENTS

The conclusive evidence that diabetes per se increases susceptibility to candidal infection derived from experiments on animals rendered diabetic by alloxan treatment (Hurley, 1966). These animals were more susceptible to cutaneous or systemic candidosis. Unfortunately, because of the time at which these studies were undertaken no detailed analysis of the immunological status was performed.

1.6.1 Median Rhomboid Glossitis

Median rhomboid glossitis, or central papillary atrophy of the tongue, was previously considered to be a developmental anomaly,

caused by a retention of the tuberculum impar (small median elevation of the tongue which arises between the distal and lateral lingual region) during embryonic development (Martin & Howe, 1983). Cooke (1975) had previously commented on the possibility of median rhomboid glossitis being a form of oral candidosis.

Additional evidence for the role of candidal species in median rhomboid glossitis was provided by the demonstration of a correlation between the incidence of central papillary atrophy in diabetics and the finding of C. albicans mycelia in smears from the lesions (Farman, 1976; Farman & Nutt, 1976; Farman, 1977). In relation to this, rats rendered diabetic by streptozotocin developed median rhomboid glossitis-like lesions after intraoral inoculation of C. albicans while similarly inoculated control animals did not develop oral candidosis (Dourov & Coremans-Pelseneer, 1987).

The prevalence of central papillary atrophy in diabetic patients has been reported to be between 14.2 and 17.7 per cent, whilst the prevalence was between 0.22 and 2.56 per cent in the general population (Farman & Nutt, 1976). As species morphologically identical to Candida mycelia were found in only 24 per cent of cases of central papillary atrophy (Farman, 1976), it appeared that the condition may not have been caused in all cases by Candida species, and a cause and effect relationship was considered unproven. In addition, the prevalence of the lesion could not be related either to diabetic treatment or to the degree of glycaemic control (Farman, 1976).

Thickening of the basement membrane and proliferation of the endothelial lining of the oral vasculature in diabetic patients have been reported as being part of generalised phenomenon (Keene, 1975).

Histological examination of biopsy specimens from diabetic patients with central papillary atrophy revealed thickening of the capillary walls within the lamina propria (Farman & Nutt, 1976). This may impair the blood supply, and then the decreased local tissue resistance could predispose to candidosis.

Due to the rarity of central papillary atrophy in the general population and its high frequency of occurrence in diabetic patients, Farman (1976) suggested that patients with this lesion should be screened for possible diabetes mellitus. However, the author admitted that central papillary atrophy was a clinical rather than a pathological entity, and that other conditions such as geographic tongue could not be excluded from the differential diagnosis.

1.6.2 Chronic Atrophic Candidosis

The aetiology of chronic atrophic candidosis is multifactorial but, infection by Candida species has been shown to be of significance (Budtz-Jorgensen, 1974). It has been proposed that diabetes mellitus promotes the growth of Candida species in the oral cavity (Knight & Fletcher, 1971) and this was a systemic predisposing factor encouraging the alteration of C. albicans from commensalism to parasitism and subsequent initiation of chronic atrophic candidosis in denture wearers (Budtz-Jorgensen, 1981). Denture wearing has been associated with a substantial increase in the frequency of oral candidal isolation in patients with diabetes mellitus (Tapper-Jones et al., 1981; Fisher et al., 1987).

A literature search revealed only occasional reports linking chronic atrophic candidosis and diabetes mellitus. Cawson (1965)

reported a case of chronic atrophic candidosis in a patient with poorly controlled diabetes mellitus. The prevalence of this condition has been shown generally to be higher in diabetics over that in denture status matched non-diabetic individuals (Lamey et al., 1988). In contrast, other studies could not demonstrate this association (Peters et al., 1966; Phelan & Levin, 1986).

1.6.3 Angular Cheilitis

C. albicans is the organism most commonly isolated from the lesions of angular cheilitis (MacFarlane & Helnarska, 1976). The observation that angular cheilitis lesions underwent resolution after local antifungal treatment of the intraoral infection alone (Cawson, 1963) has led to the suggestion that oral candidal colonisation is an aetiologic factor in the development of angular cheilitis. In the light of the substantial increase in the oral candidal colonisation in diabetic patients (Bartholomew et al., 1987), a higher incidence of angular cheilitis in diabetic patients could be expected.

Burman, Bartels and Bailey (1956) reported a case of persistent angular cheilitis in an undiagnosed diabetic patient. The lesion was dramatically improved after correction of the hyperglycaemic state. Exacerbation and remission of the lesion was noticed to be associated with the rise and fall in the patient's blood glucose in spite of antifungal therapy.

1.6.4 Chronic Mucocutaneous Candidosis

Chronic mucocutaneous candidosis is a group of conditions characterised by chronic candidal infection, usually affecting skin and nails as well as mucous membranes of the mouth and vagina (Odds,

1988). The wide clinical variation seen in affected cases has allowed differentiation to be made between those patients with and without detectable endocrine disease (Kirkpatrick, 1984).

The association of chronic mucocutaneous candidosis with one or more endocrinopathies constitutes what is known as the candida endocrinopathy syndrome (Odds, 1988). Diabetes mellitus is one of the associated endocrinopathies (Kirkpatrick & Bennett, 1971), although diabetes mellitus arising from non-autoimmune mechanism is believed not to be associated (Porter & Scully, 1986).

Chronic mucocutaneous candidosis is a rare form of candidal infection. However, according to Lehrer (1966) oral candidosis is generally classified into acute (acute pseudomembranous candidosis and acute atrophic candidosis) and chronic (chronic atrophic candidosis "denture stomatitis" and chronic hyperplastic candidosis) forms. Although this classification is far from ideal, it is nevertheless widely accepted clinically. Denture stomatitis is further classified into Type 1 which is a simple localised inflammation of the denture bearing mucosa, Type 2 a generalised simple inflammation, and Type 3 a granular type commonly involving the central part of hard palate (Newton, 1962).

CHAPTER TWO

ADHESION OF *CANDIDA* SPECIES TO EPITHELIAL AND ACRYLIC SURFACES: REVIEW OF LITERATURE

2.1 INTRODUCTION

It is generally accepted that microbial adhesion to host surfaces is an essential first step in the process of colonisation and subsequent infection (Gibbons & van Houte, 1975). During the past decade extensive research have been undertaken to elucidate the mechanisms of bacterial adhesion to host cells and surfaces but, the knowledge gained has been rarely translated into approaches for modification or prevention of infection.

Candida species, particularly *C. albicans* have been shown to adhere *in vitro* to a variety of body cells and surfaces including buccal epithelium (Kimura & Pearsall, 1978), vaginal epithelium (Sobel et al., 1981), urinary epithelium (Centeno et al., 1983), and to a fibrin platelet matrix (Samaranayake, McLaughlin, MacFarlane, 1988) as well as to dental resin materials (Samaranayake et al., 1980). Candidal adhesion to the aforementioned cells and surfaces have been studied to determine factors relevant to the pathogenesis of candidal infections. At present, the importance of candidal adhesion in pathogenesis is only partially defined and the contention that virulence of *Candida* depends on adhesion is based only on association (McCourtie & Douglas, 1984; Ghannoum & Abu Elteen, 1986). Whether or not a particular form of adhesion observed *in vitro* plays a role in colonisation or infection *in vivo* is a matter of debate. Initial studies (McCourtie & Douglas, 1984; Barrett-Bee et al., 1985)

suggested a correlation between candidal adhesion and virulence. If so, factors or mediators that affect the adhesion in vitro should be evaluated in vivo.

2.2 YEAST CELL FACTORS AFFECTING CANDIDAL ADHESION TO EPITHELIAL SURFACES

2.2.1 Candida Species and Strain Variations

C. albicans has been reported to adhere in vitro to epithelial cells in greater numbers than do other Candida species (King, Lee, Morris, 1980; Sobel et al., 1981; Ray, Digre, Payne, 1984). Some species like C. stellatoidea and C. tropicalis showed inconsistent and lesser degrees of adherence capability (King et al., 1980; Ray et al., 1984), whereas C. parapsilosis, C. guilliermondii, C. krusei and C. pseudotropicalis have been reported to show little or no adhesion capability (King et al., 1980; Ray et al., 1984). After growing different candidal species in sugar-rich medium, only C. albicans exhibited marked increase in adhesion to epithelial cells compared with the same organisms grown in a medium of relatively low sugar content, while C. guilliermondii and C. pseudotropicalis did not show any enhanced adhesion (Critchley & Douglas, 1985).

Differences in adhesion capability between strains of the same species were less obvious. Strains of C. albicans isolated from patients with candidosis showed increased adhesion in vitro over those from asymptomatic carriers (Kearns, Davies, Smith, 1983; Segal, Soroka, Schechter, 1984). However, this was not the case in all the strains studied (Kearns et al., 1983). McCourtie and Douglas (1984)

found C. albicans strains isolated from patients with candidal infections were able to show enhanced adhesion after being grown in a sugar-rich media, while strains isolated from asymptomatic carriers did not show enhanced adhesion. Recently, it was reported that some pathogenic strains of C. albicans were capable of switching reversibly between two general phenotypes (white or opaque). White cells were found to be significantly more adhesive to epithelial cells in vitro when compared with the opaque cells (Kennedy et al., 1988).

2.2.2 *Candida* Cell Viability

Although yeast cell viability is not essential for adhesion (Lee & King, 1983), viable candidal cells adhere in vitro in higher numbers to buccal epithelial cells (Kimura & Pearsall, 1978). Non-viable C. albicans with germ tubes showed no better adhesion than non-viable blastospores (Sobel et al., 1981). The non-viable candidal cells may be able to form a loose association with an epithelial cell but, viability is perhaps necessary for a stable adhesion (Vudhichammong et al., 1982).

2.2.3 *Candida* Cell Concentration

Candidal adhesion to epithelial cells in vitro is dependent upon and correlates with yeast cell concentration in the incubation medium (Kimura & Pearsall, 1978; King et al., 1980; Samaranayake & MacFarlane, 1981b).

One explanation for the reported variations in the rate of candidal adhesion to epithelial cells in different studies is the inconsistency in concentration of the yeast to epithelial cells ratio used in different adhesion assays. This ratio varied considerably

from 25:1 (Sobel et al., 1981) up to 1000:1 (King et al., 1980). However, using a very high concentration of candidal cells may lead to yeast co-adherence and clumping resulting in misleading results (Cox, 1983).

2.2.4 *Candida* Growth Phase and Temperature of Growth

C. albicans blastospores grown in stationary phase were found to adhere more than those grown in logarithmic phase (King et al., 1980). Yeast cells harvested from cultures grown at 25°C adhered significantly greater than those harvested at 37°C (Lee & King, 1983; Kennedy & Sandin, 1988). At lower temperature the yeast cells produce short germ tube-like structures which might be responsible for the increased adhesion at that temperature (Kennedy & Sandin, 1988).

2.2.5 *Candida* Cell Morphology

Germ tube formation is repeatedly reported as being associated with significantly increased adhesiveness of *C. albicans* to epithelial cells (Kimura & Pearsall, 1978; Sobel et al., 1981; Samaranayake & MacFarlane, 1982a; Kennedy & Sandin, 1988). *C. tropicalis* and *C. stellatoidea* induced to form germ tubes in tissue culture medium showed enhanced adhesion to epithelial cells when compared with the parent blastospore, while partial inhibition of germ tube formation by the enzyme cystein resulted in inhibition of yeast adhesion (Kimura & Pearsall, 1980).

Factors contributing to enhanced adhesion of *Candida* forming germ tube are not clearly understood. Ultrastructure studies have shown the germ tube region to adhere more frequently than do other portions of the fungi (Kimura & Pearsall, 1980). In addition, major

differences in the cell wall structure at the site of germ tube formation have been reported (Kennedy & Sandin, 1988). Thus current evidence has indicated that the chemical entity on the yeast cell responsible for adhesion may be concentrated on the germ tube surface (Sandin et al., 1982). Moreover, there is evidence of specific antigens synthesised during germ tube formation which are not found on the yeast form (Casanova et al., 1989). Others have suggested that there are increased numbers of adhesion sites present on filamentous yeast surfaces, or alternatively increased surface area of yeast exposed to epithelial cells may be factors responsible for enhanced adhesion (Sobel et al., 1981).

2.3 EPITHELIAL CELL FACTORS AFFECTING CANDIDAL ADHESION TO EPITHELIAL SURFACES

C. albicans adhere readily to a variety of epithelial cells but, several in vitro studies have shown differential susceptibility of candidal adhesion to epithelial cells obtained from different sources (Sandin et al., 1987a).

2.3.1 Variations in Cell Type

Studies investigating adhesion of C. albicans to different types of epithelial cells have shown inconsistent results. Candida species have been shown to adhere more to buccal epithelial cells than to vaginal epithelial cells (Sobel et al., 1981; Sandin et al., 1987a), urinary urothelial cells (Centeno et al., 1983; Sandin et al., 1987a) and corneocytes (Collins-Lech et al., 1984). Whether these variations indicate differences in receptivity of epithelial cells obtained from different tissues has yet to be determined.

2.3.2 Epithelial Cell Viability

Epithelial cell viability appears not to be necessary for adhesion of C. albicans (Sandin et al., 1987b) which have been shown to adhere equally well to viable and non-viable buccal epithelial cells (Vudhichamnong et al., 1982). However, non-viable HeLa cells permitted adhesion of C. albicans in greater numbers than did viable cells (Samaranayake & MacFarlane, 1981b).

2.3.3 Circadian and Donor to Donor Variations

Epithelial cells obtained from different donors have shown considerable variations in adhesion values (King et al., 1980; Sobel et al., 1981; Collins-Lech et al., 1984; Sandin et al., 1987a). In addition, cells from the same donor show different adhesion values from day to day (King et al., 1980; Kearns et al., 1983; Sandin et al., 1987a). In general, these differences may reflect variations in the number or nature of the available surface receptors on the epithelial cells between donors and from day to day. Therefore, a constant collection time for epithelial cells is essential if the results of adhesion is to be compared between individuals.

2.4 ENVIRONMENTAL FACTORS AFFECTING CANDIDAL ADHESION TO EPITHELIAL SURFACES

2.4.1 Temperature and Time of Contact

Candidal adhesion in vitro is time and temperature-dependent (Kimura & Pearsall, 1978). However, the effect of these factors may vary according to the media used for the adhesion assay (Kennedy & Sandin, 1988). There is evidence that candidal cells adhere optimally

at a temperature of 37°C (Kimura & Pearsall, 1978; King et al., 1980). However, others have reported that yeast incubated at 28°C adhered better than those at 37°C (Segal, Lehrer, Ofek, 1982).

Although candidal adhesion to epithelial cells can be detected after only five minutes of incubation, it seems to be optimal after 60 minutes (King et al., 1980).

2.4.2 pH of the Incubation Medium

The degree of adhesion of C. albicans to epithelial cells has been shown to vary with pH. Generally, it is enhanced under acidic than alkaline conditions (Samaranayake & MacFarlane, 1982a), probably due to enhanced germ tube formation in the acidic condition over that in an alkaline one (Sobel et al., 1981). In contrast, Cox (1983) found no significant change in adhesion when the pH was increased from 4 to 11. However, the reported optimal candidal adhesion to epithelial cells at low pH in vitro may be relevant to the in vivo data showing oral candidal colonisation to be higher in acidic saliva (Young et al., 1951).

Mammalian cells are endowed with a net negative surface potential resulting largely from ionisation of sialic acid residues of the glycocalyx (Gasic, Berwick, Sorentino, 1968). The cell surface of candidal species is likewise negatively charged. It is possible that the enhanced candidal adhesion observed under low pH conditions may be, in part, due to protonation of exposed cell surface functional groups which results in a reduction in surface electronegativity on the yeast and epithelial cells (Samaranayake & MacFarlane, 1982a).

2.4.3 Saliva

Kimura and Pearsall (1978) used whole human saliva as an adhesion medium and reported increased candidal adhesion to buccal epithelial cells over that in phosphate buffered saline. Thereafter, Samaranayake and Macfarlane (1982a) reported increased candidal adhesion to epithelial cells monolayer pretreated with whole saliva.

2.4.4 Serum

Preincubation of epithelial cells with human serum resulted in a slight, but significant, reduction in the adhesion of C. albicans (Samaranayake & MacFarlane, 1982a). In addition, when fresh human serum was added to the assay medium, candidal adhesion to corneocytes was inhibited by 25 to 50 per cent (Ray et al., 1984). Nevertheless, heat inactivated serum in the assay medium showed no inhibition and possibly slight enhancement of adhesion, which may have been due to nutritional factors provided by serum (Ray et al., 1984).

The cell wall of candidal species can activate serum complement through the alternative pathway. Ray et al. (1984) proposed that the deposition of complement from fresh serum onto the yeast cell surface may cause alterations of the physical properties of the cell wall surface which alters their adhesion to epithelial cells.

2.4.5 Dietary Carbohydrates

Several reports have described increased adhesion of Candida species grown in media containing high concentrations of certain sugars (Samaranayake & MacFarlane, 1982b; Critchley & Douglas, 1985). Addition of glucose to the incubation mixture of C. albicans and vaginal epithelial cells increased candidal adhesion (Sobel et al.,

1981). Sucrose at very low concentrations enhanced significantly the adhesion of C. albicans to epithelial cells in a concentration-dependent manner (Samaranayake & MacFarlane, 1982b).

After incubation of C. albicans in a defined concentration of different sugars, maltose was the most effective sugar, while glucose was the least effective in enhancing adhesion (Samaranayake & MacFarlane, 1982b). However, this enhancement seemed to be strain-dependent (McCourtie & Douglas, 1984) and may differ in different Candida species, or strains of the same species, incubated in the same sugar concentration (Critchley & Douglas, 1985).

Samaranayake and MacFarlane (1982b) proposed that the ability of the yeasts to produce extracellular metabolic product after incubation in sucrose-rich medium was responsible for enhanced adhesion of the organisms. That is presumably why lactose, which is not metabolised by C. albicans, exhibited no influence on candidal adhesion to epithelial cells (Samaranayake & MacFarlane, 1981b). However, the enhanced candidal adhesion after incubation in sugar-rich medium appears to be associated with the ability of the organism to produce an additional fibrillar surface layer (McCourtie & Douglas, 1981).

2.4.6 Salivary Immunoglobulins

Several studies have shown that salivary IgA (Kimura & Pearsall, 1978; Epstein et al., 1982) and secretory IgA isolated from human breast milk (Vudhichamnong et al., 1982) can inhibit adhesion of C. albicans to human oral epithelium in vitro. Salivary antibodies specific for C. albicans may be of several classes (IgA, IgG and IgM)

but, only salivary IgA anticandidal antibodies have the ability to inhibit candidal adhesion to buccal cell significantly and in a concentration-dependent manner (Epstein et al., 1982).

The reported inhibitory effect of secretory IgA is independent of its agglutination effect, as reduced candidal adhesion was shown with a subagglutination concentration of secretory IgA (Vudhichamnong et al., 1982). The effectiveness of salivary IgA as an adhesion inhibitor when used within the physiological range of concentration requires to be studied.

2.4.7 Oral Commensal Microflora

The experimental observation that C. albicans colonised epithelial cells from conventional rats in lower numbers than from germ-free rats (Liljemark & Gibbons, 1973), drew attention to the fact that indigenous oral bacteria may interfere with candidal adhesion and colonisation of oral tissues.

Liljemark and Gibbons (1973) reported that secondary inoculation of Strep. salivarius and Strep. mitior, but not Strep. mutans, inhibited intraoral candidal colonisation in germ-free rats. Similarly in vitro, candidal adhesion was significantly reduced to HeLa cells previously exposed to Strep. salivarius and Strep. mitior (Samaranayake & MacFarlane, 1982a) while strains of Strep. sanguis and Strep. milleri have shown little effect on yeast attachment (Makrides & MacFarlane, 1982). Adhesion of vaginal isolates of lactobacilli to vaginal epithelial cells reduced the subsequent adhesion of C. albicans to these cells (Sobel et al., 1981). Cox (1983) noticed reduced candidal adhesion to buccal epithelial cells which were already colonised with bacteria, and that the number of adherent

candidal cells correlated inversely with the number of adherent bacteria. In contrast, preincubation of epithelial cells with piliated strains of Escherichia coli and Klebsiella pneumoniae resulted in a significantly increased subsequent C. albicans adhesion (Centeno et al., 1983; Makrides & MacFarlane, 1983).

The mechanisms by which some bacteria inhibit, and others promote, candidal adhesion to epithelial cells is not clear. Candidal adhesion is probably influenced by competition for mucosal receptors on epithelial cells with host bacteria (Samaranayake & MacFarlane, 1982a). The fact that Strep. mutans, an organism with a relatively low affinity for epithelial cell receptors, did not significantly affect candidal adhesion (Liljemark & Gibbons, 1973; Samaranayake & MacFarlane, 1982a) supports this contention. On the other hand, pili of E. coli and K. pneumoniae could act as a bridging mechanism between epithelial cells and C. albicans, thus increasing candidal adhesion (Centeno et al., 1983; Makrides & MacFarlane, 1983).

2.5 ADHESION OF CANDIDA SPECIES TO ACRYLIC SURFACES

2.5.1 Introduction

Infection of the palatal mucosa with candidal species is generally accepted as a prominent aetiological factor in chronic atrophic candidosis (Budtz-Jorgensen, 1974) the commonest form of oral candidosis (Odds, 1988). The fact that Candida species are recovered more frequently and in higher counts from the fitting surface of the upper denture than from the palatal mucosa (Davenport, 1970) lends support to the theory that acrylic dentures may act as a reservoir of infection.

There are few studies which have investigated the adhesion of yeasts to acrylic surfaces. The study of Samaranayake and MacFarlane (1980) was the first to give informations in that field. Although many factors affect candidal adhesion to acrylic surfaces *in vitro* (Samaranayake et al., 1980), the significance of these factors *in vivo* is uncertain.

2.5.2 Effect of Dietary Carbohydrates

Candida species grown in media containing dietary sugars showed significantly increased adhesion to acrylic surfaces, in a concentration-dependent manner, than those grown in media devoid of these sugars (Samaranayake & MacFarlane, 1980; McCourtie & Douglas, 1981). However, not all sugars have had the same influence on adhesion. Galactose and sucrose were able to induce maximal enhancement (McCourtie & Douglas, 1981) while lactose and xylitol had a negligible effect on candidal adhesion (Samaranayake et al., 1980). *Candida* grown in media containing a high concentration of certain sugars undergoes changes in cell surface composition which may facilitate adhesion to acrylic surfaces (McCourtie & Douglas, 1981). Ultrastructural studies have revealed the presence of an additional fibrillar floccular layer, probably an extracellular metabolic product, when yeasts are grown in a high sugar concentration media (Samaranayake & MacFarlane, 1980; McCourtie & Douglas, 1981).

In the light of these findings one may expect denture wearers who frequently have a carbohydrate-rich diet to have a high candidal colonisation to their dentures. This is relevant to the observation of Olsen and Birkeland (1976) that a sucrose rinse initiated or aggravated denture stomatitis in a group of denture wearers.

2.5.3 Effect of Saliva

Incubation of cold-cured acrylic resin in whole saliva for 30 minutes inhibited adhesion of C. albicans (Samaranayake et al., 1980; McCourtie & Douglas, 1981; McCourtie, MacFarlane, Samaranayake, 1986a). At the same time, saliva coating of heat-cured acrylic resin was found to increase adhesion of C. albicans and to decrease adhesion of C. tropicalis (Minagi et al., 1986). Discrepancies in methodology, Candida species and strains and adhesion surfaces employed may explain these apparent contradictions.

The in vitro inhibitory effect of salivary pellicle on candidal adhesion may be paralleled by the clinical observations of increased yeast colonisation of the upper acrylic plates fitted to monkeys with reduced salivary flow (Olsen & Haanaes, 1977).

2.5.4 Effect of Serum

C. albicans exhibited enhanced adhesion to acrylic surfaces preincubated in human serum (Samaranayake et al., 1980; McCourtie et al., 1986a). The conclusion of Nyquist (1952) that trauma is an aetiological factor in chronic atrophic candidosis is relevant to these findings, where the inflammatory exudate coating the surface of the denture could enhance candidal adhesion and colonisation of the denture.

2.5.5 Effect of Oral Commensal Microflora

The role played by oral bacteria in affecting candidal adhesion to acrylic denture surfaces has been little studied and the results are conflicting. Samaranayake et al. (1980) reported a significant reduction in the number of yeast cells which adhered to

acrylic strips precoated with Strep. salivarius. In that study the bacterial adhesion was scanty and the yeast cells adhered to areas free from streptococci. In contrast, Verran and Motteram (1987) reported a significant positive correlation between the number of adherent Strep. sanguis and Strep. salivarius on acrylic surfaces and the number of adherent C. albicans. Yeast cells tended to adhere to the already adherent streptococci, which appeared to provide a suitable surface for adhesion of C. albicans. Moreover, C. albicans adhered more firmly to acrylic surfaces when the yeast was incubated simultaneously with Strep. mutans in sucrose-containing incubation medium (Branting, Sund, Linder, 1989). The same authors also showed that yeasts incubated alone with acrylic surfaces in the same medium showed only loose adhesion. Interestingly, C. albicans when preincubated with a culture supernatant of Strep. salivarius showed a significant increase in adhesion to acrylic surfaces (Samaranayake et al., 1980).

2.6 EFFECT OF SPECIFIC ANTIFUNGAL AGENTS AND CHLORHEXIDINE GLUCONATE ON CANDIDAL ADHESION TO EPITHELIAL AND ACRYLIC SURFACES

2.6.1 Effect of Specific Antifungal Agents

The evidence available for the effect of antifungal drugs on candidal adhesion to host surfaces are inconclusive. Pretreating denture acrylic with nystatin and amphotericin B in therapeutic concentrations has been shown to inhibit candidal adhesion for 48 and 24 hours respectively and the extent of inhibition seemed to be dose-

dependent (McCourtie, MacFarlane, Samaranayake, 1986b). On the other hand, C. albicans blastospores pretreated with subminimal inhibitory concentrations of amphotericin B (Brenciaglia et al., 1986), ketoconazole (Sobel & Obedeanu, 1983; Shibl, 1985), nystatin and miconazole (Shibl, 1985) showed significantly reduced adhesion to epithelial cells. In addition, pretreatment of C. albicans with subminimal inhibitiory concentrations of amphotericin B resulted in reduced adhesion of this organism to plastic surfaces and fibrin matrices in vitro (Nugent & Couchot, 1986). In contrast, Odds and Webster (1988) found that clotrimazole, itraconazole or ketoconazole had no effect on candidal adhesion to vaginal epithelial cells regardless of whether the drugs were used to pretreat the fungi or the vaginal epithelial cells.

Pretreatment of C. albicans with subminimal inhibitory concentrations of the aforementioned antifungal agents was associated with inhibition of germ tube formation (Shibl, 1985; Brenciaglia et al., 1986; Nugent & Couchot, 1986). Other researchers have reported that growth of C. albicans in the presence of subminimal inhibitory concentrations of amphotericin B reduced synthesis of surface mannan (Al-Bassam et al., 1985). This cell wall component is known to be important in mediating candidal adhesion to epithelial surfaces (Critchley & Douglas, 1987a).

2.6.2 Effect of Chlorhexidine Gluconate

Pretreating acrylic surfaces with chlorhexidine in a two per cent concentration for 15 seconds inhibited adhesion of C. albicans (Samaranayake et al., 1980; McCourtie, MacFarlane, Samaranayake,

et al., 1985). The adhesion was most inhibited when the organisms were grown in conditions known to promote adhesion such as sugar-rich media (McCourtie et al., 1985), or when adhesion was tested in the stationary growth phase (McCourtie et al., 1986b). C. albicans strains isolated from active infections were more sensitive to the inhibitory effect than those isolated from carriers whilst other species such as C. glabrata, C. parapsilosis, and C. krusei were not affected (McCourtie et al., 1985).

Although saliva and serum are known to reduce the fungicidal activity of chlorhexidine, they do not affect the ability of chlorhexidine gluconate to inhibit candidal adhesion to acrylic surfaces (McCourtie et al., 1986a).

The ability of chlorhexidine to reduce candidal adhesion to acrylic surfaces is independent of its fungicidal effect since candidal species exposed to sublethal concentration of chlorhexidine showed reduced ability to adhere to acryic (McCourtie et al., 1986b). However, this treatment has been shown to have other adverse effects on Candida causing changes in the cell wall surface leading to loss of cytoplasmic components and coagulation of the nucleoproteins, in addition to inhibition of the budding process (Bobichon & Bouchet, 1987).

A recent report has shown mouth rinsing with chlorhexidine in concentrations of 0.2 or 0.002 per cent reduced candidal adhesion to buccal epithelial cells both in vitro and in vivo (Tobgi, Samaranayake, MacFarlane, 1987).

2.7 MECHANISMS OF ADHESION

Specific adhesion involves interaction of macromolecules on the surface of the microorganism and macromolecules on the surface of the substratum, while non-specific adhesion involves hydrophobic and electrostatic forces which hold the organism in contact with the substratum (Kennedy, 1988). A clear understanding of the mechanisms of candidal adhesion is necessary for successful prevention of candidal adhesion to surfaces *in vivo*.

Candidal cell walls are composed of three main polysaccharides; mannan, glucan and chitin in addition to proteins and lipids (Sandin et al., 1982). The recent experimental evidence has indicated a role for mannoproteins in mediating yeast adhesion to epithelial cells (Sandin et al., 1982; Critchley & Douglas, 1987a) and suggested that the protein portion of the mannoprotein adhesin is more important than the carbohydrate moiety in mediating adhesion to epithelial surfaces (Critchley & Douglas, 1987a). Chitin, a candidal cell wall component which was thought to be concentrated on the inner layer of the candidal cell wall, was proposed to mediate yeast adhesion to epithelial cells (Segal et al., 1982). However, more than a single surface component may function in the process of adhesion (Kennedy, 1988) as additional studies have suggested that at least two morphologic classes of candidal adhesin exist; floccular and fibrillar adhesins. Tronchin, Poulain and Vernes (1984) noticed that adherent *Candida* cells tended to have a fibrillar cell wall coat of thin filaments arranged perpendicular to the cell surface composed mainly of mannoprotein, while non-adherent cells were devoid of this layer.

In addition to variations in candidal cell wall biochemistry, the identity of the epithelial cell receptors also remains complex. Sugars including L-fucose (Sobel et al., 1981), D-mannose (Sandin et al., 1982), aminosugars, mannosamine, glucosamine and galactosamine (Collins-Lech et al., 1984) in addition to the saccharide-like cyclic alcohol-inositol (Ghannoum et al., 1986) have all been considered as likely candidal adhesion receptors determinants. Current evidence indicates that glycoprotien receptors may be most commonly involved in C. albicans adhesion (Critchley & Douglas, 1987b) and proposed that fibronectin, the cell surface glycoprotein, functions as a receptor (Skerl et al., 1984). According to recent research, the carbohydrate moiety of the epithelial cell surface glycoprotein is more important in mediating candidal adhesion (Critchley & Dougals, 1987b). However, more than one surface component on epithelial cells may serve a receptor function (Kennedy, 1988).

The mechanisms by which yeasts adhere to acrylic surfaces are not clearly identified. Some investigators implicated cell surface components (McCourtie & Douglas, 1981), whereas others have suggested non-specific Candida surface interactions such as cell surface hydrophobicity and elecrostatic forces may mediate or participate in adhesion (Koltz, Drutz, Zajic, 1985; Miyake et al., 1986). It was found that hydrophobic yeasts adhere to hydrophobic surfaces mainly by hydrophobic interactions, whereas in less hydrophobic yeasts electrostatic interactions seemed to play a more important role in adherence to solid surfaces (Nikawa et al., 1989). However, both hydrophobic and electrostatic forces, in addition to adhesion-receptor interactions, may operate in concert to achieve irreversible adhesion either to epithelial or non-biological surfaces (Kennedy, 1988).

2.8 RELATIONSHIP OF ADHESION TO PATHOGENICITY

Microbial adhesion to epithelial cells is recognised to be the initial step in the process of colonisation of the mucosal surfaces (Gibbons & van Houte, 1975). This adhesion enables the microorganism to resist elimination by the mucosal secretions, and to proliferate under existing conditions. Epithelial cells with adherent organisms, as any other epithelial cells, exhibit a continuous desquamation process. Thus the progeny of these organisms must adhere to newly exposed cells if colonisation is to be maintained.

The ability of different Candida species to adhere to the epithelial cells (Kimura & Pearsall, 1980) may explain its ability to colonise the mucosal surfaces. Consequently, variation in adhesion capability between different species may partly explain why some species are recovered more often from the mucosal surfaces than others. This is well illustrated by C. albicans which adheres to buccal and vaginal epithelial cells to a much higher degree than the other Candida species (King et al., 1980), and at the same time is the species most commonly isolated from these sites as a commensal and pathogen (Odds, 1988). King et al. (1980) proposed a relationship between the relative ability of candidal species to adhere and their ability to colonise and subsequently infect mucosal surfaces.

C. albicans and C. stellatoidea both exhibit marked adhesion to epithelial cells and were found as cutaneous pathogens in a rodent model while non-adherent species were non-pathogenic (Ray et al., 1984). The strains of C. albicans that showed greatest adhesion to epithelial cells in vitro were the most virulent to mice in vivo (McCourtie & Douglas, 1984). In contrast, strains with reduced ability to adhere in vitro to fibrin platelet matrix were relatively

avirulent in a rabbit endocarditis model (Calderone et al., 1985). A mutant strain of C. albicans, which adhered less readily in vitro to vaginal epithelial cells than the parent strain was less virulent in an animal model of vaginal candidosis (Lehrer et al., 1986). In this context, C. albicans strains isolated from active infections gave greater adhesion values than those isolated from commensal carriers (McCourtie & Douglas, 1984; Segal et al., 1984).

Under conditions conducive to germ tube formation, C. albicans which produced germ tubes adhered better to vaginal epithelial cells in vitro, and were more virulent in a rat vaginal candidosis model than the strain which failed to produce germ tubes (Sobel et al., 1981). Candidal adhesion to cell surface is critical for mycelial penetration of the epithelial cell surfaces (Howlett & Squier, 1980). Furthermore, candidal adhesion to epithelial cells may provide a stimulus for mycelial transformation (Ray et al., 1984).

Among different Candida species, a correlation between some proposed virulence parameters such as phospholipase activity (Barrett-Bee et al., 1985), proteinase production (Ghannoum & Abu Elteen, 1986) on one side and pathogenicity and adhesion to epithelial cells on the other side has been presented. These results further emphasise the role played by adhesion mechanisms of Candida in the pathogenesis of human candidosis.

CHAPTER THREE

DIABETES MELLITUS, ORAL CANDIDAL CARRIAGE AND CANDIDAL INFECTION

3.1 INTRODUCTION AND AIMS OF THE STUDY

Whether the prevalence of oral candidal carriage in patients with diabetes mellitus is higher than in non-diabetic individuals is still a matter of debate. Several studies have shown the rate of oral candidal carriage as being greater among diabetics (Johnston et al., 1967; Tapper-Jones et al., 1981; Bhatt et al., 1983) and some studies have stated the oral candidal carriage rate as high as 80 per cent in these patients (Weinstein et al., 1960). Quantitatively oral candidal growth has also been claimed to be greater in diabetic patients than in non-diabetic individuals (Tapper-Jones et al., 1981; Agarunowa et al., 1986). However, others have not found a relationship between diabetes mellitus and oral candidal colonisation in terms of rate of carriage or quantity of growth (Mehnert & Mehnert, 1958; Peters et al., 1966).

As opposed to oral candidal carriage or quantitative candidal isolation, the incidence of oral candidal infection has been recently reported to be higher in diabetic patients than in a normal population (Lamey et al., 1988). Historically however, opinions still differ in this regard (Peters et al., 1966; Phelan & Levin, 1986). Furthermore, the genetically determined inability to secrete the water soluble glycoprotein forms of the ABO blood group antigens in saliva have been described in association with increased rate of oral candidal carriage (Burford-Mason et al., 1988) and candidal infection (May et al., 1986) in studies of non-diabetic subjects. However, the relationship

between oral candidal colonisation and secretor status in diabetic patients does not appear to have been established.

Therefore, the aims of this study were to investigate and compare the rate of oral candidal carriage, clinical candidal infection and quantity of candidal growth, in a group of patients with diabetes mellitus and a group of age, sex and denture status matched non-diabetic individuals. In addition, the possible effects of parameters such as blood glucose level, quality of glycaemic control and glycosylated haemoglobin levels on candidal colonisation were studied. Finally, the relationship between oral candidal colonisation and secretor status was also evaluated.

3.2 MATERIALS AND METHODS

3.2.1 Patients Selection

Patients with diagnosed diabetes mellitus attending the Diabetic Clinic at Glasgow Royal Infirmary were asked if they would like to participate in this investigation. The first 50 patients who agreed to participate, comprised the study group. The control group comprised 50 non-diabetic individuals who were recruited from either the Oral Medicine Unit at their first attendence to a "Migraine Clinic" or from patients attending the Department of Prosthodontics, Glasgow Dental Hospital. The latter subjects were attending for denture review assessment. The subjects of both study and control group were matched as closely as possible for age, sex and denture status. All subjects gave informed consent to participate in the study which was approved in full by the local Ethics Committee.

3.2.2 Clinical History and Oral Examination

The clinical history of each diabetic patient and control subject was recorded on a special proforma (Figure 3.1). The possession of a dental prosthesis and the patients denture wearing patterns were also noted. In the case of diabetic patients, the type of diabetes mellitus and diabetic treatment as well as the duration of the metabolic disease were recorded.

The medical and drug history, other than those used for glycaemic control, were matched in both groups as closely as possible. No individual in either group had received antibiotic or steroid therapy or had used an antiseptic mouth wash for at least three months prior to the study. Tobacco smoking and alcohol drinking habits were also matched as possible between individuals of both groups. A participant who smoked cigarettes daily at the time of sampling was classified as a smoker and who consumed alcohol on daily basis was classified as a drinker. Those individuals were considered to be non-smokers if they had never smoked, or had stopped smoking over at least the preceding six months.

Each diabetic patient and control subject underwent a routine oral clinical examination. The presence of signs of angular cheilitis or intraoral candidal infection was recorded. Patients with clinical signs of chronic atrophic candidosis had the extent of their disease classified according to Newton's classification (Newton, 1962).

3.2.3 Routine Blood Investigations

Each diabetic patient and control subject had haematological and biochemical assessment including corrected whole blood folate, ferritin, vitamin B12, haemoglobin (Hb) and a random blood glucose

estimation. For assay of corrected whole blood folate and haemoglobin, 4ml of venous blood were collected in a tube containing potassium EDTA (Sherwood Medical Industries Ltd., Crawley, West Sussex, England) while for serum vitamin B12 and ferritin estimation, 10ml blood were collected in a plain glass tube (Sherwood Medical Industries Ltd., Crawley West Sussex, England). For blood glucose determination, 2.5ml of blood were collected in a fluoride bottle (Sherwood Medical Industreis Ltd., Crawley, West Sussex, England). Corrected whole blood folate and serum vitamin B12 concentrations were determined radiometrically using SimulTRAC radioassay kit (Becton Dickinson Immunodiagnostics, Orangeburg, New York, USA) while serum ferritin level was determined immunoradiometrically using Ferritin MAb Solid Phase Component System (Becton Dikinson and Co., Orangeburg, New York, USA). Blood haemoglobin and glucose concentrations were determined by Cyanmethaemoglobin and, Glucose oxidase method on Beckman Autoanalyser respectively.

Each diabetic patient also had a blood sample assayed for glycosylated haemoglobin concentation (HbA1) by Corning method, an investigation which reflects the degree of glycaemic control during the preceding two months (Nathan et al., 1984). On the basis of latter results the quality of diabetic control of each patient was designated as "good" when the glycosylated haemoglobin level was less than 10 per cent, "fair" between 10 and 12 per cent or "poor" when it was greater than 12 per cent. This designation is somewhat arbitrary but, nevertheless, is widely used clinically (Lamey et al., 1988).

CANDIDA-DIABETES STUDY

NAME: DOB: SEX: M/F

UNIT NO: DURATION OF DIABETES: STUDY NO:

DIABETES TREATMENT: Insulin / OHA / Diet

PMH:

DRUGS: DOSE:

PDH:

BLOOG GLUCOSE: HBA1: Hb:

FERRITIN: FOLATE: VITAMIN B12:

DENTURES: Y/N CU: Y/N CL: Y/N PU: Y/N PL: Y/N

WEARING: DAY- U/L/Both DAY & NIGHT- U/L/Both NEVER- U/L/Both

SMOKING: Y/N Cigarettes/Cigar/Pipe QUANTITY:

ALCOHOL: Y/N Beer/Spirits/Others QUANTITY:

CLINICAL FINDINGS: Denture Stomatitis: Y/N Type:
Tongue:
Soft Palate:
Others:
Symptomatic Xerostomia: Y/N am/pm/both

SAMPLING: Oral Rinse: Y/N
Swab & Smear: Y/N
Saliva: Y/N
Buccal Scrabing: Y/N

RESULTS: Candidal Isolation: Rinse:
Swab :
Smear:
Secretor Status: Y/N Specify:
Mean Candidal Adhesion: Yeast/100 BEC

Figure 3.1: Proforma used for recording the clinical and medical history of the study subjects.

3.2.4 Microbiological Investigations

Swabs and smears were collected from areas of clinically infected mucosa and skin from the angles of the mouth. A plain cotton swab (Exogen Ltd., Clydebank, Scotland) was moistened in tap water and rubbed against the clinically infected tissue surface and returned immediately to its sterile tube. In cases of chronic atrophic candidosis, swabs and smears were obtained from the palatal mucosa and the fitting surface of the upper denture. The smear was taken using a sterile plastic instrument onto a microscope glass slide with the specimen then being left to air-dry.

The concentrated oral rinse technique (Samaranayake et al., 1986b) was used for oral yeast isolation. Each subject was supplied with 10ml of sterile phosphate buffered saline and requested to rinse thoroughly, following denture removal if appropriate. Although rinsing with the dentures in place was considered, it was decided to take the dentures out before rinsing according to the technique described by Samaranayake et al. (1986b). The period of rinsing was standardised at 60 seconds at which time the rinse was expectorated into the container and left on crushed ice until processed. The rinse container was then centrifuged at 1700g for 15 minutes and the supernatant discarded. The deposit was mixed with 1ml of sterile PBS and agitated on a bench vibrator for 30 seconds for disaggregation of microorganisms. A spiral plater model D (Spiral System Marketing Ltd., Maryland, USA) shown in figure 3.2 was used to dispense 50μl of the deposit onto a Sabouraud's dextrose agar plate (Gibco, Paisley, Scotland) and the swabs were streaked on similar plates. The culture plates were incubated aerobically at 37° C for 48 hours and the smears

Figure 3.2 The spiral plater model D.

were Gram-stained. All clinical examination and sampling took place between 1400 and 1600 hours.

3.2.5 Yeast Identification

Yeast growth on the culture plates (Figure 3.3) was confirmed microscopically by observing the blastospore phase in a direct wet film under a x40 objective (Olympus Ltd., London, England). Gram-stained smears were examined microscopically using oil immersion (BDH Chemicals Ltd., Poole, England) under x100 objective for the presence of yeast blastospores or hyphae. Candida albicans and other Candida species were identified by serum germ tube test (MacKenzie, 1962) and by using the yeast identification system API 20 C AUX (API Products Ltd., Basingstoke, Hampshire, England) as shown in Figure 3.4.

3.2.6 Quantification of Candidal Growth

The yeast colonies on each culture plate obtained from oral rinse were enumerated and multiplied by the 20 to calculate the initial number of colony forming units per 1ml of rinse (cfu/ml). The yeast growth obtained from a swab was semi-quantitatively estimated as a light growth when it was 10cfu or less, a growth between 11 and 50cfu was described as a moderate growth, while a growth of over 50cfu was described as a heavy growth.

3.2.7 Collection and Preparation of Saliva for Secretor Status Determination

Each subject was asked to collect about 2ml of unstimulated whole mixed saliva into a sterile universal container which was kept on crushed ice at the time of collection until processed.

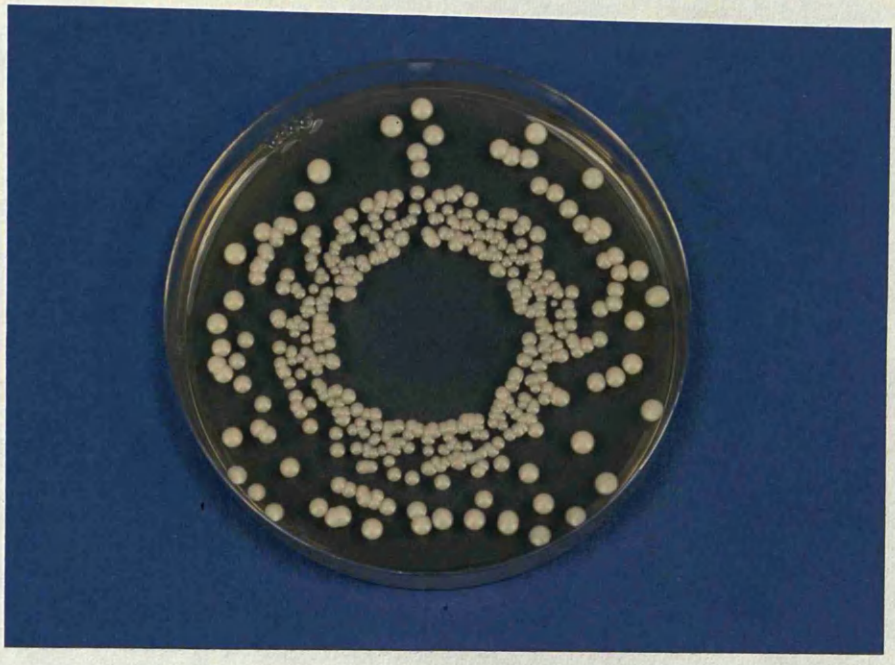

Figure 3.3 Candidal growth on the culture plate inoculated by the spiral plater.

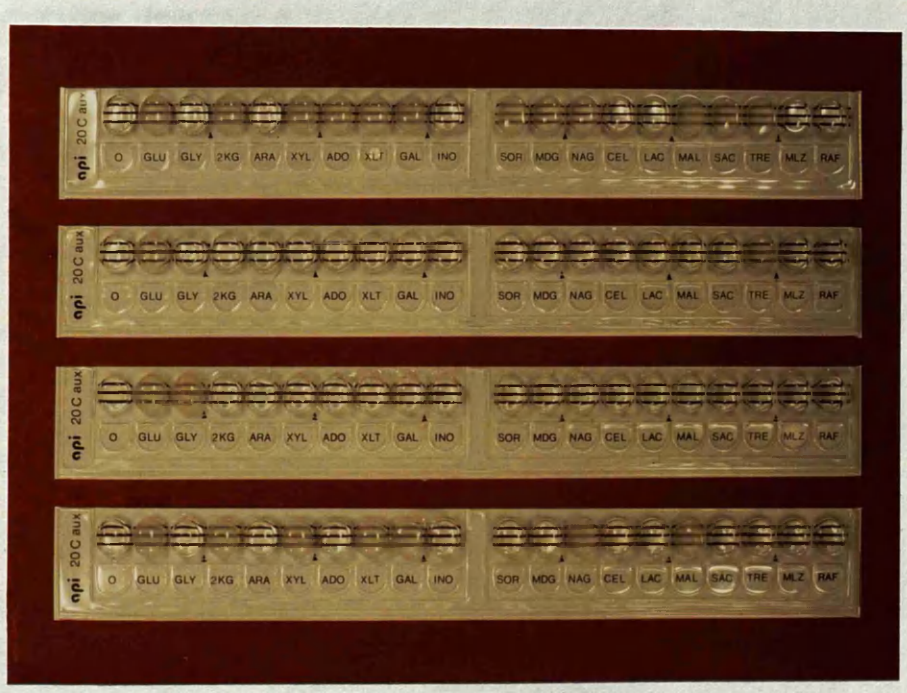

Figure 3.4 The yeast identification system API 20 C AUX.

Saliva samples were collected while patients were in a resting position, after denture removal, if appropriate. Saliva samples were prepared according to the standard techniques (Periera, Dodd, Marchant, 1969). Briefly, each sample was decanted into a sterile glass tube with an equal volume of normal saline. The tube was closed with non-absorbant cotton wool and placed in a boiling water bath for ten minutes to inactivate enzymes that may destroy secreted blood group antigens. The sample was then centrifuged at 1700g for 15 minutes to remove particulate matter and the supernatant was dispensed into a sterile bijou container (Sterilin Ltd., Feltham, England). The sample was either analysed immediately or stored frozen at a temperature of -20° C until analysis.

3.2.8 Agglutination Inhibition Technique

Secretor status was determined by the agglutination inhibition technique described by Periera et al. (1969). The anti A and anti B sera (American Hospital Supplies, Compton, England) were used at a dilution of 1/16 and 1/256 respectively. Anti H(O) serum (Ian McCombe Laboratories Supplies, Bellshill, Scotland) was used at a dilution of 1/4. These dilutions were determined after titration of the antisera with the corresponding red blood cells (Blood Transfusion Service, Glasgow) in 96 U-microwell titration plates (Gibco, Paisley, Scotland). The antisera were diluted in six per cent bovine albumin saline (Ortho Diagnostic, High Wycombe, England).

For secretor status determination the antisera were mixed with equal volumes (20μl) of saliva, diluted in two fold series in six per cent v/v bovine albumin saline up to 1/512 using a 96 U-microwell titration plate and incubated at room temperature (approximately 24° C)

for 30 minutes. Subsequently, a 20μl suspension of two per cent v/v in normal saline of corresponding red blood cells (five per cent of group O cells) were added, and the final mixture was incubated for a further two hours at room temperature. The stocks of the red blood cells were washed every other day in 10ml normal saline by centrifuging at 1700g for 20 minutes and stored at 4°C.

3.2.9 Defining Secretor Status

The individual was considered as a secretor of blood group specific antigen if his, or her, saliva inhibited agglutination of the corresponding blood cells. The titre was expressed as the reciprocal of the highest dilution of saliva showing macroscopic inhibition of agglutination. Individuals whose saliva inhibited blood cell agglutination at a titre of 1/16 or less were considered as non-secretors. Only those in whom the agglutination titre was 1/32 or more were considered as secretors (Periera et al., 1969).

3.2.10 Statistical Analysis

The proportion of subjects from whom yeasts were isolated in either group and the proportion of secretors were compared by means of the chi square test. In those instances in which the sample size gave rise to expected frequencies of less than five, Fisher's exact probability test was employed. Since the number of cfu/ml obtained from subjects of both groups were not normally distributed as determined graphically, a Mann Whitney U test was used to compare quantitative candidal isolation from each group. The quantity of candidal isolation in the groups was presented as the median of the

number of cfu/ml isolated and the the spread of data was presented by the interquartiles range (Q1, Q3). Pearson's correlation test was used to correlate candidal count to various parameters. Probabilities of less than five per cent were taken to be statistically significant.

The number of diabetic patients with clinical oral candidosis were included together with the Candida carriers when comparing oral candidal isolation rates between different groups. On the other hand, only quantity of candidal isolation from Candida carriers was compared between different groups.

3.3 RESULTS

3.3.1 Subjects of the Study

As shown in Table 3.1 the diabetic patients and control subjects were closely matched for age, sex and smoking habit as well as for denture status and denture wearing patterns.

The diabetic group comprised 19 (38%) insulin-dependent and 31 (62%) non-insulin dependent patients with a mean disease duration of 6.7 years (range four months and 32 years). For diabetic control, 19 (38%) patients were on insulin injection, six (12%) on diet control alone and 25 (50%) on a combination of diet control and oral hypoglycaemic agents.

According to the percentage of blood glycosylated haemoglobin the quality of diabetic control was designated as "good" in 25 (59.5%) patients, "fair" in 13 (31%) and "poor" in four (9.5%) patients. However, blood samples could not be obtained from eight diabetic patients. None of the control subjects were discovered to be diabetic or showed abnormally high blood glucose concentrations.

	Diabetics (n= 50)	Controls (n= 50)
Male	27 (54%)	23 (46%)
Female	23 (46%)	27 (54%)
Mean age (years)	53.7 (SD 14.9)	56.3 (SD 17.3)
Age range (years)	17 - 81	20 - 84
Denture wearers	36 (72%)	36 (72%)
Partial denture	12 (33%)	10 (28%)
Complate denture	24 (67%)	26 (72%)
Denture wearing patterns		
Day and night	13 (36%)	14 (39%)
Day only	23 (64%)	22 (61%)
Dentate	14 (28%)	14 (28%)
Smoker	16 (32%)	14 (28%)
Alcohol drinker	7 (14%)	2 (4%)
Symptomatic xerostomia	15 (30%)	8 (16%)
Am	4 (27%)	0 (0%)
Pm	5 (33%)	1 (12%)
Both	6 (40%)	7 (87%)

SD: Standard deviation of the mean.

Table 3.1 Clinical status of the study subjects.

3.3.2 Oral Candidal Carriage

Oral candidal carriage was defined as a positive oral candidal isolation but in the absence of any mucosal reaction suggestive of clinically apparent candidosis.

Twenty one (42%) diabetic patients and 20 (40%) control subjects were Candida carriers. The difference between the two groups was not significant ($p>0.05$). Altogether, Candida species were isolated from 27 (54%) diabetic patients (21 carriers and six patients with oral candidosis). However, this candidal isolation rate was not significantly different than that in control subjects ($p>0.05$). One diabetic patient and a control subject harboured more than a single Candida species; C. albicans was the most frequent species isolated from both groups, followed by C. glabrata (Table 3.2).

3.3.3 Oral Candidal Infection

Diabetic patients had a significantly higher incidence of oral candidal infection than control subjects ($p<0.02$). Indeed, six (12%) of the diabetic patients had clinical and microbiological evidence of oral candidosis compared to none of the control subjects. Table 3.3 summarises the various types of oral candidosis diagnosed in the diabetic patients.

Candida species	Frequency of isolation	
	Diabetics	Controls
C. albicans	21 (75.0%)	14 (66.7%)
C. glabrata	4 (14.3%)	3 (14.2%)
C. krusei	1 (3.6%)	1 (4.8%)
C. stellatoidea	1 (3.6%)	1 (4.8%)
C. pseudotropicalis	1 (3.6%)	1 (4.8%)
Multiple yeasts (C. albicans + C. glabrata)	1 (3.6%)	1 (4.8%)

Table 3.2 Candida species isolated from the oral cavity of the diabetic patients and the control subjects.

Case no.	Chronic atrophic candidosis			Angular cheilitis	Acute atrophic candidosis
	Type 1	Type 2	Type 3		
14			+	+	
19					+
24		+			
38			+		
41		+			
46			+		

Table 3.3 Type of oral candidosis diagnosed in the diabetic patients.

3.3.4 Quantity of Oral Candidal Isolation

Diabetic patients with oral candidosis had a significantly increased quantity of oral candidal isolation compared to the carriers ($p<0.01$; Table 3.4). Quantity of candidal isolation was not significantly different between diabetic patients and control subjects who were carriers ($p>0.05$), however, diabetic patients more frequently had a higher count of candidal isolation (Figure 3.5). In cases of chronic atrophic candidosis, swab cultures obtained from the fitting surface of upper dentures yielded a heavy candidal growth, whereas those obtained from clinically infected palatal mucosa or angles of the mouth (in cases of angular cheilitis) yielded a moderate to heavy growth.

3.3.5 Relationship of Oral Candidal Isolation to Gender

Statistical analysis of data from both diabetic and control group showed no significant difference between males and females in either rate or quantity of candidal isolation ($p>0.05$; Table 3.5).

3.3.6 Relationship of Oral Candidal Isolation to Age

The subjects of both groups were divided into ten year age groups and the number of individuals in each group who had Candida isolated from the oral cavity was analysed (Table 3.6). Chi square test showed no significant difference in the rate of candidal isolation between different age groups in either diabetic patients or control subjects ($p>0.05$). No significant correlation was found between age and the quantity of candidal isolation in diabetic patients ($r= -0.05$) and control subjects ($r= 0.23$) as shown in Tables 3.7 and 3.8 respectively.

Diabetics		Controls
Carriers (n= 21) cfu/ml	Infected (n= 6) cfu/ml	Carriers (n= 20) cfu/ml
64842	93345	62418
5280	17120	32118
3300	9120	18080
3000	6880	11040
2700	3300	2000
2200	1000	1800
1460		1280
1300		920
1140		500
800		340
500		280
500		220
240		136
180		120
160		120
140		120
140		40
100		40
100		40
80		20
40		
Median 500[a]	8000[b]	310[c]
Q1, Q3 140, 2450	2725, 36176	120, 1950

a vs b ($p<0.01$); a vs c ($p>0.05$); b vs c ($p<0.05$)

Table 3.4 Quantity of oral candidal isolation in the diabetic patients and the control subjects.

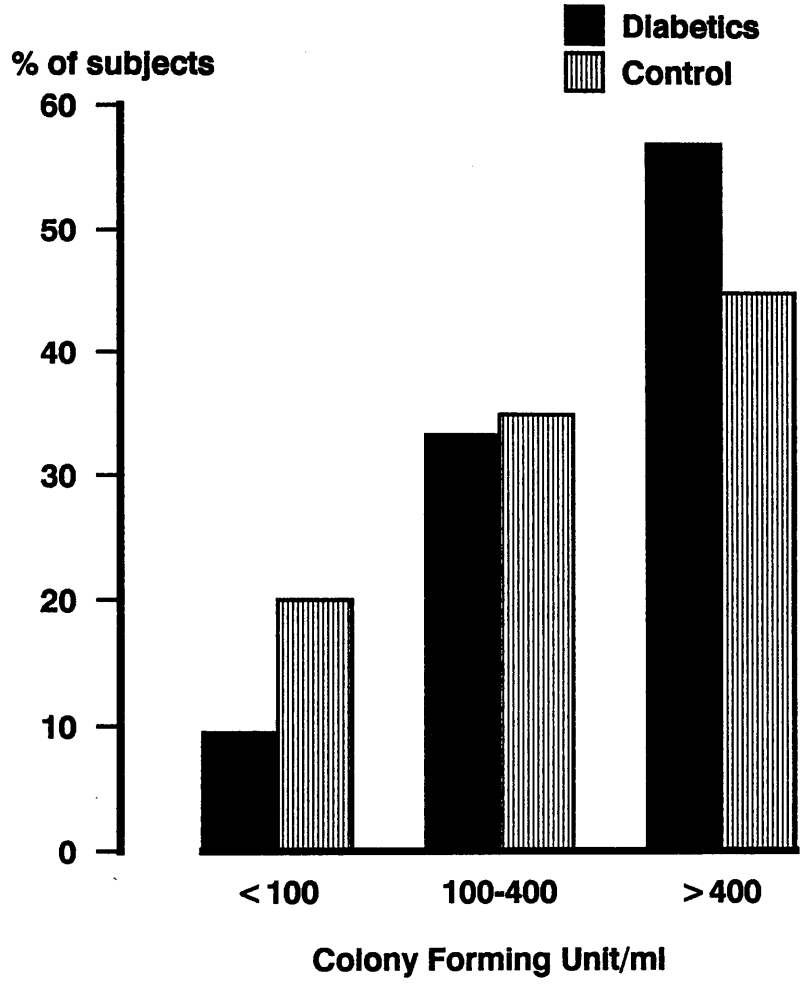

Figure 3.5 Frequency of quantitative oral candidal isolation from Candida carriers.

Group	Gender	Candidal isolation (%)	Quantity of isolation cfu/ml (Q1, Q3)
Diabetics	Male	14 (51.8%)	500 (100, 1300)
		p>0.05	p>0.05
	Female	13 (56.5%)	980 (155, 2775)
Controls	Male	8 (34.8%)	250 (120, 815)
		p>0.05	p>0.05
	Female	12 (44.4%)	810 (60, 16320)

Table 3.5 Relationship of oral candidal isolation to gender.

Age group (year)	Diabetics (n= 50)		Controls (n= 50)	
	No. of subjects	Candidal isolation (%)	No. of subjects	Candidal isolation (%)
11 - 20	1	0 (0%)	1	1 (100%)
21 - 30	4	2 (50%)	3	0 (0%)
31 - 40	5	3 (60%)	10	3 (30%)
41 - 50	9	4 (44%)	2	2 (100%)
51 - 60	15	6 (40%)	6	3 (50%)
61 - 70	11	8 (73%)	18	8 (44%)
71 - 80	4	4 (75%)	9	3 (33%)
81 - 90	1	1 (100%)	1	0 (0%)
Total	50	27 (100%)	50	20 (100%)

Table 3.6 Relationship of oral candidal isolation to age.

3.3.7 Relationship of Oral Candidal Isolation to Denture Status and Denture Wearing Patterns

The relationship of oral candidal isolation to denture status in both groups is presented in Table 3.9. Candida species were isolated at a higher rate from denture wearers compared to dentate subjects in both groups although the difference was not significant either in diabetic patients or control subjects ($p>0.05$). The difference in quantity of candidal isolation also was not significant between denture wearers and dentate subjects either in diabetic patients or control subjects ($p>0.05$).

The six diabetic patients with oral candidosis were exclusively denture wearers, with a significantly increased incidence of oral candidosis among diabetic patients who were denture wearers compared to dentate diabetic patients ($p<0.05$).

As shown in Table 3.9, the rate of candidal isolation from diabetic patients who wore dentures continuously (day and night) was significantly higher than that from those who wore dentures during the day only ($p<0.02$). This relationship was not significant in the control group ($p>0.05$).

The quantity of candidal isolation was significantly higher in control subjects who wore dentures continuously over those who wore dentures during the day only ($p<0.05$). This relationship, however, was not significant among the diabetic patients ($p>0.05$).

Statistical analysis showed that diabetic patients who wore dentures continuously had a significantly increased incidence of oral candidosis compared with those who wore dentures during the day only ($p<0.0005$).

Case no.	Age (years)	Duration of diabetes (years)	Blood glucose (mmol/l)	HbA1 (%)	Hb (g/dl)	Ferritin (ng/ml)	Folate (ng/ml)	Vit. B12 (pg/ml)	Candidal isolation (cfu/ml)
1	59	0.3	11.1	9.4	15.2	14	263	243	NG
2	53	4.0	10.8	9.5	15.2	7	210	326	NG
3	56	10.0	7.0	8.5	NDA	NDA	NDA	NDA	NG
4	66	0.5	7.0	8.6	15.5	14	200	217	NG
5	21	NDA	6.3	11.5	14.5	17	358	306	800
6	47	1.0	17.8	10.3	17.8	20	321	431	NG
7	52	1.0	20.8	11.2	14.9	7	365	394	NG
8	54	1.0	9.0	12.0	12.3	24	168	294	1460
9	17	5.0	4.5	9.4	14.3	18	411	530	NG
10	59	2.5	18.1	12.1	NDA	NDA	NDA	NDA	2200
11	67	1.0	5.7	7.3	14.3	10	227	437	2700
12	58	12.0	10.7	8.6	15.4	12	232	234	140
13	49	4.0	12.4	9.6	14.7	7	776	1022	NG
14	65	3.0	16.1	11.1	15.1	29	269	361	6880
15	53	3.0	9.4	8.5	NDA	NDA	NDA	NDA	NG
16	44	3.0	17.6	12.5	18.1	18	213	329	5280
17	71	1.0	9.9	8.7	NDA	NDA	NDA	NDA	500
18	64	3.0	7.4	7.1	14.4	20	717	345	240
19	52	7.0	8.9	8.4	11.7	9	264	419	1000
20	46	10.0	13.5	10.5	NDA	NDA	NDA	NDA	40
21	68	7.0	15.3	11.6	15.1	25	236	418	1140
22	78	6.0	7.9	NDA	NDA	NDA	NDA	NDA	1300
23	63	0.5	8.6	9.0	14.5	11	228	640	64842
24	44	5.0	9.9	9.8	13.2	12	241	322	93345
25	25	13.0	5.7	7.2	12.0	19	416	721	NG
26	33	2.0	8.7	7.0	14.0	9	779	455	NG
27	64	3.0	16.0	8.7	14.0	14	358	601	180
28	73	10.0	12.1	9.4	14.0	7	345	520	80
29	59	7.0	15.0	13.2	16.4	13	374	526	NG
30	26	6.0	10.4	7.9	14.2	9	280	405	100
31	36	14.0	4.9	9.1	15.3	15	299	463	100

Case no.	Age (years)	Duration of diabetes (years)	Blood glucose (mmol/l)	HbA1 (%)	Hb (g/dl)	Ferritin (ng/ml)	Folate (ng/ml)	Vit. B12 (pg/ml)	Candidal isolation (cfu/ml)
32	40	3.0	16.0	10.9	13.2	6	336	652	NG
33	37	7.0	16.4	12.3	13.7	11	210	452	140
34	48	3.0	11.9	10.1	14.6	3	334	433	NG
35	60	0.6	4.3	7.2	15.5	16	281	768	500
36	50	16.0	7.1	10.1	12.7	7	169	406	NG
37	65	6.0	8.4	8.6	13.4	11	320	253	NG
38	46	15.0	14.7	11.8	17.2	15	155	350	9120
39	57	3.0	5.1	9.8	11.4	15	354	301	NG
40	57	5.0	11.4	10.2	14.5	15	251	286	NG
41	40	25.0	6.8	8.0	NDA	NDA	NDA	NDA	3300
42	53	20.0	10.8	10.2	13.4	12	298	562	3300
43	52	0.5	6.8	7.5	17.2	10	352	432	NG
44	67	32.0	15.5	11.0	14.2	13	301	613	NG
45	81	4.0	11.5	11.7	13.7	3	269	179	3000
46	63	10.0	11.4	9.8	13.9	12	373	640	17120
47	63	2.0	8.0	8.3	13.1	12	262	348	160
48	46	4.0	5.5	7.8	NDA	NDA	NDA	NDA	NG
49	80	3.0	10.1	8.5	15.0	18	460	268	NG
50	23	5.0	7.0	8.9	12.6	27	459	288	NG

NDA: No data availab; NG: No growth.

Table 3.7 Relationship of oral candidal isolation to clinical and haematological status in the diabetic patients.

Case no.	Age (years)	Blood glucose (mmol/l)	Hb (g/dl)	Ferritin (ng/ml)	Folate (ng/ml)	Vit. B12 (pg/ml)	Candidal isolation (cfu/ml)
1	84	4.7	13.4	162	158	293	NG
2	55	5.7	15.3	90	139	457	220
3	54	3.7	13.8	73	162	348	NG
4	53	4.8	14.5	50	185	538	NG
5	48	3.9	12.9	21	247	495	340
6	66	2.1	14.9	49	201	413	136
7	70	2.6	13.2	29	180	263	32118
8	74	4.1	14.3	25	129	468	40
9	72	3.5	15.3	134	205	391	NG
10	52	4.0	11.5	6	204	399	1280
11	64	5.6	14.9	30	239	468	NG
12	73	5.4	13.2	97	205	307	NG
13	62	5.3	14.6	32	155	249	120
14	67	4.4	15.1	585	128	357	120
15	67	4.2	13.4	57	112	416	62418
16	69	5.6	13.9	96	162	126	NG
17	64	5.1	12.9	181	225	421	280
18	32	5.7	14.6	41	215	306	NG
19	38	4.6	13.6	51	147	343	40
20	30	4.5	14.9	121	186	150	NG
21	64	4.3	13.9	39	277	411	NG
22	73	4.9	11.4	4	159	126	18080
23	75	5.9	13.9	71	138	202	NG
24	66	5.0	17.9	432	181	334	NG
25	68	4.8	16.0	89	171	330	NG
26	78	6.1	14.7	264	181	199	920
27	63	4.3	14.1	191	196	189	NG
28	49	4.4	14.0	25	215	476	20
29	61	4.9	12.5	8	359	783	120
30	77	5.7	14.1	143	215	159	NG
31	65	6.4	13.7	69	NDA	NDA	NG

Case no.	Age (years)	Blood glucose (mmol/dl)	Hb (g/dl)	Ferritin (ng/ml)	Folate (ng/ml)	Vit. B12 (pg/ml)	Candidal isolation (cfu/ml)
32	70	4.3	12.1	NDA	NDA	NDA	NG
33	74	4.5	NDA	NDA	NDA	NDA	NG
34	79	4.7	11.4	11	315	571	NG
35	22	4.9	13.8	72	155	347	NG
36	32	5.4	14.7	131	206	366	NG
37	56	4.1	14.5	30	188	211	NG
38	31	4.5	12.2	29	204	466	11040
39	20	3.5	NDA	NDA	NDA	NDA	500
40	69	5.4	15.5	104	127	334	NG
41	57	4.7	16.0	100	112	226	40
42	38	4.8	NDA	NDA	NDA	NDA	2000
43	61	4.9	NDA	NDA	NDA	NDA	1800
44	70	5.3	13.5	51	133	234	NG
45	33	4.8	15.5	90	230	478	NG
46	35	4.7	15.7	74	272	350	NG
47	37	4.7	15.2	42	281	634	NG
48	32	4.9	14.9	37	220	429	NG
49	40	5.6	14.7	124	166	257	NG
50	30	5.3	14.0	84	154	473	NG

NDA: No data availabe; NG: No growth.

Table 3.8 Relationship of oral candidal isolation to clinical and haematological status in the control subjects.

Group		Candidal isolation (%)	Quantity of isolation cfu/ml (Q1, Q3)
DIABETICS	Denture wearers	22 (63%)	650 (165, 2015)
	Dentate	5 (36%)	140 (120, 4140)
	D & N	12 (92%)	210 (95, 1275)
	D	10 (43%)	1120 (415, 2475)
CONTROLS	Denture wearers	16 (44%)	280 (120, 11040)
	Dentate	4 (29%)	890 (155, 1820)
	D & N	8 (57%)	6420 (250, 28608)
	D	8 (36%)	120 (40, 280)

D & N : Day and night denture wearing.
D : Day only denture wearing.

Table 3.9 Relationship of oral candidal isolation to denture status and denture wearing patterns.

3.3.8 Relationship of Oral Candidal Isolation to Haematological Status

In diabetic patients and control subjects no significant correlation was detected between quantity of candidal isolation and values of corrected whole blood folate (r= -0.1 and -0.3 respectively), ferritin (r= -0.1 and -0.2 respectively), vitamin B12 (r= 0.06 and -0.1 respectively) or haemoglobin (r= -0.08 and -0.3 respectively) as presented in Tables 3.7 and 3.8 respectively.

Sixteen (39%) of the diabetic patients were iron-deficient (ferritin <12ng/ml), 26 patients had ferritin values within normal range (12 - 300ng/ml) while ferritin estimation could not be determined in eight diabetic patients. Candida species were isolated from seven (43.7%) of the iron-deficient patients (median= 1420cfu/ml; Q1, Q3= 95, 18461)) and from 15 (57.7%) of those with a normal ferritin concentration (median= 500cfu/ml; Q1, Q3= 160, 1460) without a significant difference in either rate or quantity of candidal isolation ($p>0.05$).

Three (7.1%) of the diabetic patients had low haemoglobin concentrations (<12g/dl for females and <12.5g/dl for males). Thirty nine patients had normal haemoglobin concentrations while haemoglobin level could not be determined in eight patients. Candida species were isolated from only one (33.3%) patient with a low haemoglobin concentration and from 21 (53.8%) patients with normal haemoglobin concentrations. The difference in rate of candidal isolation was not significant ($p>0.05$).

All diabetic patients had normal level of blood folate (75 - 400ng/ml) and vitamin B12 (175 - 800pg/ml). Table 3.7 presents the oral candidal isolation data and values of corrected whole blood

folate, ferritin, vitamin B12 and haemoglobin in diabetic patients.

In control subjects, four individuals (8.7%) had low ferritin levels, 42 subjects had normal ferritin concentrations while ferritin level could not be determined in four subjects. Candida species were isolated from three (75%) of the ferritin-deficient subjects (median= 1280cfu/ml; Q1, Q3= 120, 18080)) and from 14 (33.3%) of the subjects with normal ferritin levels (median= 178cfu/ml; Q1, Q3= 40, 3450). The difference was not significant in either rate or quantity of candidal isolation between subjects with low ferritin ot those with normal levels ($p>0.05$).

Three (6.5%) of the control subjects had a low haemoglobin concentration, 43 had normal haemoglobin levels while haemoglobin level could not be estimated for technical reason in four subjects. Candida species were isolated from two (66.7%) of the subjects with low haemoglobin concentrations and from 15 (34.9%) of the subjects with normal haemoglobin levels. No significant difference was detected in rate of candidal isolation between subjects who were low in haemoglobin and those with normal levels ($p>0.05$). Due to the small sample size, the quantity of candidal isolation could not be compared statistically between the two groups. None of the control subjects was folate deficient but four subjects were vitamin B12 deficient and Candida was isolated from only one of the subjects. Table 3.8 presents data of candidal isolation and values of corrected whole blood folate, ferritin, vitamin B12 and haemoglobin in control subjects.

3.3.9 Relationship of Oral Candidal Isolation to Symptomatic Xerostomia

Table 3.10 presents the relationship of oral candidal isolation and symptomatic xerostomia. Although a higher rate of candidal isolation was noticed in association with symptomatic xerostomia, neither the rate nor quantity of candidal isolation was significantly different between subjects with symptomatic xerostomia and those who were free of this complaint. This finding pertained both to diabetic patients and control subjects. In diabetic patients oral candidosis was diagnosed in three (20%) patients with symptomatic xerostomia and in another three (8.6%) patients who were free of this symptom but, there was no significant difference between the two groups ($p>0.05$).

Group	Symptomatic xerostomia	Candidal isolation (%)	Quantity of isolation cfu/ml (Q1, Q3)
DIABETICS	Yes (n= 15)	10 (66.7%)	800 (140, 3000)
		p>0.05	p>0.05
	No (n= 35)	17 (48.6%)	500 (100, 1770)
CONTROLS	Yes (n= 8)	5 (62.5%)	920 (238, 1540)
		p>0.05	p>0.05
	No (n= 42)	15 (35.7%)	220 (40, 2200)

Table 3.10 Relationship of oral candidal isolation to symptomatic xerostomia.

3.3.10 Relationship of Oral Candidal Isolation to Type of Diabetes

Candida species were isolated from 11 (58%) diabetic patients with insulin-dependent diabetes mellitus (median= 800cfu/ml; Q1, Q3= 100, 2380)) and from 16 (52%) non-insulin dependent diabetic patients (median= 500cfu/ml; Q1, Q3= 165, 2575). Statistical analysis showed no significant difference either in rate ($p>0.05$) or quantity ($p>0.05$) of candidal isolation between patients of either type of diabetes mellitus.

Oral candidosis was diagnosed in four (13%) non-insulin dependent and two (10.5%) insulin-dependent diabetic patients but, statistical analysis showed no significant difference in the incidence of oral candidosis between patients of either type of diabetes mellitus ($p>0.05$).

3.3.11 Relationship of Oral Candidal Isolation to Duration of Diabetes

Statistical analysis showed no significant correlation between quantity of candidal isolation and duration of diabetes mellitus ($r= -0.1$; Table 3.7). Diabetic patients were subdivided according to the duration of diabetes into groups of five year intervals (Table 3.11). Chi square test showed no significant difference in the rate of candidal isolation between the subgroups ($p>0.05$). However, quantity of candidal isolation was significantly higher in patients who had had diabetes for less the five years compared with those who had the disease for more than five years ($p<0.05$).

Duration of diabetes (years)	Number of patients	Candidal isolation (%)	Quantity of isolation cfu/ml (Q1, Q3)
< 5	26	13 (50%)	1460* (340, 3000)
> 5	24	14 (58%)	140* (90, 1220)
5 - 10	16	9 (56%)	120 (70, 1180)
11 - 15	4	3 (75%)	120 (100, 1250)
16 - 20	2	1 (50%)	3300
>20	2	1 (50%)	3300

*: $p<0.05$.

Table 3.11 Relationship of oral candidal isolation to duration of diabetes.

3.3.12 Relationship of Oral Candidal Isolation to Blood Glucose Level

No significant correlation was detected between quantity of candidal isolation and blood glucose concentration either in diabetic patients (r= 0.05) or control group subjects (r= 0.005) as shown in Table 3.7 and 3.8 respectively.

Diabetic patients were divided into subgroups according to blood glucose concentration. Sixteen diabetic patients had blood glucose concentrations less than 8mmol/l and Candida species were isolated from seven (43.7%) patients (median= 650cfu/ml; Q1, Q3= 205, 1650) and nine diabetic patients had blood glucose concentration between 8-10mmol/l and Candida species were isolated from six (66.6%) patients (median= 980cfu/ml; Q1, Q3= 245, 48997). Blood glucose concentration was above 10mmol/l in 25 patients and Candida species were isolated from 14 (56%) patients (median= 180cfu/ml; Q1, Q3= 100, 3000). The difference was not significant either in rate ($p>0.05$) or quantity ($p>0.05$) of candidal isolation between the subgroups.

Since results of blood glucose concentration of control subjects were within the normal range (<6mmol/l), no attempt was undertaken to assess the correlation between rate of candidal isolation and blood glucose concentration.

3.3.13 Relationship of Oral Candidal Isolation to Overall Quality of Glycaemic Control

Candida species were isolated from 13 of 24 (54%) diabetic patients with good glycaemic control (median= 210cfu/ml; Q1, Q3= 100, 1050), from six of 13 (46%) patients with fair control (median= 2070cfu/ml; Q1, Q3= 885, 3225) and from three of four (75%) patients with poor glycaemic control (median= 1460cfu/ml; Q1, Q3= 140, 5280).

Statistical analysis showed no significant difference in either rate ($p>0.05$) or quantity of candidal isolation ($p>0.05$) between diabetic patients with good, fair or poor overall glycaemic control. Analysis of the correlation between quantity of candidal isolation and percentage of glycosylated haemoglobin was also not significant ($r= 0.006$; Table 3.7).

3.3.14 Distribution of Secretors in Diabetic Patients and Control Subjects

Saliva sample analysis showed that 31 (64.6%) of the diabetic patients and 31 (62%) of the control subjects were secretors of the corresponding blood group antigens without a significant difference in proportion of secretors between the two groups ($p= 0.9$; Table 3.12). However, two diabetic patients could not provide sufficient quantity of saliva for analysis.

3.3.15 Relationship of Secretor Status to Type of Diabetes

Seven (36.8%) of the insulin-dependent and 10 (34.5%) of the non-insulin dependent diabetic patients were non-secretors. The proportion of non-secretors did not differ significantly between patients of either type of diabetes ($p>0.05$). In addition, there was no significant difference in the proportion of non-secretors between either insulin-dependent and non-insulin dependent diabetic patients compared with the controls ($p>0.05$).

3.3.16 Relationship of Oral Candidal Isolation to Secretor Status

In diabetic patients, Candida species were isolated from 13 (41.9%) of the secretors (median= 1220cfu/ml; Q1, Q3= 220, 2775) and

from 13 (76.5%) of the non-secretors (median= 1320cfu/ml; Q1, Q3= 100, 2750). The non-secretors had a significantly increased rate of candidal isolation over secretors ($p<0.05$). However, the quantity of candidal isolation was not significantly different between secretors and non-secretors ($p>0.05$).

Clinical and microbiological evidence of oral candidosis was observed in three (9.7%) of the diabetic patients who were secretors (median= 6880cfu/ml; Q1, Q3= 3300, 17120)) and in three (17.6%) of those who were non-secretors (median= 9120cfu/ml; Q1, Q3= 1000, 93345). The proportion of patients with oral candidal infection between secretors and non-secretors was not significantly different ($p>0.05$) nor was there a difference in the quantity of candidal isolation ($p>0.05$).

In control subjects, Candida species were isolated from ten (32.2%) of the secretors (median= 280cfu/ml; Q1, Q3= 100, 5870) and from ten (52.6%) of the non-secretors (median= 390cfu/ml; Q1, Q3= 100, 4260). No significant difference was detected in either rate ($p>0.05$) or quantity of candidal isolation ($p>0.05$) between secretors and non-secretors.

		Blood group antigen			
Group	**Secretors**	**A**	**B**	**AB**	**O**
Diabetics	31 (64.6%)	14 (45%)	2 (6%)	3 (10%)	12 (39%)
Controls	31 (62.0%)	10 (32%)	7 (23%)	0 (0%)	14 (45%)

Table 3.12 Distribution of secretors in the diabetic patients and the control subjects.

3.4 DISCUSSION AND CONCLUSIONS

Candida species, particularly C. albicans are common human oral commensals present in three to 47 per cent of a healthy adult population (Odds, 1988). The present finding of 40 per cent positive candidal isolates amongst the non-diabetic individuals is within this range.

The identity and frequency of Candida species isolated were comparable in the diabetic patients and control subjects. C. albicans was the predominant isolate followed by C. glabrata. This is relevant to the repeatedly reported finding that C. albicans is the most frequently isolated species in health and disease (Odds, 1988). Multiple yeast isolation was observed in only one diabetic patient (3.4%) and one non-diabetic subject (4.7%). This is lower than the multiple yeast carriage rate of 9.1 per cent in healthy individuals reported by Yamane and Saitoh (1985). In that study a discriminatory selective medium, Pagano-Levin agar, was used for isolation of multiple yeasts from the clinical specimens. That particular medium was not used in the present study because tetrazolium chloride incorporated into the medium can suppress candidal growth (Stedham, Kelley, Coles, 1966).

The finding of the present study that Candida species were isolated from 54 per cent of the diabetic patients compared to 40 per cent of the age, sex and denture status matched non-diabetic control subjects lends further support to the previous findings that Candida species are more prevalent in the oral cavity of diabetic patients compared to healthy individuals (Weinstein et al., 1960; Johnston et al., 1967; Barlow and Chattaway, 1969; Tapper-Jones et al., 1981; Lamey et al., 1988). As some of the studies selected diabetic

patients free from clinical candidal infection (Odds et al., 1978), the present study with a randomly selected patients population may present the oral clinical and microbiological state of a diabetic population more comprehensively. The present results in addition to those reported recently by Lamey et al. (1988) and Thorstensson et al. (1989) were unable to confirm the claim that diabetic patients had a quantitative increase in oral candidal carriage (Tapper-Jones et al., 1981).

The present finding of 54 per cent candidal isolation among diabetic patients using the concentrated oral rinse technique for candidal isolation is similar to the candidal isolation rate reported by Fisher et al. (1987) and Lamey et al. (1988) who used the same method of isolation. In those studies, Candida species were recovered from 51 and 57 per cent of the diabetic patients respectively. The concentrated oral rinse technique is known for its higher sensitivity in candidal isolation when compared with other isolation techniques such as imprint sampling and conventional swabing (Samaranayake et al., 1986b).

A higher candidal isolation rate from diabetic patients (75%) was recently reported by Bartholomew et al. (1987) using exfoliative cytology of swabs taken from the tongue and buccal mucosa, rather than by culture. This high isolation rate may be related to the fact that, the diabetic patients were hospital in-patients and the prevalence of oral yeasts is thought to be higher among such patients (Odds & Evans, 1980). In addition, the majority of patients studied (39 of 60) were poorly controlled diabetics and some with infections, for which they were on antibiotic therapy which in turn may encourage candidal growth

(Johnston et al., 1967). Banoczy et al. (1987) reported no significant increase in candidal isolation rate from a group of insulin-dependent diabetic women compared with age and sex matched control subjects. These authors used a special diagnostic kit for candidal isolation with a lower sensitivity limit whereby only individuals who had more than 1000cfu/ml were distinguished as carriers. In addition, the majority of subjects in the latter study were young and therefore unlikely to be wearing dentures.

To compare candidal isolation rates from different studies is fraught with a number of difficulties such as the apparent variations in candidal isolation and culture techniques used (see Table 1.1). Even those studies which had used identical candidal isolation procedures and culturing methods still have interstudy variations in local factors such as age, sex, denture wearing patterns and smoking; all factors which have been suggested as influencing oral candidal colonisation (Arendorf & Walker, 1979; 1980). Similarly, the lack of matching in these factors between diabetic patients and control subjects may be a reason why some researchers failed to record a difference in candidal isolation between the two groups (Mehnert & Mehnert, 1958; Peters et al., 1966).

Other reasons for the discrepancies in candidal isolation rates reported in different studies may be variations in the sensitivity of methods for candidal isolation and culturing techniques. In that regard, Peters et al. (1966) swabbed the teeth, gingiva, mucobuccal and mucolingual folds of 400 diabetic patients and 200 control subjects and reported no difference in candidal isolation rate between the two groups. Nevertheless, their isolation technique may not represent the entire oral cavity (eg. the tongue) so the

carriage rate may be under estimated.

However, in the present study the quantity of oral candidal isolation as presented by the cfu/ml may be an under estimation of the real intraoral candidal load. The salivary IgA secreted in higher concentration in saliva of subjects colonised with Candida (Jeganathan et al., 1987) may result in agglutination of candidal cells in the oral cavity (Lehner, 1965; Epstein et al., 1982). Accordingly, after rinsing the mouth, several agglutinated candidal blastospores in the rinse could grow and be counted as a single candidal colony.

In the present study, factors such as age, sex, type and duration of diabetes mellitus seem to bear no relationship to oral candidal isolation in diabetic patients. This conclusion was also reported by other workers in this field (Odds et al., 1978; Tapper-Jones et al., 1981; Bartholomew et al., 1987; Fisher et al., 1987; Lamey et al., 1988). However, the observed higher rate and quantity of candidal isolation, although not significant, among the female non-diabetic subjects compared to males may support the suggestion that hormonal factors may influence oral candidal colonisation (Arendorf & Walker, 1980).

Diabetes mellitus is known to predispose to many infections (Wheat, 1980) and candidosis is no exception. Genital candidal infections are more common and florid in diabetic patients (Sonck & Somersalo, 1963) and diabetes mellitus has been implicated as a factor in approximately five to 15 per cent of reported cases of systemic candidosis (Odds, 1988). Previously the author and other workers reported on the significantly increased prevalence of oral candidosis

among diabetic patients (Lamey et al., 1988) and the present study is a further confirmation. Other investigators have found more atrophic lesions of the tongue associated with Candida among diabetic patients (Farman & Nutt, 1976; Farman, 1977) while Phelan and Levin (1986) found the prevalence of denture stomatitis to be similar in diabetic and non-diabetic subjects. However, the diabetic group in the latter study included subjects with increased plasma glucose levels who were not necessarily diabetic but may have had glucose intolerance.

The pathogenicity of Candida has been reported to depend in part upon the number of organisms present (Wain, Price, Cawson, 1976). However, this does not seem the case in diabetic patients of this study, as these patients had a significantly increased incidence of oral candidosis over the matched non-diabetic individuals despite the comparable quantity of candidal growth in both groups. In an animal model of diabetes mellitus, Hurley (1966) presented evidence that diabetes mellitus decreased host resistance and predisposed to candidal infection. Later, Dourov and Coremans-Pelseneer (1987) showed that experimental oral candidosis was successfully induced in rats rendered diabetic by streptozotocin but not in healthy animals when challanged with a similar yeast inoculum. Therefore, the disease outcome may depend more on host health than on the yeast load.

Precisely why diabetes mellitus increases susceptibility of an individual to oral candidosis is not clear. Knight and Fletcher (1971) noticed increased in vitro candidal growth in saliva from diabetic patients compared to that in saliva from non-diabetic individuals. The authors proposed that a higher glucose level in the saliva of diabetics may be one factor responsible for enhancing candidal growth. However, C. albicans cultured in saliva from

diabetic patients and healthy individuals has shown comparable percentages of germ tube formation (Kumar et al., 1982). The relationship between salivary glucose concentration and *in vivo* oral candidal colonisation in diabetic patients has not been studied, as yet, and is an area worthy of investigation.

The oral microbial flora is proposed as a factor influencing candidal colonisation. Mixed aerobic microflora has been shown to inhibit oral candidal colonisation in gnotobiotic mice (Liljemark & Gibbons, 1973), and anaerobic oral flora to inhibit candidal growth (Kennedy, 1981). On the other hand, components of the oral flora like *E. coli* and *K. aerogenes* have promoted oral candidal colonisation (Makrides & MacFarlane, 1982). A prospective comprehensive study investigating the possible differences in oral microflora in diabetic patients, in relation to possibly enhanced candidal colonisation seems worthwhile.

Whether the salivary antimicrobial factors are altered in patients with diabetes mellitus is debatable (Tenovuo et al., 1986). Some investigators have reported reduced salivary lysozyme activity in diabetic patients (Velikov et at., 1985) and in experimental animals rendered diabetic by alloxan treatment (Muratsu & Morioka, 1985). Recent research has indicated that lysozyme *in vitro* has potent anticandidal activity (Tobgi et al., 1988), therefore, the increased oral colonisation with *Candida* species observed in patients with diabetes mellitus could be, in part, due to concomitant reduction in salivary lysozyme.

Several aspects of neutrophil function has been reported as being impaired in patients with diabetes mellitus (Wilson, 1986).

Intracellular candidacidal capacity of neutrophils from diabetic patients has been shown to be particularly impaired (Wilson & Reeves, 1986; Wilson et al., 1987) and this may further predispose to candidal infection.

It is of interest in the present study to note that all the diabetic patients with oral candidosis were denture wearers who wore their dentures continuously. Although the control individuals were closely matched for denture status and wearing patterns, none of the latter group had clinical oral candidosis. This tends to emphasise the role of an interaction between diabetes mellitus and local factors such as the presence of dentures, particularly when worn continuously, in promoting candidal colonisation of the mouth. However, the increased rate and quantity of candidal isolation among denture wearers, particularly those who wear dentures continuously, is consistent with that reported for denture wearers generally (Arendorf & Walker, 1979).

It is of importance to note that the control subjects of this study were recruited from individuals attending a dental hospital for treatment of non-mucosal problems and for denture assessment. This method of patient selection may not be ideal, but due to the difficulty of obtaining the required number of healthy control subjects this method was felt appropriate for the purpose of this study.

In the present study, The lack of cases of denture stomatitis among the control subjects who were denture wearers could be attributed, in part, to the fact that those subjects were recruited from a prosthodontic clinic. Such subjects may be more careful about

their denture hygiene than other denture wearers who were receiving the attention from a dentist. On the other hand, the diagnosis of denture stomatitis in about 20 per cent of the denture wearers of the diabetic subjects is relatively comparable to that reported in general population of denture wearers (Nyquist, 1952).

In the present study, more diabetic patients had a low blood ferritin concentration than control subjects. Although a relationship was suggested between the latter parameter and oral candidosis (Fletcher et al., 1975), the higher rate of candidal isolation and infection observed among diabetic patients was felt unlikely to be due to the direct effect of reduced ferritin, as no correlation was noted between haematological parameters and oral candidal status. This observation is consistent with the findings of other workers among non-diabetic individuals (Jenkins et al., 1977).

Interestingly, the concentration of blood ferritin was significantly higher in the control group when compared with that in the diabetic group generally. The precise reason(s) for this observation is not apparent at present, but could be of clinical interest. In that regard, the control subjects who harboured Candida intraorally have shown significantly lower blood ferritin concentration (though not below the lower limit of normal) than those who were Candida free in the same group. However, the relationship between blood ferritin level and oral candidal colonisation needs further clarification.

Although it is widely reported that poor glycaemic control predisposes to infections generally in diabetic patients (Rayfield et al., 1982), the relationship between oral candidal carriage, candidal

infection and glycaemic control is controversial. In order to investigate the association between glycaemic control and oral candidal colonisation in the diabetic patients of this study, random blood glucose (which reflects the degree of glycaemic control at one point in time) and glycosylated haemoglobin concentration (which reflects the degree of glycaemic control during the preceding two months) were compared between diabetics with and without positive candidal isolation. The degree of glycaemic control as assessed by either blood glucose or glycosylated haemoglobin was unrelated to rate or quantity of candidal isolation a finding which is in accordance with previous reports (Barlow & Chattaway, 1969; Tapper-Jones et al., 1981; Bartholomew et al., 1987; Fisher et al., 1987; Lamey et al., 1988). Therefore, the present results do not confirm other reports that patients with poorly controlled diabetes have a higher candidal isolation rate than those in whom the disease is well controlled (Odds et al., 1978; Hill et al., 1989).

In the study of Odds et al. (1978), glycaemic control was assessed by a scoring system including random blood glucose and the degree of fasting glucosuria. Both parameters are poor indices of glycaemic control since blood glucose concentration fluctuates from one day to another and levels of glucosuria is subjected to individual variations in renal threshold for glucose (Baird, 1987). In addition, the patients with poor glycaemic control included hospitalised in-patients who may have received antibiotics or other medications that may have enhanced candidal colonisation. However, the present investigation included only four patients with poor glycaemic control with the majority of patients having good control. Further studies

with more equal distribution of patients in different categories of diabetic control, are warranted for further investigation and statistical analysis.

Salivary analysis for ABO(H) secretor status determination has shown that 38 per cent of the non-diabetic subjects were non-secretors. This proportion is somewhat higher than the 29.4 per cent reported for other areas in Scotland (Lincoln & Dodd, 1972). The relatively small number of subjects investigated in the present study in addition to the strict definition of secretors may partly explain this discrepancy. However, this does not exclude the possibility of regional variations in the inherited ability to secrete blood group substances. For instance, Lincoln and Dodd (1972) noticed significant differences in the proportion of non-secretors between different regions in the British Isles and found the Scottish population to be among the highest in relation to non-secretor status. However, the present proportion of non-secretors is not far from the 36 per cent and 37 per cent of non-secretors reported by Macafee (1964) and Denborough, Downing and McCrea (1967) respectively.

The lack of a difference in the proportion of secretors between the diabetic patients and non-diabetic individuals observed in the present study supports the previous report of Doll, Drane and Newell (1961) and Macafee (1964) who reported that the occurrence of diabetes mellitus was independent of secretor status.

Whether patients with insulin-dependent diabetes mellitus have a higher proportion of non-secretors than patients with non-insulin-dependent diabetes (Blackwell et al., 1987) or not (Peters & Gohler, 1986) is unclear. However, the present results could not detect a difference in the proportion of non-secretors when patients

with either type of diabetes were compared with each other or when compared with the non-diabetic subjects.

The genetically determined inability to secrete the blood group specific antigens into body fluids has been described in association with several systemic infections (Blackwell, 1989). Recently Burford-Mason et al. (1988) reported an increased proportion of non-secretors among healthy subjects who were Candida carriers. In the present study, the observed significantly increased rate of positive candidal isolation among non-secretors than among secretors, in patients with diabetes mellitus, tends to support and extend these findings. A similar trend was observed among the non-diabetic individuals of the study although the difference in candidal carriage rate between secretors and non-secretors was not statistically significant.

The lack of a significant relationship between secretor status and presence of oral candidosis among the diabetic patients is consistent with that reported by Lamey et al. (1988) but contrary to the increased proportion of non-secretors among non-diabetic patients with oral candidal infections reported by Thom et al. (1989). Others too, have been unable to establish a relationship between secretor status and infections such as gonorrhoea (Kinane et al., 1983) and periodontal disease (Pradhan et al., 1971). However, the present study comprised a limited number of diabetic patients with clinical candidal infection, so a larger scale investigation may be required to investigate such an association between secretor status and candidal infection in these patients.

It has been theorised that, the blood group substances in secretions may possess the ability to occupy or interfere with binding sites present either on the surface of microorganism or on the epithelial cell surface, and retard microbial colonisation of mucosal surfaces (Blackwell, 1989). Hence the relationship between the ability to secrete these substances in saliva and candidal adhesion to oral epithelial cells warranted further investigation. In addition, salivary IgA, which has been shown to inhibit candidal colonisation to mucosal surfaces (Epstein et al., 1982), may be secreted in lower concentration in mixed saliva from non-secretors compared to secretors (Waissbluth & Langman, 1971).

In conclusion, candidal isolation rate and oral candidosis were found to be more common in patients with diabetes mellitus than in healthy individuals for reasons as yet unclear. Furthermore, all the investigations undertaken on these aspects have been so far cross-sectional in nature thus yielding limited information. The question to why a diabetic patient becomes a carrier of Candida species or develops oral candidosis while another may not is still without a convincing answer. Differences in virulence factors between different Candida species and strains may be a part of the answer while the other may be the intrinsic differences in susceptibility among individuals. Carefully designed longitudinal studies, monitoring oral candidal status in diabetic patients in different states of diabetic control and over a prolonged period of time, are required to answer these questions.

In diabetic patients, the lower proportion of secretors with positive candidal isolation suggests that the ability to secrete the ABO antigens may play a role in the innate defences of the host

against these opportunistic organisms. Further work in the area should include investigating other immunosuppressed patients such as those receiving chemotherapy or radiotherapy to determine if secretion of the blood group antigens plays a role in prevention of candidal infection. Conceivably, patients with HIV infection who develop oral candidosis earlier in the course of their disease could be those who are non-secretors.

If we accept that Candida must colonise the host surface to be able to produce its pathogenic effect (Howlett & Squier, 1980), then studies on candidal adhesion to epithelial cells from diabetic patients may be helpful to clarify differences in susceptibility to candidal carriage or infection between diabetics and non-diabetics. If, in future, this type of study has proved useful, more meticulous investigations may be designed to study interindividual variations in susceptibility among diabetic patients with different states of diabetic control.

This study and others (Lamey et al., 1988) have shown that diabetic patients with poor glycaemic control may not be more predisposed to oral candidosis than those with good control. Moreover neither type or duration of diabetes appeared to affect this predisposition. Therefore, since candidal adhesion to epithelial cells was a prerequisite for colonisation (Kimura & Pearsall, 1978) and predisposition to infection (Kennedy, 1988), additional studies were undertaken to compare candidal adhesion to epithelial cells from diabetic patients and control subjects.

CHAPTER FOUR

ADHESION OF *CANDIDA ALBICANS* TO BUCCAL EPITHELIAL CELLS OF DIABETIC PATIENTS AND NON-DIABETIC INDIVIDUALS

4.1 INTRODUCTION AND AIMS OF THE STUDY

The adhesion of microorganisms to host mucosal surfaces is a necessary prerequesite for successful microbial colonisation and infection (Gibbons & van Houte, 1975), and the role of adhesion in the pathogenesis of many fungal infections is widely appreciated (Kennedy, 1988). Various animal studies have provided evidence for a relationship between the ability of *Candida* species to adhere to a host surface and their propensity for initiating infection (McCourtie & Douglas, 1984; Calderone et al., 1985).

The predisposition of diabetic patients to cutaneous (Kandhari et al., 1969) and vaginal candidosis (Sonck & Somersalo, 1963) as well as to oral candidosis (Lamey et al., 1988) has been recognised but the reasons, as yet, not fully identified. However, as has been shown in Chapter Three, factors indicative of glycaemic control in diabetic patients, such as blood glucose concentration, glycosylated haemoglobin, and type and duration of diabetes seemed to bear no relation to the higher predisposition of diabetic patients to oral candidal infection. However, one study has shown female patients with diabetes mellitus to have higher rates of adhesion of *C. albicans* to their vaginal epithelial cells *in vitro* (Segal et al., 1984) which may explain, in part, the increased predisposition of these patients to vaginal candidosis.

In view of the reported correlation between candidal adhesion *in vitro* and the ability to initiate candidal infection *in vivo* (McCourtie & Douglas, 1984; Ray et al., 1984; Ghannoum & Abu Elteen, 1986), the present study aimed to compare the adhesion of *C. albicans* strain CDS 88 to buccal epithelial cells (BEC) from patients with diabetes mellitus and a group of age, sex and denture status matched non-diabetic control subjects in an attempt to explore whether a relationship exists between the higher predisposition of such patients to oral candidal infection and increased candidal adhesion *in vitro*. The opportunity was also taken to investigate the effect of parameters such as oral candidal colonisation, secretor status, blood glucose level and haematological status, as well as quality of diabetic control as factors which might influence candidal adhesion to oral epithelial cells.

4.2 MATERIALS AND METHODS

4.2.1 Patients Selection

The subjects of this study (the same groups investigated in Chapter Three) comprised 50 randomly selected outpatients with diagnosed diabetes mellitus and 50 age, sex and denture status matched non-diabetic control subjects. Diabetic patients were recruited from the Diabetic Clinic at Glasgow Royal Infirmary while control subjects were recruited from either the Oral Medicine Unit at their first attendence to a Migraine Clinic or from patients routinely attending the Department of Prosthodontics, Glasgow Dental Hospital for denture assessment. All participants gave informed consent to participate in the study which was approved in full by the local Ethics Committee.

4.2.2 Clinical History and Oral Examination

The clinical history of each participant was recorded on a special proforma (see Figure 3.1). The type of diabetes mellitus, diabetic treatment and duration of the disease were recorded for the diabetic patients. For each participant, the possession of a dental prosthesis and whether the prosthesis was worn continuously was also recorded.

The medical and drug history (other than that used for glycaemic control) was matched in both groups as closely as possible. No individual in either group was, or had in the previous three months, received antibiotic or steroid therapy or used an antiseptic mouth wash and no female subject was known to be a pregnant. Tobacco smoking and alcohol consumption were also matched as closely as possible between subjects of both groups.

Each participant underwent a routine oral clinical examination and the presence of oral candidosis was recorded. Subjects with chronic atrophic candidosis had their disease extent described according to the classification of Newton (Newton, 1962).

4.2.3 Routine Blood Investigations

These investigations were described in Section 3.2.3. Briefly, a venous blood sample was obtained from the subjects for estimation of corrected whole blood folate, ferritin, haemoglobin (Hb), vitamin B12 and (biochemically) random blood glucose concentration. Glycosylated haemoglobin (HbA1) was also estimated for the diabetic patients.

4.2.4 Microbiological Investigations

The microbiological investigations were described in detail in Section 3.2.4. Briefly, The concentrated oral rinse technique described by Samaranayake et al. (1986b) was used for oral yeast isolation. Swabs and smears were obtained from areas of clinically infected mucosa and from the angles of the mouth if appropriate. The swabs and oral rinses were inoculated onto Sabouraud's dextrose agar (Gibco, Paisley, Scotland) and incubated aerobically at 37°C for 48 hours. The smears were taken using plastic instruments and samples applied to glass microscope slides and stained by Gram technique.

4.2.5 Yeast Identification and Quantification

C. albicans and other Candida species were identified by serum germ tube formation technique (MacKenzie, 1962) and using the identification system API 20 C AUX (API Products Ltd., Basingstoke, Hampshire, England). Details of yeast identification and quantification were given in Sections 3.2.5 and 3.2.6 respectively.

4.2.6 Secretor Status Determination

Secretor status was determined by agglutination inhibition technique described by Periera et al. (1969). Details of saliva collection, the agglutination inhibition technique itself and defining the secretor status were described in Sections 3.2.7 to 3.2.9.

4.2.7 Collection of Buccal Epithelial Cells

Buccal epithelial cells (BEC) for the adhesion assay were obtained from each subject by gently scraping the right and left buccal mucosa separately with a sterile cotton swab (Exogen Ltd.,

Clydebank, Scotland). BEC from each subject were pooled by agitating the swabs in 10ml PBS (0.1M pH 7.2) in a universal container (Sterilin Ltd., Feltham, England) which was kept on crushed ice till the time of processing. PBS of the same osmolarity and pH was used throughout the study. All clinical examinations and samplings took place between 1400 and 1700 hours.

4.2.8 Preparation of Buccal Epithelial Cell Suspension

BEC were washed twice in 10ml PBS by centrifugation at 200g for ten minutes (MSE Centaur 2 Centrifuge. Fisson's Ltd., Crawley, England) to remove loosely attached microorganisms and debris. The cells were then suspended in PBS to give a final BEC concentration of 10^5 per ml determined by haemocytometer counting (Hawksley and Sons Ltd., Lancing, England).

4.2.9 Source, Identification and Maintenance of *Candida Albicans* Strain CDS 88

C. albicans strain CDS 88 used throughout this study was originally isolated from the oral cavity of an asymptomatic carrier in Glasgow Dental Hospital. The species was identified by serum germ tube formation technique (MacKenzie, 1962) and using the yeast identification system API 20 C AUX and subsequently freeze-dried. The strain was maintained on slopes of Sabouraud's dextrose agar at $4^{\circ}C$ and the culture was replaced monthly by a new one freshly grown from freeze-dried stock. The strain used has never been subcultured throughout the study.

4.2.10 Preparation of Candida Albicans Suspension

To prepare the candidal cells for the adhesion assay, a loopful of the stock culture was inoculated into 10ml of Sabouraud's broth (Gibco, Paisley, Scotland) containing 500mM sucrose in a universal container, and incubated overnight (18-24 hours) at 37°C in an orbital shaker incubator (Gallenkamp, Loughborough, England) operating at 100 revolutions per minute. A growth medium containing 500mM sucrose was known to enhance candidal adhesion (McCourtie & Douglas, 1984) and C. albicans in the stationary phase of growth adhere to a higher degree than when in the logarithmic growth phase (King et al., 1980). The culture was then harvested by centrifugation at 400g for five minutes and the deposit washed twice in aliquots of 10ml PBS by centrifugation at 400g for five minutes. A final yeast cells suspension of 10^7 per ml was prepared by appropriate dilution of the deposit in PBS followed by haemocytometer counting.

4.2.11 The Candidal Adhesion Assay

The in vitro adhesion of the C. albicans strain CDS 88 to BEC from each diabetic patient and control subject was studied as described by Kimura and Pearsall (1978) with minor modifications.

For the adhesion assay, equal volumes (0.5ml) of BEC suspension and C. albicans suspension were mixed in a sterile bijou container (Sterilin Ltd., Feltham, England) and incubated on an orbital shaker operating at 80 revolutions per minute at 37°C for one hour. In each experiment, a control container was included that contained 0.5ml BEC suspension and 0.5ml PBS to determine the background count of possible indigenous yeasts. Following incubation, the BEC were harvested on a polycarbonate filter of 25mm diameter and

12μm pore-size (Nuclepore Gmbh, Germany) mounted on a DEFT (direct epifluorescent filter technique) manifold (Micromeasurements Ltd., Saffron Waden, Essex, England) shown in Figure 4.1. A negative pressure of 5mm Hg was applied to the manifold, and the filter with retained BEC were washed gently with 30ml of PBS in aliquots of 5ml to remove unattached yeast cells. The pore size of 12μm was small enough to retain the BEC without gross distortion but large enough to allow unattached yeast cells to be washed through the filter unit, leaving only the BEC with adherent yeast on the filter. The filter with washed BEC was then removed from the filter unit, mounted on a 1.0mm thick, 26 X 76mm glass microscope slide (Chance Proper Ltd., Smethwick, Warley, England) and left for one hour to air-dry then Gram-stained. The filter and the stained BEC, on the glass slide, were left overnight to air-dry then a 22 X 22mm glass cover slip (Chance Proper Ltd., Smethwick, Warley, England) was fixed on the filter with Harleco resin (Kodak Chemicals, England).

The DEFT manifold (Figure 4.1) consists of a stainless steel housing on which up to five filters can be mounted in filter units consisting of a sintered glass base beneath a filter tower, between which the filters are sandwiched. The weight of the filter tower forms a seal against the filter and the negative pressure is applied to the manifold from a Venturi pump drawing the suspension in the filter tower through the filter. The BEC are thus deposited evenly over the exposed area of the filter, determined by the internal diameter of the filter tower.

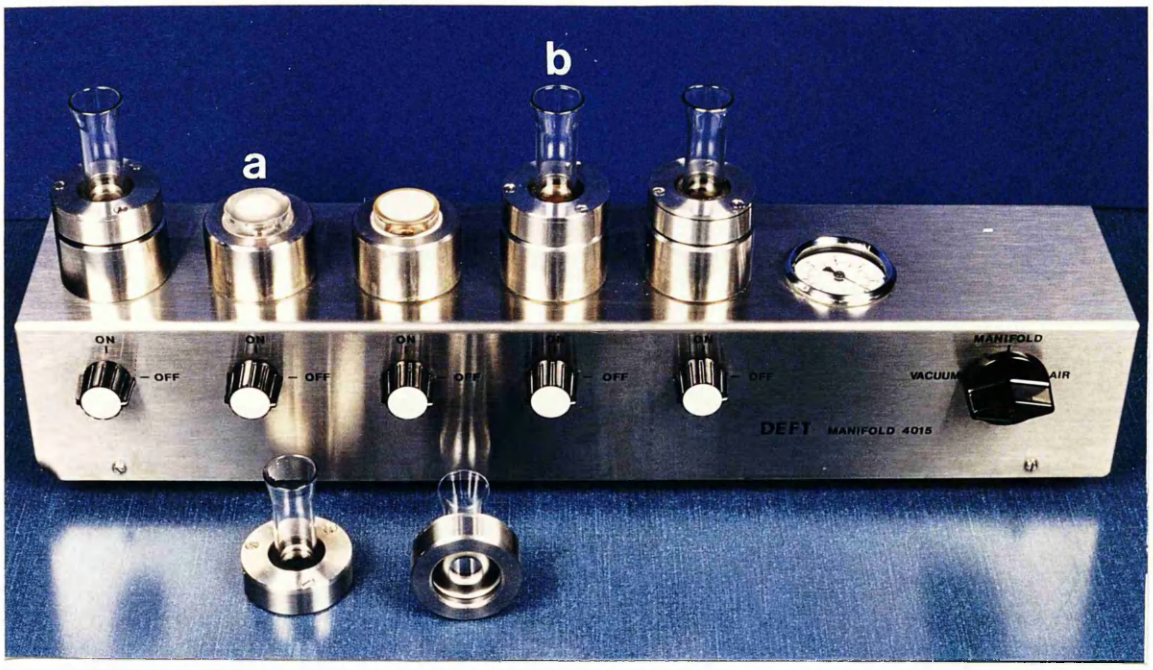

Figure 4.1 DEFT manifold filter unit showing (a) the sintered glass base and (b) the filter tower.

4.2.12 Counting of Adherent Yeast Cells

The glass slides holding the filters were coded and read "blindly". The number of C. albicans blastospores attached to one hundred individual BEC were counted under a x40 objective. To ensure consistent counting of fields, on each occasion the count was started at the top left hand side of the filter and consecutive fields counted till 100 BEC were obtained. Only morphologically normal epithelial cells with a round entire edge and cells that were flat, but not folded, were counted in the field. Clumps of BEC were excluded and pseudohyphae, if present, were not counted. To obtain the correct final count of the adherent yeast cells the background count of the control assay, if present, was subtracted from the total count.

4.2.13 Statistical Analysis

Mean candidal adhesion (MCA) together with the standard deviation of the mean (SD) to BEC from the groups was compared by Student's t test for unpaired data. Pearson's correlation test was used to correlate MCA to various parameters. Probabilities of less than five per cent were taken to be statistically significant.

4.3 RESULTS

4.3.1 Subjects of the Study

The diabetic patients and control subjects were closely matched for age, sex, denture status and denture wearing patterns (Table 4.1).

	Diabetics (n= 50)	Controls (n= 50)
Male	27 (54%)	23 (46%)
Female	23 (46%)	27 (54%)
Mean age (years)	53.7 SD 14.9	56.3 SD 17.3
Age range (years)	17 - 81	20 - 84
Denture wearers	36 (72%)	36 (72%)
Dentate	14 (28%)	14 (28%)
Smokers	16 (32%)	14 (28%)
Alcohol drinkers	7 (14%)	2 (4%)

Table 4.1 Clinical status of the study subjects.

Study no.		Total yeast counted Yeast / 100 BEC		Background count Yeast / 100 BEC		Adherent *Candida* Yeast / 100 BEC	
1	26	406	308	0	0	406	308
2	27	210	235	0	0	210	235
3	28	806	310	0	8	806	302
4	29	144	368	0	0	144	368
5	30	400	350	0	2	400	348
6	31	443	352	0	0	443	352
7	32	857	431	0	0	857	431
8	33	549	491	4	0	545	491
9	34	645	519	0	0	645	519
10	35	637	418	0	1	637	417
11	36	367	305	0	0	367	305
12	37	566	271	0	0	566	271
13	38	535	423	0	0	535	423
14	39	330	376	0	0	330	376
15	40	530	333	0	0	530	333
16	41	1053	234	28	0	1025	234
17	42	691	284	2	0	689	284
18	43	469	256	1	0	468	256
19	44	472	256	1	0	471	256
20	45	262	183	0	0	262	183
21	46	261	250	0	0	261	250
22	47	229	169	0	0	229	169
23	48	480	156	0	0	480	156
24	49	257	287	10	0	247	287
25	50	560	461	0	0	560	461

Table 4.2 Raw data for candidal adhesion to BEC from diabetic patients.

Study no.		Total yeast counted Yeast / 100 BEC		Background count Yeast / 100 BEC		Adherent *Candida* Yeast / 100 BEC	
1	26	253	375	0	37	253	338
2	27	228	503	0	0	228	503
3	28	421	359	0	0	421	359
4	29	317	380	0	0	317	380
5	30	428	330	0	0	428	330
6	31	211	395	0	0	211	395
7	32	417	339	0	0	417	339
8	33	295	267	0	0	295	267
9	34	369	213	0	0	369	213
10	35	484	440	11	0	473	440
11	36	231	483	0	0	231	483
12	37	190	408	0	0	190	408
13	38	153	245	0	0	153	245
14	39	173	131	0	0	173	131
15	40	220	107	0	0	220	107
16	41	125	78	0	0	125	78
17	42	200	43	0	0	200	43
18	43	220	77	0	0	220	77
19	44	371	61	0	0	371	61
20	45	393	156	0	0	393	156
21	46	244	157	0	0	244	157
22	47	282	201	0	0	282	201
23	48	134	212	0	0	134	212
24	49	149	218	0	0	149	218
25	50	143	106	0	0	143	106

Table 4.3 Raw data for candidal adhesion to BEC from control subjects.

4.3.2 Adhesion of *Candida Albicans* to Buccal Epithelial Cells from Diabetic Patients and Non-diabetic Control Subjects

The number of *C. albicans* blastospores of the strain used (CDS 88) adherent to 100 BEC of 50 diabetic patients and 50 control subjects was counted. Then the mean candidal adhesion (MCA) to a single BEC together, with the standard deviation from the mean (SD) for each group, was calculated. The value derived was 4.02 (SD 1.85) yeast / BEC for the diabetic patients and 2.6 (SD 1.2) yeast / BEC for the control subjects (Figure 4.2). Statistical analysis showed that MCA in the diabetic patients was significantly higher than in control subjects ($p<0.001$). As shown in Figure 4.3, there was increased frequency of candidal adhesion values over 300 yeast / 100 BEC in diabetic patients compared to the control subjects.

The study also attempted to determine whether a qualitative difference existed in affinity of BEC to the *C. albicans* strain used between diabetic patients and control subjects. The number of BEC with one or more adherent *C. albicans* blastospores (*Candida* +ve cells) and the number of BEC with no adherent blastospores (*Candida* -ve cells) (Figure 4.4) was counted, and the mean number of *Candida* +ve and *Candida* -ve cells per 100 BEC from each group then calculated.

The affinity of BEC from diabetic patients to the *C. albicans* strain used was higher than that of the control subjects. The mean number of *Candida* +ve cells in diabetic patients (63.2 SD 11.2) was significantly higher ($p<0.001$) than in control subjects (54.4 SD 13.8). The mean number of *Candida* -ve cells in diabetic patients (37 SD 11.2) was significantly decreased ($p<0.001$) compared to control subjects (45.7 SD 13.7).

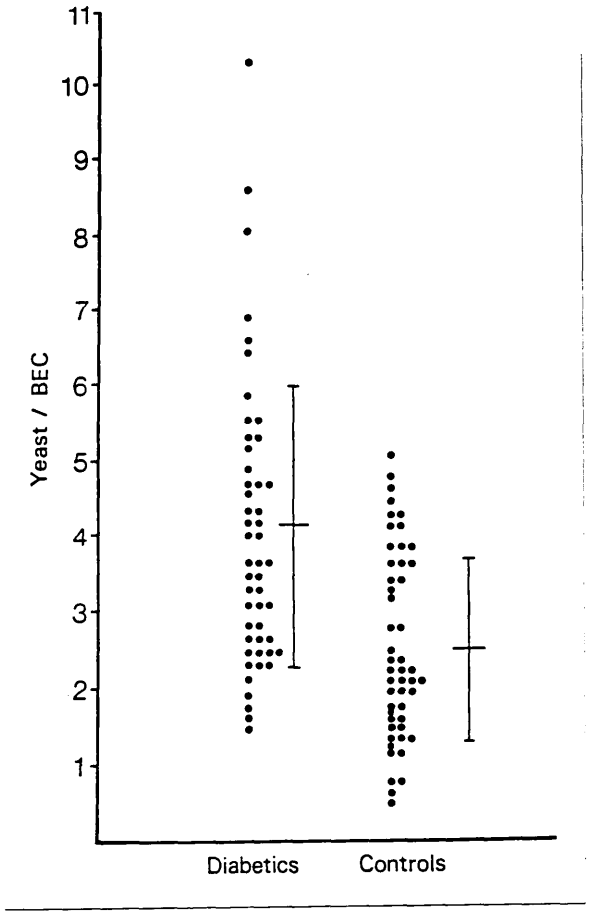

Figure 4.2 Mean candidal adhesion to buccal epithelial cells from the diabetic patients and the control subjects. The vertical bars present the standard deviation of the mean.

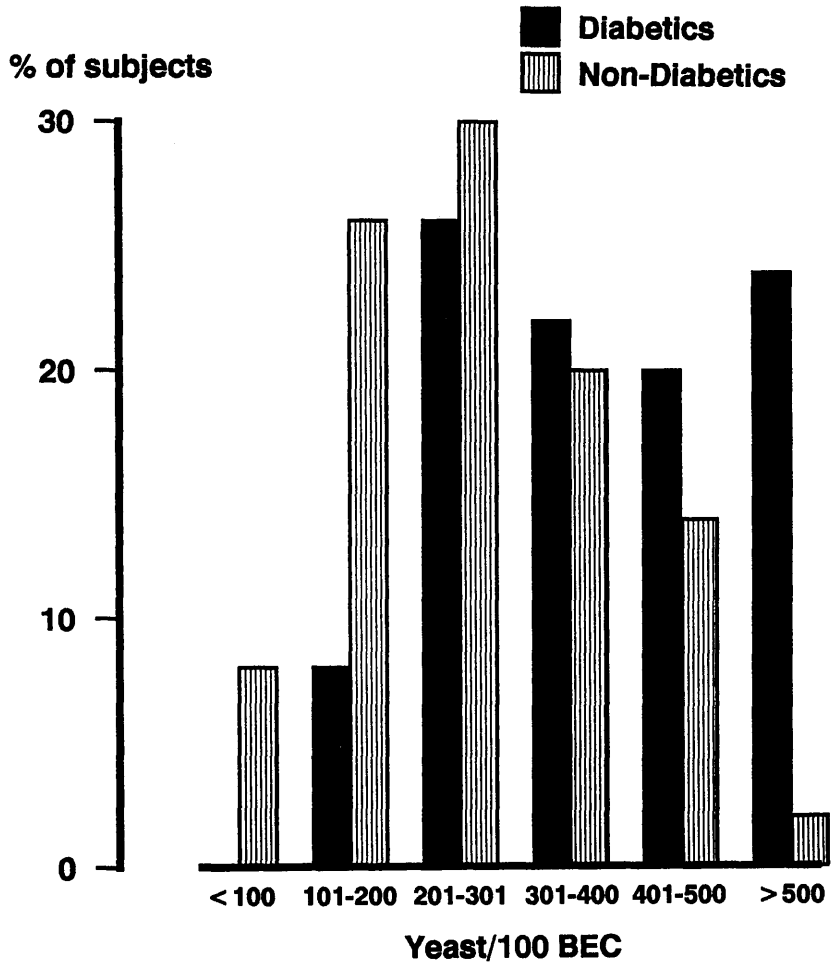

Figure 4.3 Relative frequency of subjects with different candidal adhesion values to 100 buccal epithelial cells.

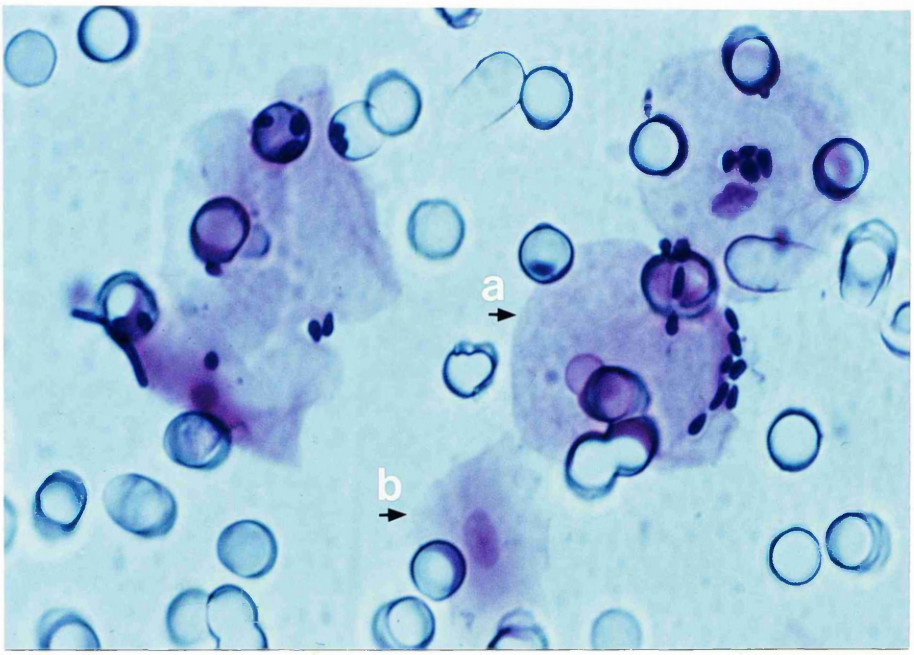

Figure 4.4 Candida albicans blastospores adhering to buccal epithelial cells. (a) Candida +ve cell. (b) Candida -ve cell.

As shown in Tables 4.2 and 4.3, there was considerable interindividual variations in candidal adhesion to BEC among subjects of both groups. In the diabetic patients, candidal adhesion ranged from 144 to 1025 yeast / 100 BEC. The range in the control group was 43 to 503 yeast / 100 BEC.

4.3.3 Relationship of Candidal Adhesion to Age

The diabetic patients and control subjects were divided into two subgroups according to age (Table 4.4). The diabetic patients who were below 60 years of age had significantly increased candidal adhesion than those aged 60 years or over ($p= 0.004$).

In the diabetic patients, a significant inverse correlation between MCA and age was detected ($r= -0.3$; $p<0.05$). However, this was not significant in control subjects ($r= -0.04$; $p>0.05$) as shown in Tables 4.5 and 4.6 respectively.

Group	Age group (Years)	No. (%)	Mean candidal adhesion Yeast / BEC (SD)
Diabetics	< 60	33 (66)	4.6 (1.9)
	> 60	17 (34)	3.1 (1.4)
Controls	< 60	22 (44)	2.8 (1.4)
	> 60	28 (56)	2.4 (1.1)

Table 4.4 Mean candidal adhesion in different age groups.

Case no.	Age (years)	Duration of diabetes (years)	Blood glucose (mmol/l)	HbA1 (%)	Hb (g/dl)	Ferritin (ng/ml)	Folate (ng/ml)	Vit. B12 (pg/ml)	Candidal isolation (cfu/ml)	MCA (Yeast/BEC)
1	59	0.3	11.1	9.4	15.2	14	263	243	NG	4.06
2	53	4.0	10.8	9.5	15.2	7	210	326	NG	2.10
3	56	10.0	7.0	8.5	NDA	NDA	NDA	NDA	NG	8.06
4	66	0.5	7.0	8.6	15.5	14	200	217	NG	1.44
5	21	NDA	6.3	11.5	14.5	17	358	306	800	4.00
6	47	1.0	17.8	10.3	17.8	20	321	431	NG	4.43
7	52	1.0	20.8	11.2	14.9	7	365	394	NG	8.57
8	54	1.0	9.0	12.0	12.3	24	168	294	1460	5.45
9	17	5.0	4.5	9.4	14.3	18	411	530	NG	6.45
10	59	2.5	18.1	12.1	NDA	NDA	NDA	NDA	2200	6.37
11	67	1.0	5.7	7.3	14.3	10	227	437	2700	3.67
12	58	12.0	10.7	8.6	15.4	12	232	234	140	5.66
13	49	4.0	12.4	9.6	14.7	7	776	1022	NG	5.35
14	65	3.0	16.1	11.1	15.1	29	269	361	6880	3.30
15	53	3.0	9.4	8.5	NDA	NDA	NDA	NDA	NG	5.30
16	44	3.0	17.6	12.5	18.1	18	213	329	5280	10.25
17	71	1.0	9.9	8.7	NDA	NDA	NDA	NDA	500	6.89
18	64	3.0	7.4	7.1	14.4	20	717	354	240	4.68
19	52	7.0	8.9	8.4	11.7	9	264	419	1000	4.71
20	46	10.0	13.5	10.5	NDA	NDA	NDA	NDA	40	2.62
21	68	7.0	15.3	11.6	15.1	25	236	418	1140	2.61
22	78	6.0	7.9	NDA	NDA	NDA	NDA	NDA	1300	2.29
23	63	0.5	8.6	9.0	14.5	11	228	640	64842	4.80
24	44	5.0	9.9	9.8	13.2	12	241	322	93345	2.47
25	25	13.0	5.7	7.2	12.0	19	416	721	NG	5.60
26	33	2.0	8.7	7.0	14.0	9	779	455	NG	3.08
27	64	3.0	16.0	8.7	14.0	14	358	601	180	2.35
28	73	10.0	12.1	9.4	14.0	7	345	520	80	3.02
29	59	7.0	15.0	13.2	16.4	13	374	526	NG	3.68
30	26	6.0	10.4	7.9	14.2	9	280	405	100	3.48
31	36	14.0	4.9	9.1	15.3	15	299	463	100	3.52

Case no.	Age (years)	Duration of diabetes (years)	Blood glucose (mmol/l)	HbA1 (%)	Hb (g/dl)	Ferritin (ng/ml)	Folate (ng/ml)	Vit. B12 (pg/ml)	Candidal isolation (cfu/ml)	MCA (Yeast/BEC)
32	40	3.0	16.0	10.9	13.2	6	336	652	NG	4.31
33	37	7.0	16.4	12.3	13.7	11	210	452	140	4.91
34	48	3.0	11.9	10.1	14.6	3	334	433	NG	5.19
35	60	0.6	4.3	7.2	15.5	16	281	768	500	4.17
36	50	16.0	7.1	10.1	12.7	7	169	406	NG	3.05
37	65	6.0	8.4	8.6	13.4	11	320	253	NG	2.71
38	46	15.0	14.7	11.8	17.2	15	155	350	9120	4.23
39	57	3.0	5.1	9.8	11.4	15	354	301	NG	3.76
40	57	5.0	11.4	10.2	14.5	15	251	286	NG	3.33
41	40	25.0	6.8	8.0	NDA	NDA	NDA	NDA	3300	2.34
42	53	20.0	10.8	10.2	13.4	12	298	562	3300	2.84
43	52	0.5	6.8	7.5	17.2	10	352	432	NG	2.56
44	67	32.0	15.5	11.0	14.2	13	301	613	NG	2.56
45	81	4.0	11.5	11.7	13.7	3	269	179	3000	1.83
46	63	10.0	11.4	9.8	13.9	12	373	640	17120	2.50
47	63	2.0	8.0	8.3	13.1	12	262	348	160	1.69
48	46	4.0	5.5	7.8	NDA	NDA	NDA	NDA	NG	1.56
49	80	3.0	10.1	8.5	15.0	18	460	268	NG	2.87
50	23	5.0	7.0	8.9	12.6	27	459	288	NG	4.61

NDA: No data availab; NG: No growth.

Table 4.5 Relationship of mean candidal adhesion to oral candidal isolation and clinical and haematological status of the diabetic patients.

Case no.	Age (years)	Blood glucose (mmol/l)	Hb (g/dl)	Ferritin (ng/ml)	Folate (ng/ml)	Vit. B12 (pg/ml)	Candidal isolation (cfu/ml)	MCA (Yeast/BEC)
1	84	4.7	13.4	162	158	293	NG	2.53
2	55	5.7	15.3	90	139	457	220	2.28
3	54	3.7	13.8	73	162	348	NG	4.21
4	53	4.8	14.5	50	185	538	NG	3.17
5	48	3.9	12.9	21	247	495	340	4.28
6	66	2.1	14.9	49	201	413	136	2.11
7	70	2.6	13.2	29	180	263	32118	4.17
8	74	4.1	14.3	25	129	468	40	2.95
9	72	3.5	15.3	134	205	391	NG	3.69
10	52	4.0	11.5	6	204	399	1280	4.73
11	64	5.6	14.9	30	239	468	NG	2.31
12	73	5.4	13.2	97	205	307	NG	1.90
13	62	5.3	14.6	32	155	249	120	1.53
14	67	4.4	15.1	585	128	357	120	1.73
15	67	4.2	13.4	57	112	416	62418	2.20
16	69	5.6	13.9	96	162	126	NG	1.25
17	64	5.1	12.9	181	225	421	280	2.00
18	32	5.7	14.6	41	215	306	NG	2.20
19	38	4.6	13.6	51	147	343	40	3.71
20	30	4.5	14.9	121	186	150	NG	3.93
21	64	4.3	13.9	39	277	411	NG	2.44
22	73	4.9	11.4	4	159	126	18080	2.82
23	75	5.9	13.9	71	138	202	NG	1.34
24	66	5.0	17.9	432	181	334	NG	1.49
25	68	4.8	16.0	89	171	330	NG	1.43
26	78	6.1	14.7	264	181	199	920	3.38
27	63	4.3	14.1	191	196	189	NG	5.03
28	49	4.4	14.0	25	215	476	20	3.59
29	61	4.9	12.5	8	359	783	120	3.80
30	77	5.7	14.1	143	215	159	NG	3.30
31	65	6.4	13.7	69	NDA	NDA	NG	3.95

Case no.	Age (years)	Blood glucose (mmol/l)	Hb (g/dl)	Ferritin (ng/ml)	Folate (ng/ml)	Vit. B12 (pg/ml)	Candidal isolation (cfu/ml)	MCA (Yeast/BEC)
32	70	4.3	12.1	NDA	NDA	NDA	NG	3.39
33	74	4.5	NDA	NDA	NDA	NDA	NG	2.67
34	79	4.7	11.4	11	315	571	NG	2.13
35	22	4.9	13.8	72	155	347	NG	4.40
36	32	5.4	14.7	131	206	366	NG	4.83
37	56	4.1	14.5	30	188	211	NG	4.08
38	31	4.5	12.2	29	204	466	11040	2.45
39	20	3.5	NDA	NDA	NDA	NDA	500	1.31
40	69	5.4	15.5	104	127	334	NG	1.07
41	57	4.7	16.0	100	112	226	40	0.78
42	38	4.8	NDA	NDA	NDA	NDA	2000	0.43
43	61	4.9	NDA	NDA	NDA	NDA	1800	0.77
44	70	5.3	13.5	51	133	234	NG	0.61
45	33	4.8	15.5	90	230	478	NG	1.56
46	35	4.7	15.7	74	272	350	NG	1.57
47	37	4.7	15.2	42	281	634	NG	2.01
48	32	4.9	14.9	37	220	429	NG	2.12
49	40	5.6	14.7	124	166	257	NG	2.18
50	30	5.3	14.0	84	154	473	NG	1.06

NDA: No data availabe; NG: No growth.

Table 4.6 Relationship of mean candidal adhesion to oral candidal isolation and clinical and haematological status of the control subjects.

4.3.4 Relationship of Candidal Adhesion to Gender

In the diabetic patients, MCA was not statistically different between males and females (Table 4.7), whereas in non-diabetic subjects MCA was significantly higher in females ($p<0.02$) compared to males (Table 4.8).

4.3.5 Relationship of Candidal Adhesion to Denture Status and Denture Wearing Patterns

Statistical analysis showed no significant difference in MCA between dentate and denture wearers in either diabetic patients (Table 4.7) or control subjects (Table 4.8). Nevertheless, continuous denture wearing (day and night) in the control subjects, but not in the diabetic patients, was associated with a significantly increased MCA compared to day denture wearing ($p<0.02$; Table 4.8). However, in the control subjects, 71.4% of those who wore dentures continuously were females.

4.3.6 Relationship of Candidal Adhesion to Tobacco Smoking

The subjects of the study were subdivided into smokers and non-smokers according to the subject's social history. Any subject who smoked daily was considered as a smoker. MCA was not significantly different between smokers and non-smokers either in the diabetic patients (Table 4.7) or control subjects (Table 4.8).

4.3.7 Relationship of Candidal Adhesion to Alcohol Consumption

The subjects of the study were subdivided into alcohol users and non-drinkers according to the subject's social history.

Group	No.	Mean candidal adhesion Yeast / BEC (SD)	p	
Male	27	4.2 (1.8)	0.5	NS
Female	23	3.8 (1.9)		
Dentate	14	4.7 (2.0)	0.1	NS
Denture	36	3.7 (1.7)		
D and N	13	3.5 (1.0)	0.5	NS
D	23	3.9 (2.0)		
Smokers	16	3.5 (1.3)	0.1	NS
Non-smokers	34	4.3 (2.0)		
Drinkers	7	3.8 (1.2)	0.6	NS
Non-drinkers	43	4.0 (1.9)		
Dry mouth	15	4.3 (2.1)	0.5	NS
Not dry	35	3.9 (1.7)		

D and N : Day and night denture wearing.
D : Day only denture wearing.
NS : Not significant.

Table 4.7 Mean candidal adhesion in subgroups of diabetic patients.

Group	No.	Mean candidal adhesion Yeast / BEC (SD)	p	
Male	23	2.1 (1.1)	0.01	S
Female	27	3.0 (1.2)		
Dentate	14	2.7 (1.5)	0.7	NS
Denture	36	2.5 (1.1)		
D and N	14	3.1 (1.1)	0.01	S
D	22	2.2 (1.0)		
Smokers	14	2.6 (1.3)	1.00	NS
Non-smokers	36	2.6 (1.2)		
Drinkers	2	2.4 (1.1)	0.9	NS
Non-drinkers	48	2.6 (1.2)		
Dry mouth	8	2.7 (1.4)	0.7	NS
Not dry	42	2.5 (1.2)		

D and N: Day and night denture wearing.
D : Day only denture wearing.
S : Significant.
NS : Not significant.

Table 4.8 Mean candidal adhesion in subgroups of control subjects.

Any subject who drank alcohol daily was considered as a drinker. MCA was not significantly different between alcohol drinkers and non-drinkers either in the diabetic patients (Table 4.7) or control subjects (Table 4.8).

4.3.8 Relationship of Candidal Adhesion to Symptomatic Xerostomia

Statistical analysis showed no significant difference in MCA between those who reported symptomatic mouth dryness and those who did not report this symptom either in the diabetic patients (Table 4.7) or control subjects (Table 4.8).

4.3.9 Relationship of Candidal Adhesion to Haematological Status

Relationship to Haemoglobin Concentration

In the diabetic patients, the correlation was not significant between MCA and blood haemoglobin level ($r= 0.2$; $p= 0.2$; Table 4.5). Three diabetic patients had low haemoglobin levels (<12g/dl for females and <12.5g/dl for males) with MCA 4.7 (SD 0.9) while 39 patients had normal haemoglobin levels with MCA 3.9 (SD 1.8). The difference between the two groups was not significant ($p= 0.3$). Haemoglobin estimation could not be determined in eight patients.

In the control subjects, a significant inverse correlation was detected between haemoglobin levels and MCA ($r= - 0.3$; $p= 0.03$; Table 4.6). Three control subjects had low haemoglobin levels with MCA 3.2 (SD 1.3) while 43 had normal blood haemoglobin levels with MCA 2.5 (SD 1.1). The difference in MCA between the two groups was not significant ($p= 0.5$). However, due to the small sample size, statistical analysis was inappropriate.

Relationship to Ferritin Concentration

No significant correlation was detected between MCA and ferritin concentration in either diabetic (r= 0.14) or control group subjects (r= -0.16) as shown in Tables 4.5 and 4.6 respectively.

In the diabetic patients, 16 patients were iron-deficient (ferritin <12ng/ml) with MCA 4.0 (SD 1.7) while 26 patients had normal blood ferritin concentrations with MCA 3.9 (SD 1.8). The difference in MCA between the two groups was not significant (p= 0.9). Ferritin concentration could not be determined in eight patients.

In the control group, four subjects were iron-deficient with MCA 3.4 (SD 1.1) while 42 subjects had normal blood iron concentrations with MCA 2.5 (SD 1.1). The difference in MCA between the two groups was not significant (p= 0.2). However, ferritin concentration could not be determined in four subjects.

Relationship to Corrected Whole Blood Folate Concentration

No significant correlation was detected between MCA and corrected whole blood folate concentration in either the diabetic patients (r= -0.09) or control group subjects (r= 0.3) as shown in Tables 4.5 and 4.6 respectively. Subjects of both groups had normal folate levels.

Relationship to Blood Vitamin B12 levels

No significant correlation was detected between MCA and vitamin B12 concentration in either diabetic patients (r= 0.16) or control group subjects (r= 0.1) as shown in Tables 4.5 and 4.6 respectively.

Vitamin B12 concentration was normal in diabetic patients. However, four control subjects were vitamin B12-deficient (<175pg/ml) with MCA 2.8 (SD 1.1) while 42 had normal concentrations with MCA 2.5 (SD 1.1). The difference between the two groups was not significant (p= 0.7). Vitamin B12 concentration could not be determined in four control subjects.

4.3.10 Relationship of Candidal Adhesion to Type of Diabetes

The diabetic patients group comprised 19 patients with insulin-dependent diabetes mellitus (MCA 4.1, SD 2.0) and 31 patients with non-insulin dependent diabetes mellitus (MCA 4.0, SD 1.8). The difference in MCA between patients of either type of diabetes was not significant (p= 0.8).

4.3.11 Relationship of Candidal Adhesion to Duration of Diabetes

No significant correlation was detected between MCA and duration of diabetes mellitus (r= -0.18; Table 4.5). Diabetic patients were divided according to the duration of the disease into groups of five year intervals (Table 4.9). Patients with diabetes of less than five years duration had a significantly increased MCA over those who had the disease for more than 14 years (p= 0.03).

4.3.12 Relationship of Candidal Adhesion to Blood Glucose Concentration

No significant correlation was detected between MCA and blood glucose concentration in either diabetic (r= 0.25) or control group subjects (r= -0.19) as shown in Tables 4.5 and 4.6 respectively.

Duration of diabetes (Years)	No.	Mean candidal adhesion Yeast / BEC (SD)
< 5	26	4.2 (2.1)
5 - 9	11	3.7 (1.3)
10 - 14	7	4.4 (2.0)
>14	5	3.0 (0.7)

Table 4.9 Relationship of mean candidal adhesion to duration of diabetes mellitus.

% HbA1	No.	Mean candidal adhesion Yeast / BEC (SD)	p
< 10	25	3.8 (1.5)[a]	0.9
10 - 12	13	3.8 (1.7)[b]	
> 12	4	6.0 (2.8)[c]	0.05
< 12	38	3.8 (1.5)	

a vs c (P= 0.2).
b vs c (P= 0.2).

Table 4.10 Relationship of mean candidal adhesion to glycosylated haemoglobin per cent (%HbA1).

The diabetic patients were further subdivided into two groups according to blood glucose concentration and MCA was compared between those who had a random blood glucose concentration of 10mmol/dl or less (25 patients; MCA 3.9, SD 1.7), and those who had a blood glucose concentration above 10mmol/dl (25 patients; MCA 4.1, SD 2.0). The difference was not significant between the two subgroups (p= 0.8).

4.3.13 Relationship of Candidal Adhesion to Glycosylated Haemoglobin

No significant correlation was detected between MCA and percentage of glycosylated haemoglobin in diabetic patients (r= 0.2; Table 4.5).

The diabetic patients were further subdivided into three subgroups according to the percentage of glycosylated haemoglobin concentration as shown in Table 4.10. A Mann-Whitney U test showed diabetic patients with a glycosylated haemoglobin of 12 per cent or higher had a marginally significant increased MCA compared with those who had a glycosylated haemoglobin below 12 per cent.

4.3.14 Relationship of Candidal Adhesion to Oral Yeast Colonisation

The data relating to oral candidal colonisation aspects of this study were presented fully in Chapter Three. Briefly, oral candidal carriage was detected in 21 (42%) of diabetic patients while another six (12%) patients had clinical and microbiological evidence of oral candidosis. In the control group, 20 (40%) of subjects were Candida carriers while oral candidosis was not detected clinically in any of the control subjects. Oral candidal carriage rate was not significantly different between the diabetic patients and control subjects, whereas oral candidosis was significantly increased in the

diabetic patient group ($p<0.02$). C. albicans was the most commonly isolated species from both diabetic patients (75%) and control subjects (67%).

No significant correlation was detected between MCA and quantity of oral candidal isolation either in the diabetic patients ($r= -0.08$) or control subjects ($r= 0.08$) as shown in Tables 4.5 and 4.6 respectively.

Finally, in diabetic patients, no significant difference was detected in MCA between patients who were Candida carriers, non-carriers or those with oral candidal infection (Table 4.11). Similarly, in control subjects, the difference in MCA between Candida carriers and non-carriers was also not significant (Table 4.12).

4.3.15 Relationship of Candida Adhesion to Secretor Status

The secretor status of the diabetic patients and control subjects were described in details in Chapter Three.

In the diabetic group, 31 (64.6%) patients were secretors of blood group substances in saliva with MCA 3.8 (SD 1.8) and 17 (33.4%) patients were non-secretors with MCA 4.5 (SD 2.0). Although MCA in the diabetic patients who were non-secretors was higher than in secretors, nevertheless, the difference was not statistically significant ($p= 0.2$).

In the control group, 31 (62%) subjects were secretors with MCA 2.5 (SD 1.3) and 19 (38%) subjects were non-secretors with MCA 2.6 (SD 1.1). The difference in MCA between secretors and non-secretors was also not statistically significant ($p= 0.9$).

Oral candidal status	No.	Mean candidal adhesion Yeast / BEC (SD)	p
Carriers	21	4.1 (2.0)[a]	0.9
Non-carriers	23	4.1 (1.9)[b]	0.8
Carriers + Infected	27	3.9 (1.9)	
Infected	6	3.3 (1.0)[c]	

a vs c (P= 0.16).
b vs c (P= 0.15).

Table 4.11 Relationship of mean candidal adhesion to oral candidal colonisation in diabetic patients.

Oral candidal status	No.	Mean candidal adhesion Yeast / BEC (SD)	p
Carriers	20	2.5 (1.3)	0.9
Non-carriers	30	2.6 (1.2)	

Table 4.12 Relationship of mean candidal adhesion to oral candidal colonisation in control subjects.

4.4 DISCUSSION AND CONCLUSIONS

The majority of the studies in the field of candidal adhesion have focussed on the adhesion process itself. Although the mechanisms involved were repeatedly studied they were, however, often not fully identified. In addition, the biological attributes of the yeast cells or the significance of their growth environment which might influence adhesion were also investigated extensively (Kennedy, 1988). Some studies have described variations in the adhesion capability of different Candida species and strains (Kearns et al., 1983; McCourtie & Douglas, 1984). However, there have been few investigations examining variations in the ability of epithelial cells from different sources to bind yeast cells (Sandin et al., 1987a) which was the main aim of the present study.

Accumulating evidence has indicated a relationship between microbial adhesion to the host surfaces and propensity to cause infection (Gibbons & van Houte, 1975). The experimental studies have shown a good correlation between candidal adhesion to epithelial cells in vitro and pathogenicity. C. albicans strains which had higher adhesion rates to epithelial cells were more virulent in animal models of disseminated candidosis (McCourtie & Douglas, 1984; Barrett-Bee et al., 1985; Ghannoum & Abu Elteen, 1986). C. albicans and C. stellatoidea which adhered markedly to corneocytes were particularly pathogenic in a rodent model of cutaneous candidosis in contrast to non-pathogenic species which showed little or no adherence (Ray et al., 1984). Moreover, a rabbit Candida endocarditis model (Calderone et al., 1985) and a murine model of vaginal candidosis (Lehrere et al., 1986) were established by using a C. albicans strain which was able to adhere well in vitro to fibrin platelet clots and vaginal

epithelial cells respectively, while a mutant of the same strain which showed a reduced ability to adhere was not pathogenic.

C. albicans is an opportunistic yeast known to reside commensally in the oral cavity, gastrointestinal tract and urogenital system of many individuals (Odds, 1988). Although the pathogenesis of clinical fungal infection involves a complex series of interactions which may not be fully reproduced during in vitro experiments, nevertheless, candidal adhesion to host surfaces still appears to be of prime importance. Adherence of C. albicans to oral epithelial cells might not be neccessary either for yeasts survival or growth, it is an important first step in surface colonisation by counteracting the influence of dislodging forces like the continuous secretion of saliva, food movement and muscular contraction in the oral cavity. This "adherability" enables the yeast to proceed to colonisation and subsequent infection. In that regard, Howlett and Squier (1980) have shown that candidal adhesion to the epithelial cells was necessary for the invasion to occur while yeasts which did not adhere were unable to invade the epithelium.

Comparing results of adhesion of the present investigation with those of other workers can be problematical due to differences in methods used to study candidal adhesion in vitro. These variations include differences in strains of C. albicans used (or concentration), epithelial cell source, candidal growth media and culture environment (Kennedy & Sandin, 1988) all of which indicate the need for a universal standardised adhesion assay.

Buccal epithelial cells were used in this study as a substratum for candidal adhesion for the following reasons : (I) The

ease of collection of a reasonable number of cells. (II) Cell size and morphology which is suitable for counting procedures. (III) Comparatively few indigenously attached bacteria. (IV) Buccal mucosa is a site for candidal infection *in vivo*. *C. albicans* was chosen as the test organism because it adheres to buccal epithelial cells to a greater degree than other *Candida* species (King et al., 1980) and is the most commonly species isolated from cases of oral candidosis (Odds, 1988).

Adhesion of *C. albicans* to epithelial cells is generally the result of interaction between *Candida* adhesion site(s) and complementary epithelial cell receptor(s). Critchley and Douglas (1987b) have shown that epithelial receptors are composed of glycosides containing a protein and carbohydrate moiety which may be particularly required for *C. albicans* adhesion. Since all adhesion assays in the present study were conducted under the same environmental conditions, using the same growth medium, the same thorough washing technique and a single candidal strain, any quantitative differences in candidal adhesion between the groups studied should reflect true differences in the number and / or nature of the available receptors on the epithelial cell membrane.

The finding that *C. albicans* was the most commonly isolated species in both diabetic and control subjects is consistent with the generally accepted idea that *C. albicans* is recovered more commonly from mucosal surfaces than other species (Odds, 1988). This may be due to the ability of this particular species to adhere to buccal and vaginal epithelial cells *in vitro* to a much higher degree than other candidal species (King et al., 1980).

The increased susceptibility of women with diabetes mellitus to vaginal candidosis has been previously linked with an increased *in vitro* adhesion of *C. albicans* to vaginal epithelial cells collected from these patients (Segal et al., 1984). The present study is the first to report a significantly increased candidal adhesion to buccal epithelial cells of diabetic patients compared with non-diabetic individuals. Theoretically, this may mean the presence of higher number of receptors for *Candida* on epithelial cells from diabetic patients. In addition, the possible qualitative difference in receptors on epithelial cell surface between diabetic patients and non-diabetic subjects should be taken into consideration. Interestingly, the number of cells that had no adherent *Candida* was considerably less in the diabetic patients. This suggests that the proportion of epithelial cells having receptors for *Candida* is greater in diabetic patients when compared with non-diabetics. These observations may partly explain the increased predisposition of diabetic patients to oral candidal infection. However, the additional finding of considerable interindividual variations in candidal adhesion between subjects in either group is consistent with that reported in other studies of normal individuals (Sobel et al., 1981; Sandin et al., 1987a). If other studies confirmed the increased candidal adhesion to epithelial cells from diabetic patients, this will open the way for further research to explain this phenomenon. Although the present study has not identified specific reason for the increased candidal adhesion in diabetic patients, multiple mechanisms are possible.

Long standing diabetes is frequently associated with permanent and irreversible functional and structural changes in the

cells of the body (Baird, 1987). Glucose in blood is known to form glycosylation products with proteins at a rate proportional to the glucose concentration. Hence the amount of glycosylation products, whether on haemoglobin, basement membranes or endothelial cells are much increased in diabetic patients (Brownlee, Cerami, Vlassara, 1988). It would therefore be interesting to investigate the possibility of alterations in the glycosylation process in diabetic patients, which may affect carbohydrate moieties on the epithelial cells in a way that might modify their receptivity to yeast cells. A phenomenon such as this may account for the significant increase in candida "positive" cells observed in the diabetics when compared with the controls. It is also worth noting that candidal cells adhered in greater numbers to epithelial cells from those diabetic patients with high percentages of glycosylated haemoglobin (over 12 per cent - indicating poor glycaemic control) than to diabetics with lower glycosylated haemoglobin levels and thus better glycaemic control. Segal et al. (1984) explained the increased candidal adhesion to vaginal epithelial cells from diabetic women as being due to the increased glycogen content of these cells and the higher proportion of intermediate epithelial cells with larger surface area.

C. albicans grown in media supplemented with glucose exhibited changes in the cell wall structure resulting in enhanced adhesion to epithelial cells (McCourtie & Douglas, 1981; Samaranayake & MacFarlane, 1982b). Therefore it is tempting to speculate that a higher glucose concentration in the saliva of diabetic patients (Thorstensson et al., 1989), may modify the surface of oral epithelial cell in a way which increases their receptivity to Candida.

Oral commensal microflora has been shown to affect candidal adhesion to oral epithelium (Liljemark & Gibbons, 1973). Strep. salivarius, Strep. mitior (Samaranayake & MacFarlane, 1982a) and lactobacilli (Sobel et al., 1981) all have been shown to reduce candidal adhesion to epithelial cells. In addition, Cox (1983) noticed reduced candidal adhesion to buccal epithelial cells already colonised with bacteria.

Bacterial competition for receptors on epithelial cells or production of bacterial metabolites affecting these receptors are possible mechanisms (Samaranayake & MacFarlane, 1982a). However, certain piliated bacteria such as E. coli can enhance, rather than reduce, candidal adhesion to epithelial cells (Makrides & MacFarlane, 1983). Probably the mannose-sensitive pili from attached bacteria could recognise mannan-containing moities on the surface of C. albicans, bind to them and facilitate candidal adhesion to epithelial cells. There is, therefore, a need for subsequent studies quantifying oral bacterial flora and investigating its influence on candidal adhesion to epithelial cells from diabetic patients and control subjects. However, neither type of diabetes mellitus, duration of disease, or blood glucose concentration seems to affect candidal adhesion in diabetic patients. Perhaps the metabolic effect of diabetes on epithelial cells therefore occurs early in the course of the disease and may be irreversable. In addition, the measurement of a single blood glucose level at one point in time is too crude to be expected to correlate with candidal adhesion.

Salivary pH has been reported frequently to be lower in diabetic patients than that in non-diabetic individuals (Banoczy et al., 1987). A culture medium of low pH has also been shown to enhance

candidal adhesion to epithelial cells (Samaranayake & MacFarlane, 1982a). Mammalian cells and yeast cells, are both endowed with a net negative surface potential. Therefore, prior to candidal adhesion to epithelial cells, each must overcome the electrostatic barrier of their negatively-charged functional groups (Kennedy, 1988). It is tempting to speculate that the low pH in the oral cavity of diabetic patients acts to reduce surface electronegativity on the epithelial cell surface due to protonation of the exposed cell surface functional groups and, theoretically, may facilitate candidal adhesion in diabetic patients.

Since lysozyme, the potent fungicidal salivary factor, is known to have a highly cationic charge, it may avidly bind either to the yeast or the epithelial cell surface (or both) and thereby, interfere with yeast adhesion to the epithelial cell surface (Tobgi et al., 1988). However, lysozyme in saliva of diabetic patients may not be as influencial in retarding candidal adhesion, as it is in non-diabetic individuals, since it has been shown to have significantly reduced activity in saliva of diabetic patients (Velikov et al., 1985) and in hamsters rendered diabetic by alloxan (Muratsu & Morioka, 1985).

Previous studies of yeast adhesion to buccal cells of healthy adults have shown that age of the donors does not have an influence on yeast adhesion (Cox, 1983). The present results in the non-diabetic subjects tend to agree with this conclusion. However, why diabetics aged below 60 years had a higher mean candidal adhesion than older patients is not at present explicable, and the clinical importance is uncertain. Whether functional or quantitative differences exist in

epithelial cell receptors for Candida in diabetics of different ages warrants study.

In health, gender does not affect candidal adhesion to buccal epithelial cells (Cox, 1983; Sandin et al., 1987b) which agrees with the present results in the diabetic patients. Quite why candidal adhesion was found to be higher in non-diabetic females compared to males is uncertain, although a possible hormonal influence cannot be excluded. However, it is noteworthy that more than 70 per cent of control female subjects wore their dentures continuously and continuous denture wearing in the non-diabetic subjects was associated with a significant increase in candidal adhesion compared with those who wore dentures during the day only. Although this latter finding is an observation rather than an explanation, it could be relevant to the results presented in Chapter Three since clinical oral candidosis was diagnosed only in continuous denture wearers, which is in accordance with that reported in denture wearers generally (Budtz-Jorgensen, 1974). In addition to the multiple factors predisposing denture wearers to oral candidal infection (Budtz-Jorgensen, 1974), the present observation of effect of denture wearing pattern suggests that the continuous wearing of dentures, in some way, has an influence on oral epithelial cells integrity rendering them more receptive to yeast cells.

There is little information in the literature on the effect of saliva on candidal adhesion to oral epithelial cells. Samaranayake and MacFarlane (1982a) reported that pretreatment of HeLa cell monolayers with mixed saliva enhanced adherence of C. albicans. The present study could not detect any difference in candidal adhesion between those patients who reported symptomatic xerostomia and those

who did not report this symptom. However, the present *in vitro* finding is difficult to interpret since only the outer surface of buccal cells would be directly coated with saliva leaving the deeper cell surface saliva-free. In addition, subsequent washing of the buccal epithelial cells may dilute or remove some of the salivary components on the cell surface.

The relationship of haematological status including levels of haemoglobin, ferritin, folate and vitamin B12 on *in vitro* candidal adhesion has not as yet been reported. These factors were shown to have no apparent influence on candidal adhesion in diabetic patients. The observed significant positive correlation between reduced haemoglobin level and *in vitro* candidal adhesion in the diabetic patients is far from explained. However, this finding may be reasonable considering the reported increased incidence of oral candidosis in patients with iron deficiency (Fletcher et al., 1975; Challacombe, 1986). This warrants further study to include patients with selected haematological deficiencies.

The present study did not demonstrate a difference in mean candidal adhesion between carriers of *Candida* species and non-carriers or patients with oral candidosis. This finding is in contrast to the results of Cox (1983) who found a significantly increased candidal adhesion to epithelial cells both from *Candida* colonised subjects and from patients with oral candidosis compared with controls. However, 95 per cent of the colonised and 80 per cent of diseased subjects in that study were receiving antibiotic therapy at the time of testing. Other recent research in this field has shown antibiotic therapy to increase candidal adhesion to oral epithelial cells (Velichko &

Karaev, 1988). This is probably because antibiotic therapy suppresses indigenous oral bacteria competing with yeasts for nutrients (Knight & Fletcher, 1971) and adhesion sites on the epithelial cell surface (Centeno et al., 1983).

The lack of correlation between yeast carriage and in vitro candidal adhesion implies that other factors in addition to candidal adhesion such as presence of dental prostheses or denture wearing patterns (Arendorf & Walker, 1979) or high carbohydrate intake (Samaranayake, 1986) may promote carriage of candidal species in the oral cavity. Although carbohydrate intake was not quantified in diabetic subjects, it would be expected to be low since most of the diabetic patients had good glycaemic control as measured by glycosylated haemoglobin level.

Considering subjects with proven oral candidal colonisation, the present study has shown that candidal adhesion to buccal epithelial cells was comparable both in infected and non-infected individuals. Thus factors such as host immune status and other environmental determinants, including saliva composition, may be more important in determining why adherence was achieved and resulted in clinical infection in some individuals. In other words, the propensity for the adhered yeast cells to behave as commensal organisms or proceed to clinical infection could depend on a dynamic balance of various parameters acting either in favour or against the host. This is in line with oral candidosis being described by the epithet "disease of the diseased".

The result of comparing candidal adhesion to buccal epithelial cells isolated from secretors and non-secretors, was not significant and, therefore, not in agreement with the finding reported

by May et al. (1986) that epithelial cells from secretors bind Candida in fewer number than those epithelial cells derived from non-secretors.

In the saliva of non-secretors, Lewis^a antigen (a blood group antigen found predominantly on the cells of non-secretors) may adsorb to epithelial cells and function as a receptor for some strains of C. albicans (Blackwell, 1989). Recently May, Blackwell and Weir (1989) have shown that not all C. albicans blastospores have the ability to recognise Lewis^a antigen on epithelial cells from non-secretors as a receptor, which was probably the case with the strain used in the present study.

If future studies confirm the present finding of increased candidal adhesion to buccal epithelial cells in diabetic patients, the precise additional factors responsible could be clearly identified and their importance in vivo established. This may clear the way for a greater understanding of the mechanisms of mucosal candidal infection in patients with systemic disease such as diabetes, and the information thus gained could be translated to a clinical setting.

CHAPTER FIVE

THE *IN VITRO* AND *IN VIVO* EFFECT OF 0.2% CHLORHEXIDINE GLUCONATE ON ADHESION OF *CANDIDA ALBICANS* TO BUCCAL EPITHELIAL CELLS FROM DIABETIC PATIENTS AND NON-DIABETIC INDIVIDUALS

5.1 INTRODUCTION AND AIMS OF THE STUDY

Chlorhexidine gluconate in a concentration of 0.2 per cent is used widely in clinical practice as an antiseptic oral rinse. It has proven to be effective against a broad spectrum of oral microorganisms and therefore, has been shown to be useful in the control of supragingival plaque and gingivitis (Salem et al., 1987). The antifungal effect of chlorhexidine has been demonstrated in several clinical trials where it was used successfully in a regimen for the treatment of chronic atrophic candidosis in otherwise healthy individuals (Budtz-Jorgensen & Loe, 1972; Olsen, 1975a). Indeed, the therapeutic effect of chlorhexidine in cases of chronic atrophic candidosis was comparable to that of the specific antifungal agent amphotericin B (Olsen, 1975b). The application of 0.2% chlorhexidine to the fitting surface of upper denture and palatal mucosa four times daily has also proved useful in reducing candidal colonisation to these surfaces (Olsen, 1975a).

In addition to its fungicidal properties (Bobichon & Bouchet, 1987), chlorhexidine, when used as a mouth rinse, has been reported to render the host surfaces less receptive to candidal colonisation. Pretreating acrylic surfaces *in vitro* with 2% chlorhexidine reduced the adherence of *Candida* species (Samaranayake et al., 1980; McCourtie et al., 1986a). A recent study, albeit on a limited number

of healthy individuals, has shown a significant reduction in the adhesion of C. albicans to buccal epithelial cells exposed to 0.2% chlorhexidine (Tobgi et al., 1987). It is conceivable that, diabetic patients who as a disease group are predisposed to oral candidosis and have higher rates of candidal adhesion to their epithelial cells (described in Chapters Three and Four), may benefit from the use of a 0.2% chlorhexidine mouth rinse. This benefit could be derived by both reducing candidal adhesion to their oral mucosa and the direct fungicidal activity of the agent. However, this hypothesis has to date not been investigated.

Since the inherited inability to secrete blood group antigens in saliva has been linked with susceptibility to oral candidosis (Thom et al., 1989) and diabetic patients who are non-secretors may have an increased propensity to harbour yeast intraorally, the aims of the present in vitro and in vivo investigations were to answer several specific questions. Firstly, would exposure of buccal epithelial cells (BEC) from healthy adult individuals to 0.2% chlorhexidine gluconate, for one minute reduce candidal adhesion ? Secondly, if so, whether similar treatment of BEC from diabetic patients results in a comparable reduction in candidal adhesion and finally, whether such a treatment results in an equivalent reduction in candidal adhesion to BEC from non-secretors and secretors.

5.2 MATERIALS AND METHODS

5.2.1 Patients Selection

Twelve patients with diabetes mellitus and 12 non-diabetic

subjects gave informed consent to participate in the study which was approved by the local Ethics Committee. All subjects from both patient and control group participated jointly in both *in vitro* and *in vivo* experiments.

The diabetic patients were recruited from the outpatient Diabetic Clinic at Glasgow Royal Infirmary to the Oral Medicine Unit at Glasgow Dental Hospital where the clinical sampling was performed. The non-diabetic subjects were postgraduate students or staff at Glasgow Dental Hospital and School who volunteered to participate in the study.

5.2.2 Clinical History and Oral Examination

A verbal medical history was taken from each subject. The type of diabetes mellitus and duration of the disease were recorded for the diabetic patients. For each subject, the wearing of a dental prosthesis was recorded as was whether the prosthesis was worn continuously (day and night). Subjects who either consumed alcohol regularly or who smoked cigarettes daily were considered as smokers and drinkers respectively.

Each subject underwent a routine oral clinical examination to detect the presence of clinical oral candidal infection.

5.2.3 Secretor Status Determination

Secretor status was determined by the agglutination inhibition technique described by Periera et al. (1969) using unstimulated mixed saliva. Details of saliva collection, the agglutination inhibition technique itself and criteria for defining secretor status were described in Sections 3.2.7 to 3.2.9.

5.2.4 Collection and Preparation of Buccal Epithelial Cells to Study the Effect of Exposure to 0.2% Chlorhexidine *In Vitro* on Candidal Adhesion *In Vitro*

For the *in vitro* experiment, BEC were collected from each subject by gently rubbing both the right and left buccal mucosa each with a sterile cotton swab (Exogen Ltd., Clydebank, Scotland). Each swab was then agitated in 10ml phosphate buffered saline (PBS 0.1M, pH 7.2) in a universal container (Sterilin Ltd., Feltham, England) which was retained on crushed ice till the time of processing. Throughout the study, PBS of the same osmolarity and pH was used. Buccal epithelial cells obtained from the right buccal mucosa were used as the control for the experiment (control cells), while those obtained from the left mucosa were allocated for the *in vitro* experiment (test cells).

BEC were pelleted by centrifugation (MSE Centaur 2 Centrifuge. Fisson's Ltd., Crawley, Sussex, England) at 400g for five minutes. Subsequently, the BEC pellet from the left buccal mucosa was exposed to 10ml 0.2% chlorhexidine gluconate ("Corsodyl", ICI Pharmaceuticals, Macclefield, England) while BEC from the right buccal mucosa were treated similarly with 10ml PBS. After one minute incubation at room temperature, BEC were harvested on polycarbonate filters of 25mm diameter and 12μm pore-size (Nuclepore Gmbh, Germany) mounted on a DEFT (direct epifluorescent filter technique) manifold (Micromeasurements Ltd., Saffron Walden, Essex, England) as shown in Figure 4.1. This method provided rapid separation of BEC from chlorhexidine and thus attempted to simulate the *in vivo* conditions.

The filter and the retained BEC were then agitated gently in 10ml PBS in a universal container to detach the BEC from the filter

which therefore became suspended in PBS. Subsequently, the filter was discarded and the BEC washed twice in 10ml PBS by centrifugation at 400g for five minutes. Microscopic examination has shown none of the BEC had naturally attached yeasts after the PBS wash. A final BEC suspension of 10^5 cell per ml was prepared by appropriate dilution of the deposit in PBS following haemocytometer counting (Hawksley and Sons Ltd., Lancing, England).

5.2.5 Collection and Preparation of Buccal Epithelial Cells to Study the Effect of 0.2% Chlorhexidine Rinse *In Vivo* on Candidal Adhesion *In Vitro*

In the assay *in vivo*, BEC were collected from each subject by gently rubbing the right buccal mucosa with a sterile cotton swab, which was then agitated in 10ml PBS in a universal container retained on crushed ice. These cells acted as the control BEC for the experiment. Subsequently, each subject was asked to rinse the mouth thoroughly (after denture removal if appropriate) with 10ml 0.2% chlorhexidine gluconate. After one minute, the subject expectorated the rinse and BEC were immediately obtained by gently rubbing the left buccal mucosa with a sterile cotton swab which was then suspended in 10ml PBS in a universal container, and was placed on crushed ice until processing.

BEC washed twice in 10ml PBS by centrifugation at 400g for five minutes were then resuspended in PBS at a concentration of 10^5 cell per ml as determined by haemocytometer counting. Microscopic examination showed that none of the BEC had indigenously adherent yeasts after the PBS wash. All sampling was carried out between 1400

and 1700 hours. The *in vitro* and *in vivo* experiments were repeated twice for each subject with a two weeks interval between sampling to avoid any possible carry-over effect of the chlorhexidine mouth wash. Also, as further standardisation, BEC were collected at the same hour of the day to exclude any possible influence of diurnal variations.

5.2.6 Source, Identification and Maintenance of *Candida Albicans* Strain CDS 88

C. albicans strain CDS 88 used throughout the study was originally isolated from the oral cavity of an asymptomatic carrier of *C. albicans* from the Oral Medicine Unit at Glasgow Dental Hospital. The species was identified by the serum germ tube formation technique (MacKenzie, 1962) and using the identification system API 20 C AUX (API Products Ltd., Basingstoke, Hampshire, England), and then freeze-dried. The strain was maintained on slopes of Sabouraud's dextrose agar (Gibco, Paisley, Scotland) at 4° C and the culture was replaced monthly by a new one freshly-grown from freeze-dried stock.

5.2.7 Preparation of *Candida Albicans* Suspension

To prepare the yeast cells for the adhesion assay, a loopful of the stock culture was inoculated into 10ml Sabouraud's broth (Gibco, Paisley, Scotland) containing 500mM sucrose in a universal container, and incubated overnight (18-24 hours) at 37°C in an orbital shaker incubator (Gallenkamp, Loughborough, England) operating at 100 revolutions per minute. Sabouraud's broth containing 500mM sucrose is known to enhance candidal adhesion (McCourtie & Douglas, 1981) and yeast cells in the stationary phase of growth have been shown to adhere more readily to epithelial cells than yeast cells in the

logarithmic phase (King et al., 1980). The culture was then harvested by centrifugation at 400g for five minutes and the pellet washed twice in 10ml PBS by centrifugation at 400g for five minutes. A final yeast cells suspension of 10^7 per ml was prepared by appropriate dilution of the deposit in PBS followed by haemocytometer counting.

5.2.8 The Candidal Adhesion Assay

The in vitro candidal adhesion assay described by Kimura and Pearsall (1978) was used in this study with few modifications.

For the adhesion assay, equal volumes (0.5ml) of BEC suspension and C. albicans suspension were mixed in a sterile bijou container (Sterilin Ltd., Feltham, England) and incubated on an orbital shaker operating at 80 revolutions per minute at 37°C for one hour. The BEC were then harvested on polycarbonate filters of 25mm dimater and 12μm pore-size mounted on a DEFT manifold. A negative pressure of 5mm Hg was applied to the manifold from a Venturi pump drawing the suspension in the filter tower through the filter, and the filter with the retained BEC were washed gently with 30ml PBS in aliquots of 5ml to eliminate unattached yeast cells. The pore size of 12μm was small enough to retain the BEC without gross distortion but large enough to allow unattached yeast cells to be washed through the filter unit, leaving only the BEC with the adherent yeast cells on the filter. The filter with the washed BEC were then removed from the filter unit, mounted on a 1mm thick, 26 X 76mm glass microscope slide (Chance Proper Ltd., Smethwick, England) and left for one hour to air-dry and then Gram-stained. The filter and the stained BEC on each glass slide were left overnight to air-dry then a 22 X 22mm glass

cover slip (Chance Proper Ltd., Smethwick, England) was fixed on the filter with Harleco resin (Kodak Chemicals, England).

5.2.9 Counting of Adherent Yeast Cells

The glass slides holding the filters were coded and counting was undertaken in a "blind" manner. The number of C. albicans blastospores which adhered to 100 individual BEC in randomly selected fields were counted microscopically under x40 objective (Olympus Ltd., London, England). Only morphologically normal epithelial cells were counted. These were flat cells, with an intact surface membrane which was not folded. Clumps of BEC were excluded and pseudohyphae, if present, were not counted.

5.2.10 Statistical Analysis

The percentage reduction in candidal adherence for each subject was calculated as follows:

$$\% \text{ reduction in adherence} = \frac{Ac - At}{Ac} \times 100$$

Ac = Yeast adhered to 100 BEC of control cells.

At = Yeast adhered to 100 BEC of test cells.

Student's t test for paired data was used to evaluate differences in candidal adherence between subjects of a group, whereas Student's t test for unpaired data was used to evaluate differences in candidal adhesion between different groups as well as between different experiments. Probabilities of less than five per cent were taken to be statistically significant.

5.3 RESULTS

5.3.1 Subjects of the Study

The clinical status of study subjects is presented in Table 5.1. The diabetic group comprised of eight patients (66.7%) with insulin-dependent diabetes mellitus and four patients (33.3%) with non-insulin dependent diabetes mellitus. The mean duration of the disease was 8.98 (SD 8) years, ranging from three months to 22 years.

None of the subjects had received antibiotic or steroid therapy, or had used a mouth wash or tooth paste containing chlorhexidine for at least three months before commencing the study. None of the subjects had either signs or symptoms suggestive of oral candidal infection.

5.3.2 The Effect of Exposure of BEC to 0.2% Chlorhexidine Gluconate *In Vitro* on Candidal Adhesion

Pretreating BEC for one minute with 0.2% chlorhexidine gluconate *in vitro* resulted in a significant reduction in candidal adhesion in both non-diabetic subjects (Table 5.2; $p<0.0001$) and diabetic patients (Table 5.3; $p<0.0001$). However, BEC from one control subject showed an overall increase in candidal adhesion after 0.2% chlorhexidine treatment.

	Diabetics (n= 12)	Non-diabetics (n= 12)
Male	6 (50%)	8 (67%)
Female	6 (50%)	4 (33%)
Mean age (years)	37.3 SD 13.8	31.9 SD 4.9
Age range (years)	15 - 57	22 - 39
Denture wearers	3 (25%)	1 (8%)
D and N	3 (100%)	1 (100%)
Dentate	9 (75%)	11 (92%)
Symptomatic xerostomia	4 (33%)	0 (0%)
Smokers	3 (25%)	2 (17%)
Alcohol drinkers	0 (0%)	0 (0%)

D and N : Day and night denture wearing.

SD : Standard deviation of the mean.

Table 5.1 Clinical status of the chlorhexidine study subjects.

In diabetic patients and non-diabetic subjects, variations were observed in mean percentage reduction in candidal adhesion between individuals. The overall mean percentage reduction in candidal adhesion in non-diabetic subjects was 33.1 per cent (95% confidence intervals 21.6 , 46.9) ranged from 6.5 per cent to 62.6 per cent. The mean percentage reduction in candidal adhesion in diabetic patients as a whole was 21.6 per cent (95% confidence intervals 15.6 , 27.6) ranged from 8.9 per cent to 36.8 per cent. However, when the test was repeated in two separate occasions, intraindividual variations were also observed in percentage reduction in candidal adhesion to BEC in both groups. Although the mean percentage reduction in candidal adhesion was higher in non-diabetic subjects than in diabetic patients, the difference was not statistically significant ($p>0.05$).

5.3.3 The Effect of 0.2% Chlorhexidine Gluconate Rinse *In Vivo* on Candidal Adhesion

Rinsing the mouth for one minute with 0.2% chlorhexidine gluconate resulted in a significant reduction in candidal adhesion to BEC in non-diabetic subjects (Table 5.4; $p<0.05$) and diabetic patients (Table 5.5; $p<0.002$). However, BEC obtained from one non-diabetic subjects and two diabetic patients showed overall increased candidal adhesion after chlorhexidine rinsing. In agreement with the results of the *in vitro* experiments, in the *in vivo* experiment both inter and intraindividual variations were observed in the percentage reduction in candidal adhesion in individuals of both groups. The mean percentage reduction in candidal adhesion in non-diabetic subjects was 20.1 per cent with a range from 6.4 per cent to 51.2 per cent (95%

confidence intervals 7.5, 32.8). In the diabetic patients, the mean percentage reduction in candidal adhesion to BEC was 14.8 per cent (95% confidence intervals -1.5 , 31) with overall results ranging from 0.2 per cent to 42.8 per cent. Although the mean percentage reduction in candidal adhesion was higher in non-diabetic subjects than in diabetic patients, the difference was not statistically significant ($p>0.5$).

5.3.4 Comparison Between *In Vitro* and *In Vivo* Results

One minute exposure of BEC to 0.2% chlorhexidine *in vitro* resulted in a higher mean percentage reduction in candidal adhesion to BEC than *in vivo* one minute mouth rinsing with 0.2% chlorhexidine (Figure 5.1). Nevertheless, statistical analysis showed no significant difference between *in vitro* and *in vivo* experiments either in diabetic patients or non-diabetic subjects ($p>0.05$).

5.3.5 Relationship Between Percentage Reduction in Candidal Adhesion to BEC and Secretor Status

No significant difference was detected in mean percentage reduction in candidal adhesion to BEC between secretors and non-secretors. This finding held true for both the diabetic patients (Table 5.6) and non-diabetic subjects (Table 5.7) and also for the results of both the *in vitro* and *in vivo* experiments.

Case no.	MCA: Yeast / 100 BEC (SD) Control		Test		% Reduction in adhesion	
1	254		150		40.9	
	173		97		43.9	
Mean	**213.5**	**(57.3)**	**123.5**	**(37.5)**	**42.4**	**(2.1)**
2	206		186		9.7	
	303		203		33.0	
Mean	**254.5**	**(68.6)**	**194.5**	**(12.0)**	**21.4**	**(16.5)**
3	141		136		3.5	
	241		108		55.2	
Mean	**191.0**	**(70.7)**	**122.0**	**(19.8)**	**29.4**	**(36.6)**
4	103		47		54.4	
	241		108		55.2	
Mean	**172.0**	**(97.6)**	**77.5**	**(43.1)**	**54.8**	**(0.6)**
5	107		77		28.0	
	317		204		35.6	
Mean	**212..**	**(103.9)**	**140.5**	**(89.8)**	**45.1**	**(13.5)**
6	67		100		+49.2	
	281		160		43.0	
Mean	**174.0**	**(151.0)**	**130**	**(42.4)**	**+ 3.1**	**(65.2)**
7	317		148		53.3	
	330		171		48.2	
Mean	**323.5**	**(9.2)**	**159.5**	**(16.3)**	**50.7**	**(3.6)**
8	177		87		50.8	
	247		218		11.7	
Mean	**212.0**	**(49.5)**	**152.5**	**(92.5)**	**31.2**	**(27.6)**
9	153		133		13.0	
	150		150		0.0	
Mean	**151.5**	**(2.1)**	**141.5**	**(12.5)**	**6.5**	**(9.2)**
10	182		88		51.6	
	249		139		44.2	
Mean	**215.5**	**(47.4)**	**113.5**	**(36.1)**	**47.9**	**(3.7)**
11	112		87		22.3	
	228		177		22.4	
Mean	**170.0**	**(82.0)**	**132.0**	**(63.6)**	**22.3**	**(0.1)**
12	296		99		66.5	
	211		87		58.8	
Mean	**253.5**	**(60.1)**	**93.0**	**(8.5)**	**62.6**	**(5.4)**
Mean	214.5		131.6		33.1	
SD	48.4		30.5		19.9	

+ : % increase in candidal adhesion.

Table 5.2 The effect of exposure to 0.2% chlorhexidine gluconate *in vitro* on adhesion of *C. albicans* to buccal epithelial cells from non-diabetic subjects.

Case no.	MCA: Yeast / 100 BEC (SD) Control		Test		% Reduction in adhesion	
1	169		128		24.3	
	280		151		46.0	
Mean	**224.5**	**(78.5)**	**139.5**	**(16.3)**	**35.1**	**(15.3)**
2	156		109		30.1	
	244		239		2.0	
Mean	**200.0**	**(62.2)**	**174.0**	**(91.9)**	**19.9**	**(2.5)**
3	274		248		9.5	
	203		168		17.2	
Mean	**238.5**	**(50.2)**	**208.0**	**(56.6)**	**13.3**	**(5.4)**
4	316		271		14.2	
	275		235		14.5	
Mean	**295.5**	**(29.0)**	**253.0**	**(25.5)**	**14.4**	**(0.2)**
5	240		142		40.8	
	247		239		3.2	
Mean	**243.5**	**(4.9)**	**190.5**	**(86.6)**	**22.0**	**(26.6)**
6	345		209		39.4	
	295		194		34.2	
Mean	**320.0**	**(35.4)**	**201.5**	**(10.6)**	**36.8**	**(3.7)**
7	311		219		29.6	
	370		295		20.3	
Mean	**340.5**	**(41.7)**	**257.0**	**(53.7)**	**24.5**	**(6.6)**
8	300		260		13.3	
	173		165		4.6	
Mean	**236.5**	**(89.8)**	**212.5**	**(67.2)**	**8.9**	**(6.1)**
9	553		432		21.9	
	310		253		18.4	
Mean	**432.0**	**(172.0)**	**342.5**	**(126.5)**	**20.1**	**(2.5)**
10	272		252		7.3	
	247		213		13.8	
Mean	**259.5**	**(17.7)**	**232.5**	**(27.6)**	**10.5**	**(4.6)**
11	416		191		54.0	
	430		389		9.5	
Mean	**423.0**	**(9.9)**	**290.0**	**(140.0)**	**31.7**	**(31.5)**
12	368		246		33.1	
	438		352		19.6	
Mean	**403.0**	**(49.0)**	**299.0**	**(75.0)**	**26.3**	**(9.5)**
Mean	301.3		233.3		21.6	
SD	81.7		57.7		9.5	

Table 5.3 The effect of exposure to 0.2% chlorhexidine gluconate *in vitro* on adhesion of *C. albicans* to buccal epithelial cells from diabetic patients.

Case no.	MCA: Yeast / 100 BEC (SD) Control		Test		% Reduction in adhesion	
1	216		146		32.3	
	138		100		27.5	
Mean	**177.0**	**(55.2)**	**123.0**	**(32.5)**	**29.9**	**(3.5)**
2	167		153		8.4	
	132		175		32.6	
Mean	**149.5**	**(24.7)**	**164.0**	**(15.6)**	**12.1**	**(28.9)**
3	71		86		+21.1	
	182		120		34.0	
Mean	**126.5**	**(78.5)**	**103.0**	**(24.0)**	**6.4**	**(38.9)**
4	156		216		+38.5	
	206		184		10.4	
Mean	**181.0**	**(35.4)**	**200.0**	**(22.6)**	**+13.9**	**(34.8)**
5	184		118		35.9	
	185		120		35.1	
Mean	**184.5**	**(0.7)**	**119.0**	**(1.4)**	**35.5**	**(0.6)**
6	67		69		+ 2.9	
	251		148		41.0	
Mean	**159.0**	**(130.0)**	**108.5**	**(55.9)**	**19.0**	**(31.0)**
7	144		101		29.9	
	538		321		40.3	
Mean	**341.0**	**(279.0)**	**211.0**	**(156.0)**	**35.1**	**(1.5)**
8	240		134		44.2	
	235		98		58.3	
Mean	**237.5**	**(3.5)**	**116.0**	**(25.5)**	**51.2**	**(9.9)**
9	193		193		0.0	
	170		149		12.3	
Mean	**181.5**	**(16.3)**	**171.0**	**(31.1)**	**6.9**	**(8.7)**
10	164		92		43.9	
	179		136		24.0	
Mean	**171.5**	**(10.6)**	**114.0**	**(31.1)**	**33.9**	**(14.1)**
11	160		106		33.7	
	238		183		23.1	
Mean	**199.0**	**(55.2)**	**144.5**	**(54.4)**	**28.4**	**(7.5)**
12	286		198		30.8	
	129		111		13.9	
Mean	**207.5**	**(111.0)**	**154.5**	**(61.5)**	**22.3**	**(11.9)**
Mean	192.9		139.8		20.1	
SD	54.5		35.9		19.9	

+ : % increase in candidal adhesion.

Table 5.4 The effect of 0.2% chlorhexidine gluconate rinse *in vivo* on adhesion of *C. albicans* to buccal epithelial cells from non-diabetic subjects.

Case no.	MCA: Yeast / 100 BEC (SD) Control	Test	% Reduction in adhesion
1	468	328	29.9
	233	202	13.3
Mean	**351.5 (166.0)**	**265 (89.1)**	**21.6 (11.7)**
2	297	269	9.4
	606	385	36.5
Mean	**452.5 (218.0)**	**327 (82.0)**	**23.0 (19.2)**
3	370	223	39.7
	191	103	46.0
Mean	**280.5 (126.6)**	**163 (84.9)**	**42.8 (4.4)**
4	350	280	20.0
	621	743	+19.6
Mean	**485.0 (192.0)**	**512 (327.0)**	**0.2 (28.2)**
5	336	473	+40.8
	367	379	+ 3.3
Mean	**351.5 (21.9)**	**426 (66.5)**	**+22.0 (26.5)**
6	300	210	30.0
	171	126	26.3
Mean	**235.5 (91.2)**	**168 (59.4)**	**28.1 (2.6)**
7	300	188	37.3
	296	139	34.8
Mean	**298.0 (2.8)**	**163.5 (34.5)**	**36.0 (1.7)**
8	402	301	25.1
	393	215	45.3
Mean	**397.5 (6.4)**	**258 (60.8)**	**35.2 (14.4)**
9	178	130	27.0
	336	252	25.0
Mean	**257.0 (111.7)**	**191 (86.3)**	**26.7 (1.4)**
10	84	132	+57.1
	105	138	+31.4
Mean	**94.5 (14.8)**	**135 (4.2)**	**+44.2 (18.2)**
11	365	265	27.4
	362	306	15.5
Mean	**363.5 (2.1)**	**285.5 (29.0)**	**21.4 (8.4)**
12	132	76	42.4
	145	182	+25.5
Mean	**138.5 (9.2)**	**129 (75.0)**	**8.5 (48.0)**
Mean	308.9	251.9	14.8
SD	116.8	120.8	25.6

+ : % increase in candidal adhesion.

Table 5.5 The effect of 0.2% chlorhexidine gluconate rinse *in vivo* on adhesion of *C. albicans* to buccal epithelial cells from diabetic patients.

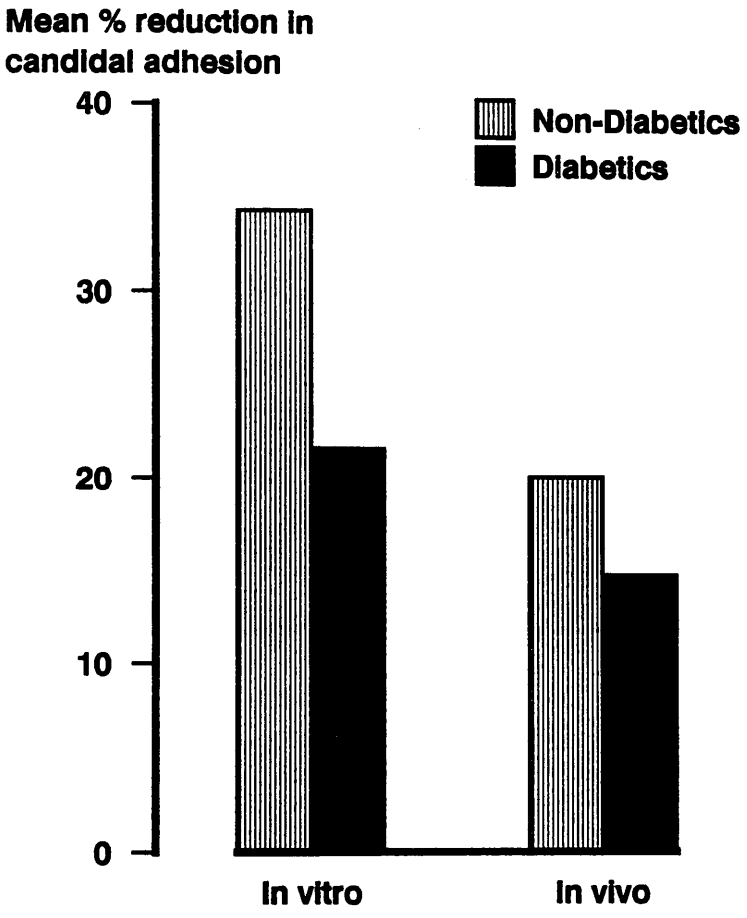

Figure 5.1 Comparison between *in vitro* and *in vivo* effect of 0.2% chlorhexidine gluconate on candidal adhesion to buccal epithelial cells.

Experiment	Mean % reduction in candidal adhesion ± SD		p
	Secretors (n= 9)	Non-secretors (n= 3)	
In vitro	18.5 ± 8.3	31.0 ± 6.2	0.05
In vivo	10.2 ± 28.2	28.5 ± 7.3	0.1

Table 5.6 Relationship between mean percentage reduction in candidal adhesion and secretor status in the diabetic patients.

Experiment	Mean % reduction in candidal adhesion ± SD		p
	Secretors (n= 8)	Non-secretors (n= 4)	
In vitro	38.3 ± 21.0	26.3 ± 17.2	0.2
In vivo	25.9 ± 18.8	8.6 ± 19.0	0.3

Table 5.7 Relationship between mean percentage reduction in candidal adhesion and secretor status in the non-diabetic subjects.

5.4 DISCUSSION AND CONCLUSIONS

The results of the present investigations have demonstrated that the adhesion of the strain of C. albicans tested was substantially reduced by pretreatment of the buccal epithelial cells in vitro, or rinsing the mouth, with 0.2% chlorhexidine gluconate for one minute.

Olsen (1975a; b) showed that 0.2% chlorhexidine gluconate mouth rinsing reduced the number of yeast cells on the palatal epithelium and also reduced palatal inflammation in a group of patients with chronic atrophic candidosis. Lamb and Martin (1983) have shown that when chlorhexidine is incorporated into acrylic dental appliances, it diffuses in saliva and can both eliminate and prevent palatal candidosis in rats. Also, Langslet et al. (1974) reported that 0.2% chlorhexidine gluconate was effective in treatment of children with oral candidosis when it was used either as a mouth wash or painted directly onto the lesions. This regimen of mouth rinsing was also able to resolve oral candidal infections in immunocompromised patients (Ferretti et al., 1987). In addition to the previously documented fungicidal effect of chlorhexidine (Bobichon & Bouchet, 1987; Salem et al., 1987), the results of the present investigation suggest that part of the clinical improvement reported by the use of chlorhexidine in earlier studies could be the result of reduced adhesion of candidal species to the epithelial cell surfaces. Therefore, the present data provides evidence that mouth rinsing with 0.2% chlorhexidine gluconate for one minute may have a role in the management of oral candidosis. The regular use of chlorhexidine could be useful in preventing or reducing the incidence of oral candidosis in patients with diabetes mellitus but clearly this would need to be

proven in clinical trials.

Due to the highly cationic property of chlorhexidine, it binds avidly to the negatively charged surfaces including epithelial cells. In addition, it has been shown to adsorb to hydroxyapatite (Moran & Addy, 1985) and salivary proteins (Hjeljord, Rolla, Bonesvoll, 1973). Several studies have concluded that about 30 per cent of the total chlorhexidine dose is retained in the mouth for 24 hours after one minute rinsing (Hjeljord et al., 1973; Bonesvoll et al., 1974).

The adsorption of chlorhexidine to the epithelial cell surface may be a factor in the reduced candidal adhesion observed in the present study. Previously, the reduced adherence of Candida species to acrylic surfaces pretreated with chlorhexidine has been reported (McCourtie et al., 1985; McCourtie et al., 1986a; b) but, the mechanisms have never been fully explained. However, it is conceivable that the binding of chlorhexidine gluconate to the epithelial cell surface is a process which, in some way, alters candidal adhesion.

Previous research has shown rapid release of chlorhexidine adsorbed in the oral cavity in the first hour after mouth rinsing (Bonesvoll et al., 1974). When C. albicans cells were exposed to a subminimal inhibitory concentration of chlorhexidine gluconate for a short time, alterations in the cell wall occurred (Bobichon & Bouchet, 1987) and reduced adhesion to acrylic surfaces was observed (McCourtie et al., 1986a; b). It could be that the chlorhexidine released from the buccal epithelial cells in the assay medium of the present study was sufficient to induce surface changes on C. albicans cells responsible for the observed reduced adherence to epithelial cells,

and similar mechanisms may also pertain in vivo.

It is possible that chlorhexidine released from the buccal epithelial cells into the adhesion assay medium affected the viability of candidal cells and thus reduced candidal adhesion. Kimura and Pearsall (1978) have shown viable candidal cells to adhere in higher number than non-viable ones (Kimura & Pearsall, 1978). However, due to the low concentration of chlorhexidine expected in the assay medium and the short incubation time, this assumption should be considered with caution. Nevertheless, testing the viability of candidal cells, before and after the adhesion assay, is recommended in further studies. In the present study, a single mouth rinse with 0.2% chlorhexidine produced a significant inhibitory effect on candidal adherence to buccal epithelial cells. This raises the question of whether daily mouth rinsing with 0.2% chlorhexidine gluconate would produce comparable or additive inhibitory effects on candidal adhesion.

As to why chlorhexidine showed less of an adherence inhibitory effect in diabetic patients compared with non-diabetic subjects is presently not clear and, therefore, the clinical importance of this observation is uncertain. The lower salivary pH reported frequently in diabetic patients (Banoczy et al., 1987) may partially explain the present findings since the adsorption of chlorhexidine to surfaces is less under acidic conditions (Hjeljord et al., 1973; Morran & Addy, 1985), probably due to a reduction in anionic influence of the recipient surface (Morran & Addy, 1985). However, additional possible explanations include differences in epithelial cell surface properties in diabetics or alterations in salivary composition.

It is also unclear why the adherence inhibitory effect of chlorhexidine was more pronounced in vitro than in vivo, although again there are several theoretical possibilities. Chlorhexidine in the oral cavity has been shown to bind to a variety of structures including salivary proteins (Hjeljord et al., 1973), teeth (Park, Katz, Stookey, 1984), the oral microbial flora (Davies, 1973) in addition to epithelial cells surfaces. Clearly, apart from the epithelial cells, the other aforementioned variables were not included in the in vitro study. As a consequence, probably less chlorhexidine was available to interact with epithelial cells in vivo than in vitro. In addition, chlorhexidine bound to the salivary film on epithelial cells in vivo is likely to have been partly removed by post treatment washing. Relevent to that possibility is the observation of Moran and Addy (1985) who exposed saliva-treated and saliva-untreated polymethylmethacrylate to chlorhexidine. After washing the polymethylmethacrylate, they noticed a relatively reduced uptake of chlorhexidine to saliva-treated samples than to untreated samples. In addition, the ability of chlorhexidine to inhibit candidal adhesion has been shown to be dependent on concentration (McCourtie et al., 1985). Almost certainly any 0.2% chlorhexidine gluconate held intraorally becomes diluted by saliva and consequently exhibits less of an adherence inhibitory effect.

The present investigation has shown considerable inter and intraindividual variations in reduction of candidal adhesion to epithelial cells after exposure to chlorhexidine both in vitro and in vivo. This may be explained partly by a differing ability of epithelial cells to bind chlorhexidine. The magnitude of this ability

seems to change from day to day. In that regard, Bonesvoll et al. (1974) studied 12 subjects and found the oral retention of 0.2% chlorhexidine to range between 32 per cent and 47 per cent after a one minute rinse. The same experiment was repeated at weekly intervals on some of the individuals on five seperate occasions and considerable interindividual variations in chlorhexidine retention were noted between experiments. However, this does not explain why epithelial cells from some subjects showed increased, rather than decreased, candidal adhesion after exposure to chlorhexidine. Whether this has any clinical significance is not certain, but it is tempting to speculate that in those individuals in whom chlorhexidine was adsorbed to the epithelial surfaces in small amounts it was therefore insufficient to inhibit candidal adhesion although it may have decreased the net negative charge on epithelial cell surfaces. A mechanism such as this could theoretically reduce the electrostatic repulsion forces between negatively charged yeast cells and epithelial cells and subsequently enhance candidal adhesion.

Although candidal adhesion to epithelial cells has been shown to be increased in non-secretors of blood group antigens in saliva compared with secretors (Blackwell, 1989), the present results indicated that exposure to chlorhexidine gluconate reduces candidal adhesion to epithelial cells irrespective of the secretor status.

CHAPTER SIX

THE *IN VITRO* AND *IN VIVO* EFFECT OF THE ANTIFUNGAL AGENT NYSTATIN ON ADHESION OF *CANDIDA ALBICANS* TO BUCCAL EPITHELIAL CELLS FROM DIABETIC PATIENTS AND NON-DIABETIC INDIVIDUALS

6.1 INTRODUCTION AND AIMS OF THE STUDY

The polyene nystatin was the first specific antifungal agent to be developed and hence is the drug most commonly used clinically to treat cases of oral candidosis (Odds, 1988). However, even when drug treatment has been effective clinically, recurrence of candidosis commonly occurs after cessation of medication.

Although nystatin is a potent fungicidal agent (Barkvoll & Attramadal, 1989), its possible other actions, for example on adherence mechanisms of *C. albicans*, have rarely been investigated but may be important therapeutically.

Inhibition of candidal adhesion to oral epithelial cells could be advantageous in reducing candidal colonisation and hence oral candidosis, particularly in susceptible patients such those with diabetes mellitus. Recent research has indicated that exposure of acrylic denture base materials to therapeutic concentrations of nystatin, *in vitro*, effectively inhibits adherence of *Candida* species (McCourtie et al., 1986b).

The purposes of the present study were firstly to investigate whether exposure of buccal epithelial cells (BEC) to therapeutic doses of nystatin, *in vitro* and *in vivo*, would result in inhibition of adhesion of *C. albicans*. The second aim was to establish whether inhibition of adhesion was comparable in diabetic patients and non-

diabetic subjects. Finally, since the inherited inability to secrete ABO(H) blood group antigens in saliva has been linked with relatively increased candidal adhesion to epithelial cells (Blackwell, 1989), this study was also designed to investigate whether nystatin therapy would reduce candidal adhesion to epithelial cells from non-secretors as well as from secretors.

6.2 MATERIALS AND METHODS

6.2.1 Patients Selection

Twelve randomly selected patients with diagnosed diabetes mellitus and 12 non-diabetic subjects gave informed consent to participate in the study which was approved by the local Ethics Committee. All subjects from both patients and control groups participated jointly both in the *in vitro* and *in vivo* experiments.

The diabetic patients were recruited from the outpatient Diabetic Clinic at Glasgow Royal Infirmary and refered to the Oral Medicine Unit at Glasgow Dental Hospital where the clinical sampling was performed. The non-diabetic subjects were postgraduate students or staff at Glasgow Dental Hospital and School who willingly volunteered to participate in the study.

6.2.2 Clinical History and Oral Examination

A verbal clinical and medical history was taken from each subject. In the patients group, the type of diabetes mellitus and duration of the disease were recorded. For each subject, the wearing of a dental prosthesis was recorded as was whether the prosthesis was

worn continuously (day and night). Subjects who either consumed alcohol or who smoked cigarettes daily were considered as drinkers and smokers respectively. Each subject underwent a routine oral clinical examination to detect the presence of clinical oral candidal infection.

6.2.3 Secretor Status Determination

Secretor status was determined by the agglutination inhibition technique described by Periera et al. (1969) using unstimulated mixed saliva. Details of saliva collection, the agglutination inhibition technique itself and criteria for defining secretor status were as described in Sections 3.2.7 to 3.2.9.

6.2.4 Preparation of Nystatin Solution

Pilot studies showed that the solubility of nystatin pastilles in distilled water was poor. Therefore, nystatin powder (E.R. Squibb and Sons Ltd., Hounslow, Middlesex, England) was used. Nystatin powder was obtained in sterile vials each containing 500,000 international units (iu) and was dissolved in propylene glycol (The British Drug House Ltd., BDH Laboratory Chemical Devision, Poole, England) as recommended by the manufacturer. Five ml of propylene glycol was injected by a sterile syringe into the vial which was then vibrated until dissolution of the powder occurred thus producing a final drug concentration of 100,000iu / ml. Throughout the period of the experiment a new solution was prepared weekly and stored at temperature of 4°C when not in use and the old solution discarded.

6.2.5 Collection and Preparation of Buccal Epithelial Cells to Study the Effect of Exposure to Nystatin *In Vitro* on Candidal Adhesion *In Vitro*

BEC for the *in vitro* experiment were collected from each subject by gently rubbing the right and left buccal mucosa individually with a sterile cotton swab (Exogen Ltd., Clydebank, Scotland). Each swab was then agitated in 10ml phosphate buffered saline (PBS 0.1M, pH 7.2) in a universal container (Sterilin Ltd., Feltham, England) which was kept on crushed ice until the time of processing. Throughout the study, PBS of the same osmolarity and pH was used. BEC obtained from the right buccal mucosa were used as the control for the experiment (control cells), while cells obtained from the left buccal mucosa were allocated for the *in vitro* experiment (test cells).

BEC were pelleted by centrifugation (MSE Centaur 2 Centrifuge. Fisson's Ltd., Crawley, Sussex, England) at 400g for five minutes. Subsequently, the BEC from the left buccal mucosa were exposed to 1ml nystatin solution (100,000iu) at room temperature while cells from the right buccal mucosa were treated similarly with 1ml propylene glycol. After ten minutes exposure time (the average time needed by a subject to suck completely one nystatin pastille), BEC were harvested on a polycarbonate filter of 25mm diameter and of 12μm pore-size (Nuclepore Gmbh, Germany) mounted on a DEFT (direct epifluorescent filter technique) manifold (Micromeasurement Ltd., Saffron Walden, Essex, England) as shown in Figure 4.1. This method provided rapid and complete separation of BEC from the nystatin solution.

The filter and the retained BEC were then agitated gently in 10ml PBS in a universal container to detach the BEC from the filter and thus become suspended in the PBS. Subsequently, the filter was discarded and the BEC washed twice in 10ml PBS by centrifugation at 400g for five minutes. Microscopic examination demonstrated that none of the BEC had indigenously adherent commensal yeast organisms after the PBS wash. A final BEC suspension of 10^5 cell per ml was prepared by appropriate dilution of the deposit in PBS following haemocytometer counting (Hawksley and Sons Ltd., Lancing, England).

6.2.6 Studying the Effect of Propylene Glycol on Candidal Adhesion to Buccal Epithelial Cells

A pilot experiment was undertaken on BEC obtained from six apparently healthy non-diabetic males to detect if propylene glycol, used in the dissolving of the nystatin powder, had any effect on candidal adhesion. BEC were collected from each subject by gently scraping the right and left buccal mucosae each with a sterile cotton swab followed by agitation of the swabs in 10ml PBS in a universal container. BEC were washed twice in 10ml PBS by centrifugation at 400g for five minutes. After the second wash the supernatant was discarded and BEC from the left buccal mucosa (test cells) were exposed to 1ml propylene glycol while those from the right mucosa (control cells) were treated similarly with PBS. After ten minutes at room temperature, BEC were washed twice in PBS by centrifugation at 400g for five minutes and a final BEC suspension of 10^5 per ml was prepared by dilution in PBS follwed by haemocytometer counting. The adhesion assay was then conducted as described in Section 6.2.10.

6.2.7 Collection and Preparation of Buccal Epithelial Cells to Study the Effect of Nystatin *In Vivo* on Candidal Adhesion *In Vitro*

BEC for the assay *in vivo* were collected from each subject by gently rubbing the right buccal mucosa with a sterile cotton swab which was then agitated in 10ml PBS in a universal container placed on crushed ice. These cells acted as the control BEC for the experiment (control cells). Subsequently, each subject was asked to suck one nystatin pastille (100,000iu, E.R. Squibb and Sons Ltd., Hounslow, Middlesex, England) slowly in the mouth, after denture removal if appropriate. Each subject was specifically asked to allow pastille dissolution to occur as near the buccal mucosae as possible. After the subject had dissolved the pastille completely (approximately ten minutes), BEC were immediately obtained from the left buccal mucosa (test cells) by gentle scraping with a sterile cotton swab and suspended in 10ml PBS in a universal container placed on crushed ice.

BEC were washed twice in 10ml PBS by centrifugation at 400g for five minutes and then suspended in PBS at a concentration of 10^5 cell per ml as determined by haemocytometer counting. Microscopic examination showed that none of the BEC had attached yeast cells after the PBS wash. All samplings were carried out between 1400 and 1700 hours. The *in vivo* and *in vitro* experiments were repeated twice for each subject with a two weeks lapse to avoid any possible carry-over effect of nystatin, and the BEC were collected at the same hour of the day to minimise any possible diurnal variations.

6.2.8 Source, Identification and Maintenance of *Candida Albicans* Strain CDS 88

C. albicans strain CDS 88 used throughout the study was originally isolated from the oral cavity of an asymptomatic carrier in Glasgow Dental Hospital. Species identification and maintenance were described in details in Section 5.2.6.

6.2.9 Preparation of *Candida Albicans* Suspension

C. albicans suspension for the adhesion assay was prepared as described in detail in Section 5.2.7. Briefly, a loopful of the stock culture was inoculated into 10ml Sabouraud's broth (Gibco, Paisley, Scotland) containing 500mM sucrose in a universal container, and incubated overnight (18-24 hours) at 37° C in an orbital shaker incubator (Gallenkamp, Loughborough, England) operating at 100 revolutions per minute. The culture was then harvested by centrifugation at 400g for five minutes and the deposit washed twice in 10ml PBS by centrifugation at 400g for five minutes. A final yeast cells suspension of 10^7 per ml was prepared by appropriate dilution of the deposit in PBS followed by haemocytometer counting.

6.2.10 The Candidal Adhesion Assay

The *in vitro C. albicans* adhesion assay described by Kimura and Pearsall (1978) with minor modification was used throughout as decribed in detail in Section 5.2.8. Briefly, equal volumes (0.5ml) of BEC suspension and *C. albicans* suspension were mixed in a sterile bijou container (Sterilin Ltd., Feltham, England), and incubated on an orbital shaker operating at 80 revolutions per minute at a temperature of 37° C for one hour. Subsequently, BEC were harvested on 25mm

diameter 12μm pore-size polycarbonate filters mounted on a DEFT manifold. The BEC were then washed gently in 30ml PBS in aliquots of five ml to remove unattached yeast cells. Each filter with the washed BEC was then removed and mounted on a 1mm thick, 26 X 76mm glass microscope slide (Chance Proper Ltd., Smethwick, England) and left for one hour to dry at room temperature before being Gram-stained. The filter and the stained BEC on the glass slide were left overnight to air-dry and then a 22 X 22mm glass cover slip (Chance Proper Ltd., Smethwick, England) was fixed on the filter with Harleco resin (Kodak Chemicals, England).

6.2.11 Counting of Adherent Yeast Cells

The glass slides holding the filters were coded and "blind" conditions were used in counting. The number of C. albicans blastospores adherent to 100 individual BEC in randomly selected fields were counted microscopically under x40 objective (Olympus Ltd., London, England). Only morphologically normal epithelial cells, with an intact surface membrane which was not folded, were counted in the selected fields. Clumps of BEC were excluded and pseudohyphae, if present, were not counted.

6.2.12 Statistical Analysis

The percentage reduction in candidal adhesion was calculated as follows:

$$\% \text{ reduction in adhesion} = \frac{Ac - At}{Ac} \times 100$$

Ac = Yeast adhered to 100 BEC of control cells.

At = Yeast adhered to 100 BEC of test cells.

Student's t test for paired data was used to evaluate differences in candidal adherence in either experiment between control and test BEC in the same group. Student's t test for unpaired data was used to evaluate differences in candidal adhesion between different groups as well as between *in vitro* and *in vivo* experiments. Since data of candidal adhesion obtained from secretors and non-secretors were known not to be normally distributed, data were analysed by a Mann-Whitney U test. A probability of less than five per cent was taken to be statistically significant.

6.3 RESULTS

6.3.1 Subjects of the Study

The clinical status of the study subjects is presented in Table 6.1. The diabetic group comprised nine patients (75%) with insulin-dependent diabetes mellitus and three patients (25%) with non-insulin dependent diabetes mellitus. The mean duration of the disease was 10.3 (SD 7.8) years, with a range of three months and 22 years.

None of the subjects was receiving antibiotic or steroid therapy, or had used a mouth wash for at least three months before commencing the study. None of the subjects had either signs or symptoms suggestive of oral candidal infection or had previously received antifungal therapy.

	Diabetics (n= 12)	Non-diabetics (n= 12)
Male	7 (58.3%)	9 (75.0%)
Female	5 (41.7%)	3 (25.0%)
Mean age (years)	37.5 SD 14.5	33.5 SD 4.5
Age range (years)	15 - 56.5	26 - 40
Denture wearers	3 (25.0%)	2 (16.7%)
D and N	2 (66.7%)	2 (100.0%)
Symptomatic xerostomia	3 (25.0%)	0 (00.0%)
Smokers	2 (16.7%)	2 (16.7%)
Alcohol drinkers	0 (00.0%)	0 (00.0%)

D and N : Day and night denture wearing.

Table 6.1 Clinical status of nystatin study subjects.

6.3.2 Effect of Propylene Glycol on Candidal Adhesion to Buccal Epithelial Cells

Pretreating BEC with propylene glycol did not result in a significant effect on candidal adhesion to BEC (p= 0.5; Table 6.2).

Subject no.	Candidal adhesion (Yeast / 100 BEC)	
	Control	Test
1	60	46
2	242	234
3	77	117
4	210	198
5	147	169
6	214	223
Mean	158.3	164.5
SD	76.4	71.7

Table 6.2 Effect of propylene glycol on candidal adhesion to buccal epithelial cells.

6.3.3 The Effect of Exposure of Buccal Epithelial Cells to Nystatin *In Vitro* on Candidal Adhesion

Pretreating BEC for ten minutes with nystatin (100,000iu) *in vitro* resulted in a significant reduction in candidal adhesion in both non-diabetic subjects (Table 6.3; $p<0.002$) and diabetic patients (Table 6.4; $p<0.002$). However, BEC from one non-diabetic subject showed an overall increased candidal adhesion after the nystatin treatment.

The overall mean percentage reduction in candidal adhesion was not statistically different between the two groups ($p>0.05$). It was 25.2 per cent in non-diabetic subjects compared to 29.6 per cent in diabetic patients.

6.3.4 The Effect of Nystatin Pastille Dissolution on Candidal Adhesion

Dissolving a nystatin pastille (100,000iu) in the oral cavity produced an inconsistent and fluctuating effect on candidal adhesion to BEC in subjects of both groups (Tables 6.5 and 6.6). Overall results showed no significant difference in candidal adhesion to BEC before and after a nystatin pastille intake both in non-diabetic subjects (Table 6.5; $p= 0.6$) and diabetic patients (Table 6.6; $p= 0.1$).

Case no.	MCA: Yeast / 100 BEC (SD) Control	Test	% reduction in adhesion
1	159	140	11.9
	88	88	0.0
Mean	**123.5 (50.2)**	**114.0 (36.8)**	**5.9 (8.4)**
2	147	64	56.5
	89	49	44.9
Mean	**118.0 (41.0)**	**56.5 (10.6)**	**50.7 (8.2)**
3	136	96	29.4
	274	92	66.4
Mean	**205.0 (92.6)**	**94.0 (2.8)**	**47.9 (26.2)**
4	784	419	46.5
	504	499	1.0
Mean	**644.0 (198.0)**	**459.0 (56.6)**	**23.7 (32.2)**
5	375	325	13.3
	234	236	+ 0.8
Mean	**295.5 (87.0)**	**280.5 (62.9)**	**6.2 (9.9)**
6	180	142	21.1
	508	323	36.4
Mean	**344.0 (231.9)**	**232.5 (128.0)**	**28.7 (10.8)**
7	152	103	32.3
	694	479	31.0
Mean	**423.0 (383.2)**	**291.0 (265.9)**	**31.6 (0.9)**
8	394	218	44.7
	363	335	7.7
Mean	**378.5 (21.9)**	**276.5 (82.7)**	**26.2 (26.2)**
9	154	123	20.1
	312	190	39.1
Mean	**233.0 (111.7)**	**156.5 (47.4)**	**29.6 (13.4)**
10	519	302	41.8
	324	203	37.3
Mean	**421.5 (137.9)**	**252.5 (70.0)**	**39.5 (3.2)**
11	420	249	40.7
	237	212	10.5
Mean	**328.5 (129.4)**	**230.5 (26.2)**	**25.6 (21.3)**
12	158	128	19.0
	325	476	+46.5
Mean	**241.5 (118.1)**	**302.0 (246.1)**	**+13.7 (46.3)**
MEAN	313.0	228.8	25.2
SD	146.6	110.4	18.3

+ : % increase in candidal adhesion.

Table 6.3 The effect of exposure to nystatin *in vitro* on adhesion of *C. albicans* to buccal epithelial cells from non-diabetic subjects.

Case no.	MCA: Yeast / 100 BEC (SD) Control	Test	% reduction in adhesion
1	250	104	58.4
	337	215	36.2
Mean	**293.5 (61.5)**	**159.5 (78.5)**	**47.3 (15.7)**
2	184	149	19.0
	420	253	41.0
Mean	**306.5 (173.2)**	**201.0 (73.5)**	**30.0 (15.5)**
3	137	88	35.8
	172	212	+23.2
Mean	**154.5 (24.7)**	**150.0 (87.7)**	**6.3 (41.7)**
4	402	172	57.2
	386	180	53.4
Mean	**394.0 (11.3)**	**176.0 (5.6)**	**55.3 (2.7)**
5	177	80	54.8
	177	130	26.5
Mean	**177.0 (0.0)**	**105.0 (35.3)**	**40.6 (20.0)**
6	171	162	2.3
	156	158	+ 1.3
Mean	**163.5 (10.6)**	**162.5 (6.4)**	**0.5 (2.5)**
7	150	139	7.3
	240	172	28.3
Mean	**195.0 (63.6)**	**155.5 (23.3)**	**17.8 (14.8)**
8	448	167	62.7
	273	221	19.0
Mean	**360.5 (123.7)**	**194.0 (38.2)**	**40.8 (30.9)**
9	254	142	44.0
	431	495	+14.8
Mean	**342.5 (125.1)**	**318.5 (249.6)**	**14.6 (41.6)**
10	257	113	56.0
	264	156	40.9
Mean	**260.5 (4.9)**	**134.5 (30.4)**	**48.4 (10.7)**
11	361	344	4.7
	237	193	18.6
Mean	**299.0 (87.7)**	**258.5 (106.8)**	**11.6 (9.8)**
12	472	270	42.8
	329	192	41.6
Mean	**400.5 (101.1)**	**231.0 (55.1)**	**42.2 (0.8)**
MEAN	278.9	188.0	29.6
SD	88.8	59.9	18.6

+ : % increase in candidal adhesion.

Table 6.4 The effect of exposure to nystatin <u>in vitro</u> on adhesion of <u>C. albicans</u> to buccal epithelial cells from diabetic patients.

Case no.	MCA: Yeast / 100 BEC (SD) Control	Test	% reduction in adhesion
1	220	193	12.3
	344	178	48.2
Mean	**282.0 (87.7)**	**185.5 (10.6)**	**30.2 (25.4)**
2	232	169	27.1
	224	168	25.0
Mean	**228.0 (5.6)**	**168.5 (0.7)**	**26.0 (1.5)**
3	388	489	+26.0
	172	330	+91.9
Mean	**280.0 (152.7)**	**409.5 (112.4)**	**+58.9 (46.6)**
4	296	368	+24.3
	477	348	27.0
Mean	**368.5 (128.0)**	**358.0 (14.1)**	**1.3 (36.3)**
5	418	467	+11.7
	392	430	+ 9.7
Mean	**405.0 (18.4)**	**448.5 (26.2)**	**+10.7 (1.4)**
6	311	290	6.7
	357	402	+12.6
Mean	**334.0 (32.5)**	**346.0 (79.2)**	**+ 2.9 (13.6)**
7	588	860	+46.2
	686	835	+24.3
Mean	**637.0 (69.3)**	**847.5 (17.7)**	**+35.2 (15.5)**
8	327	317	3.0
	256	251	1.9
Mean	**291.5 (50.2)**	**284.0 (46.7)**	**2.4 (0.8)**
9	510	387	24.1
	441	424	3.8
Mean	**475.5 (48.8)**	**405.5 (26.2)**	**13.9 (14.3)**
10	513	244	52.4
	174	241	+38.5
Mean	**343.5 (239.7)**	**242.5 (2.1)**	**6.9 (46.3)**
11	259	304	+17.4
	380	353	7.1
Mean	**319.5 (85.5)**	**328.5 (34.6)**	**+ 5.1 (17.3)**
12	693	436	37.0
	603	366	39.3
Mean	**648.0 (63.6)**	**401.0 (49.5)**	**38.1 (1.6)**
MEAN	358.9	368.7	0.5
SD	136.5	175.5	27.2

+ : % increase in candidal adhesion.

Table 6.5 The effect of nystatin pastille dissolution on adhesion of C. albicans to buccal epithelial cells from non-diabetic subjects.

Case no.	MCA: Yeast / 100 BEC (SD) Control		Test		% reduction in adhesion	
1	289		156		46.0	
	217		254		+17.0	
Mean	**253.0**	**(50.9)**	**205.0**	**(69.3)**	**14.5**	**(44.5)**
2	141		184		+30.5	
	214		198		7.5	
Mean	**177.5**	**(51.6)**	**191.0**	**(9.9)**	**11.5**	**(26.9)**
3	108		165		+52.8	
	349		259		25.8	
Mean	**228.5**	**(170.4)**	**212.0**	**(66.5)**	**+13.5**	**(55.6)**
4	283		166		41.3	
	298		170		42.9	
Mean	**290.5**	**(10.6)**	**168.0**	**(2.8)**	**42.1**	**(1.1)**
5	157		85		45.8	
	275		106		61.4	
Mean	**216.0**	**(88.4)**	**95.5**	**(14.8)**	**53.6**	**(11.8)**
6	148		134		9.4	
	296		179		39.5	
Mean	**222.0**	**(104.6)**	**156.5**	**(31.8)**	**24.4**	**(21.3)**
7	234		308		+31.6	
	157		242		+45.1	
Mean	**195.5**	**(54.4)**	**275.0**	**(46.7)**	**+42.8**	**(15.9)**
8	164		256		+56.0	
	256		194		24.2	
Mean	**210.0**	**(65.0)**	**225.0**	**(43.8)**	**+15.9**	**(56.7)**
9	394		498		+26.4	
	790		788		0.2	
Mean	**592.0**	**(280.0)**	**643.0**	**(205.0)**	**+13.1**	**(18.8)**
10	220		203		7.7	
	243		211		13.2	
Mean	**231.5**	**(16.3)**	**207.5**	**(5.6)**	**10.4**	**(3.9)**
11	241		230		4.6	
	467		413		11.6	
Mean	**354.0**	**(159.8)**	**321.5**	**(129.4)**	**8.1**	**(4.9)**
12	475		305		35.8	
	402		406		+ 1.0	
Mean	**438.5**	**(51.6)**	**355.5**	**(71.4)**	**17.4**	**(26.0)**
MEAN	284.1		254.6		6.1	
SD	122.0		141.4		27.0	

+ : % increase in candidal adhesion.

Table 6.6 The effect of nystatin pastille dissolution on adhesion of C. albicans to buccal epithelial cells from diabetic patients.

6.3.5 Relationship Between the Effect of Nystatin Pretreatment on Candidal Adhesion and Secretor Status

The effect of nystatin pretreatment on candidal adhesion was not statistically different between secretors and non-secretors. This finding held true for non-diabetic subjects (Table 6.7) and diabetic patients (Table 6.8) and both in the in vitro and in vivo experiments.

Experiment	Mean % reduction in candidal adhesion ± SD		p
	Secretors (n= 10)	Non-secretors (n= 2)	
In vitro	23.0 ± 19.4	35.5 ± 5.6	0.1
In vivo	3.4 ± 27.3	+14.1 ± 29.8	0.5

+ : % increase in candidal adhesion.

Table 6.7 The relationship between the effect of nystatin pretreatment on candidal adhesion and secretor status in non-diabetic subjects.

Experiment	Mean % reduction in candidal adhesion ± SD		
	Secretors (n= 9)	Non-secretors (n= 3)	p
In vitro	28.8 ± 20.3	32.0 ± 15.4	0.7
In vivo	13.0 ± 24.7	+14.6 ± 26.7	0.3

+ : % increase in candidal adhesion.

Table 6.8 The relationship between the effect of nystatin pretreatment on candidal adhesion and secretor status in diabetic patients.

6.4 DISCUSSION AND CONCLUSIONS

In spite of the considerable array of antifungal agents developed in recent years, nystatin remains the mainstay of chemotherapy for superficial candidosis due to its potent and specific antifungal activity. The fact that nystatin absorption from the gastrointestinal tract is negligible makes oral ingestion of this drug a safe procedure in the treatment of oral candidosis (Odds, 1988). This treatment usually involves sucking nystatin pastilles containing 100,000iu and/or applying suspension or cream formulation of this drug containing 100,000iu / ml on the lesion and to the fitting surface of the denture if relevant (Cawson, 1963).

Nystatin is known to cause membrane damaging effects to Candida cells by binding to sterols in the cell membrane (Hamilton-Miller, 1973). Recent studies have shown pretreatment of C. albicans with nystatin resulted in inhibition of adhesion to buccal epithelial cells (Brenciaglia et al., 1986; Macura, 1988) as well as to denture

acrylic materials (McCourtie et al., 1986b) and plastic surfaces (Vuddhakul et al., 1988). On the other hand, when candidal adhesion was investigated after treatment of the host (rather than the yeast cells) with nystatin, candidal adhesion was considerably reduced to acrylic pretreated with nystatin (McCourtie et al., 1986b). However, the present study is the first to report the ability of nystatin to inhibit adherence to pretreated buccal epithelial cells.

Candidal adhesion to epithelial cells surfaces is recognised as an essential first step in the process of candidal colonisation and subsequent infection (Kennedy, 1988). The present study has demonstrated that exposure of buccal epithelial cells to therapeutic doses of nystatin *in vitro*, effectively reduced adhesion of *C. albicans* although the mechanism(s) involved remains to be determined. However, when the *in vitro* study was simulated by *in vivo* experimentation by dissolving a single nystatin pastille in the oral cavity of the subjects, no difference in candidal adhesion to buccal epithelial cells was noted before and after nystatin intake. These results were, however, obtained in subjects free of an oral candidal infection. Whether nystatin treatment would lead to reduction in candidal adhesion to epithelial cells from patients with oral candidosis is worthy of further study.

As to why nystatin treatment *in vitro* inhibited candidal adhesion is not clear as yet. Nystatin has a preferential binding to ergosterol in the fungal cell membrane (Hamilton-Miller, 1973), which is not a component of mammalian cells. However, nystatin can bind to cholesterol that is present in membranes of human cells (Epstein, 1990). It is tempting to speculate that nystatin binding to cholesterol in the buccal epithelial cell wall may have led to

structural changes and thus induced inhibition of candidal adhesion.

McCourtie et al. (1986b) have shown the inhibitory effect of nystatin on adhesion to pretreated acrylic surfaces to be dose-dependent. The lack of an inhibitory effect of nystatin on adherence *in vivo* in the present study may be due to the diluting and flushing action of saliva in the oral cavity. Thus, intraoral nystatin would probably not have been bathing the buccal mucosa continuously as opposed to the conditions existed *in vitro*. Moreover, due to the intraoral dissolution of the nystatin pastille and the continuous swallowing of saliva, the buccal mucosa may have been exposed to a reduced concentration of nystatin than that was available to buccal epithelial cells *in vitro*. Due to these reasons, it may be worth studying the effect of saliva alone on nystatin-mediated inhibition of candidal adherence. Conventionally, nystatin pastilles (100,000iu) are taken four time daily for four to six weeks to control oral candidosis (Cawson, 1963). In view of the *in vitro* results of the present study, it is tempting to speculate that the frequent and regular usage of nystatin pastilles topically may have an additive effect on reducing adherence of *Candida* species.

On reviewing the literature, it would appear that the influence of antifungal agents on candidal adhesion to host surfaces, has not been conclusively studied (Odds & Webster, 1988). Methodological differences between researchers in addition to variations in the concentration of the drugs used makes comparisons between different studies difficult and, highlights the need for the adoption of standardised methods to study the effect of antifungal drugs on microbial adhesion.

The inherited inability to secrete blood group antigens in saliva has been described in association with increased candidal adhesion to epithelial cells and a predisposition to candidosis (Blackwell, 1989). The results of the present study suggested that nystatin treatment *in vitro* reduced candidal adhesion comparably in both secretors and non-secretors. Nevertheless, this study has indicated that after oral dissolution of a single nystatin pastille, the non-secretors tend to show increased, rather than decreased, candidal adherence. This finding held true for both non-diabetic subjects and diabetic patients. However, the difference was not statistically significant between secretors and non-secretors and one reason for this may be due to the relatively small number of non-secretors used in the present study. More studies with larger groups of secretors and non-secretors are required to identify differences between the two groups.

REFERENCES

For references from 1 to 6 see page 225.

AGARUNOWA, J.S., NOWOSILZEW, G.I., SUTEEWA, T.G., KOSLOWSKAJA, E.A. (1986) Risk of candidosis in patients with endocrinological disorders.
Mykosen, **29**, 474-479.

AL-BASSAM, T., POULIN, D., GIUMMELLY, P., LEMATRE, J., BONALY, R. (1985) Chemical and antigenic alterations of Candida albicans cell walls related to the action of amphotericin B subinhibitory doses.
Journal of Antimicrobial Chemotherapy, **15**, 263-269.

ARENDORF, T.M., WALKER, D.M. (1979) Oral candidal populations in health and disease.
British Dental Journal, **147**, 267-272.

ARENDORF, T.M., WALKER, D.M. (1980) The prevalence and intraoral distribution of Candida albicans in man.
Archives of Oral Biology, **25**, 1-10.

ARNOLD, R.R., BREWER,M., GAUTHIER, J. (1980) Bactericidal activity of human lactoferrin : Sensitivity of a variety of microorganisms.
Infection and Immunity, **28**, 893-898.

BAGDADE, J.D., ROOT, R.K., BULGER, R.J. (1974) Impaired leukocyte function in patients with poorly controlled diabetes.
Diabetes, **23**, 9-15.

BAGDADE, J.D., STEWART, M., WALTERS, E. (1978) Impaired granulocyte adherence. A reversible defect in host defense in patients with poorly controlled diabetes.
Diabetes, **27**, 677-681.

BAIRD, J.D. (1987) Diabetes mellitus. In: Davidson's Principle and Practice of Medicine. 14th edition, ed. McLeod, J. pp. 461-488, Churchill Livingstone. Edinburgh, London, Melbourne, New York.

BANOCZY, J., ALBRECHT, M., RIGO, O., EMBER, G., RITLOP, B. (1987) Salivary secretion rate, pH, lactobacilli and yeast counts in diabetic women.
Acta Diabetologica Latina, **24**, 223-228.

BARKVOLL, P., ATTRAMADAL, A. (1989) Effect of nystatin and chlorhexidine digluconate on Candida albicans.
Oral Surgery, Oral Medicine, Oral Pathology, **67**, 279-281.

BARLOW, A.J.E., CHATTAWAY, F.W. (1969) Observations on the carriage of Candida albicans in man.
British Journal of Dermatology, **81**, 103-106.

BARRETT-BEE, K., HAYES, Y., WILSON, R.G., RYLEY, J.F. (1985) A comparison of phospholipase activity, cellular adherence and pathogenicity of yeasts.
Journal of General Microbiology, **131**, 1217-1221.

BARTELS, H.A. (1965) Local and systemic factors in oral moniliasis.
New York Journal of Dentistry, **35**, 283-285.

BARTHOLOMEW, G.A., RODU, R., BELL, D.S. (1987) Oral candidiasis in patients with diabetes mellitus: A thorough analysis.
Diabetes Care, **10**, 607-612.

BHATT, G.H., DOSHI, J.J., BILIMORIA, K.F (1983) Candida in the oral cavity of diabetics and non-diabetics.
Journal of the Indian Dental Association, **55**, 367-369.

BLACKWELL, C.C. (1989) The role of ABO blood groups and secretor status in host defences.
FEMS Microbiology Immunology, **47**, 341-349.

BLACKWELL, C.C., JAMES, V., WEIR, D.M., GEMMILL, J.D., PATRICK, A.W., COLLIER, A., CLARKE, B.F. (1987) Secretor status of patients with insulin dependent diabetes mellitus.
British Medical Journal, **295**, 1024-1025.

BOBICHON, H., BOUCHET, P. (1987) Action of chlorhexidine on budding Candida albicans: Scanning and transmission electron microscopic study.
Mycopathologia, **100**, 27-35.

BONESVOLL, P., LOKKEN, P., ROLLA, G., PAUS, P.N. (1974) Retention of chlorhexidine in the human oral cavity after mouth rinses.
Archives of Oral Biology, **19**, 209-212.

BRANTING, C., SUND, M.L., LINDER, L.E. (1989) The influence of Streptococcus mutans on adhesion of Candida albicans to acrylic surfaces in vitro.
Archives of Oral Biology, **34**, 347-353.

BRENCIAGLIA, M.I., GHEZZI, M.C., CIPRIANI, P., MANCINI, C., TRANCASSINI, M. (1986) The influence of antifungal drugs on adhesion of Candida albicans to buccal epithelial cells.
Chemiotherapia, **5**, 200-203.

BROWNLEE, M., CERAMI, A., VLASSARA, H. (1988) Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications.
New England Journal of Medicine, **318**, 1315-1321.

BUDTZ-JORGENSEN, E. (1974) The significance of Candida albicans in denture stomatitis.
Scandinavian Journal of Dental Research, **82**, 151-190.

BUDTZ-JORGENSEN, E. (1975) Effect of triamcinolone acetonide on experimental oral candidiasis in monkeys.
Scandinavian Journal of Dental Research, **83**, 171-178.

BUDTZ-JORGENSEN, E. (1978) Clinical aspects of Candida infection in denture wearers.
Journal of the American Dental Association, **96**, 474-479.

BUDTZ-JORGENSEN, E. (1981) Oral mucosal lesions associated with the wearing of removable dentures.
Journal of Oral Pathology, **10**, 65-80.

BUDTZ-JORGENSEN, E., LOE, H. (1972) Chlorhexidine as a denture disinfectant in the treatment of denture stomatitis.
Scandinavian Journal of Dental Research, **80**, 457-464.

BURFORD-MASON, A.P., WEBER, J.C.P., WILLOUGHBY, J.M.T. (1988) Oral carriage of Candida albicans, ABO blood group and secretor status in healthy subjects.
Journal of Medical and Veterinary Mycology, **26**, 49-56.

BURMAN, L.R., BARTELS, H.A., BAILEY, R. (1956) Oral moniliasis in a diabetic patient.
Journal of Oral Medicine, **21**, 177-179.

BYBEE, J.D., ROGERS, D.E. (1964) The phagocytic activity of polymorphonuclear leukocytes obtained from patients with diabetes mellitus.
Journal of Laboratory and Clinical Medicine, **64**, 1-13.

CALDERONE, R.A., CHILAR, R.L., LEE, D.D., HOBERG, K., SCHELD, W.M. (1985) Yeast adhesion in the pathogenesis of endocarditis due to Candida albicans: Studies with adherence negative mutant.
Journal of Infectious Diseases, **152**, 710-715.

CASANOVA, M., GIL, M.L., CARDENOSO, L., MARTINEZ, J.P., SENTANDREU, R. (1989) Identification of wall-specific antigens synthesized during germ tube formation by Candida albicans.
Infection and Immunity, **57**, 262-271.

CASEY, J.I., HEETER, B.J., KLYSHEVICH, K.A. (1977) Impaired response of lymphocytes of diabetic subjects to antigen of Staphylococcus aureus.
Journal of Infectious Diseases, **136**, 495-501.

CAWSON, R.A. (1963) Denture sore mouth and angular cheilitis. Oral candidiasis in adults.
British Dental Journal, **115**, 441-449.

CAWSON, R.A. (1965) Thrush in adult outpatients.
Dental Practitioner, **15**, 361-364.

CENTENO, A., DAVIS, C.P., COHEN, M.S., WARREN, M.M. (1983) Modulation of Candida albicans attachment to human epithelial cells by bacteria and carbohydrates.
Infection and Immunity, **39**, 1354-1360.

CHALLACOMBE, S.J. (1986) Haematological abnormalities in oral lichen planus, candidiasis, leukoplakia and non-specific stomatitis.
International Journal of Oral and Maxillofacial Surgery, **15**, 72-80.

CHANDRASEKAR, P.H., MOLINARI, J.A. (1985) Oral candidiasis: forerunner of acquired immunodeficiency syndrome (AIDS).
Oral Surgery, Oral Medicine, Oral Pathology, **60**, 532-534.

CHILGREN, R.A., QUIE, P., MEUWISSEN, H.J., HONG, R. (1967) Chronic mucocutaneous candidiasis, deficiency of delayed hypersensitivity and selective local antibody defect.
Lancet, **2**, 688-693.

COHEN, B.E., ELIN, R.J. (1974) Enhanced resistance to certain infections in vitamin A treated mice.
Plastic and Reconstructive Surgery, **54**, 192-194.

COLLINS, M.S., PAPPAGIANIS, D. (1974) Lysozyme enhanced killing of Candida albicans and Coccidioides immitis by amphotericin B.
Sabouraudia, **12**, 329-340.

COLLINS-LECH, C., KALEBFLEISCH, J.H., FRANSON, T.R., 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.

COOKE, B.E.D. (1975) Median rhomboid glossitis. Candidiasis and not developmental anomaly.
British Journal of Dermatology, **93**, 399-405.

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

CRITCHLEY, I.A., DOUGLAS, L.J. (1985) Differential adhesion of pathogenic Candida species to epithelial and inert surfaces.
FEMS Microbiology Letters, **28**, 199-203.

CRITCHLEY, I.A., DOUGLAS, L.J. (1987a) Isolation and characterization of an adhesin from Candida albicans.
Journal of General Microbiology, **133**, 629-636.

CRITCHLEY, I.A., DOUGLAS, L.J. (1987b) Role of glycosides as epithelial cell receptors for Candida albicans.
Journal of General Microbiology, **133**, 637-643.

DAVENPORT, J.C. (1970) The oral distribution of Candida in denture stomatitis.
British Dental Journal, **129**, 151-156.

DAVIES, A. (1973) The mode of action of chlorhexidine.
Journal of Periodontal Research, **8 (Supplement 12)**, 68-75.

DAVIES, R.R., DENNING, T.J.V. (1972) Candida albicans and the fungicidal activity of the blood.
Sabouraudia, **10**, 301-312.

DELESPESSE, G., DUCHATEAU, J., BASTENIE, P.A., LAUVAUX, J.P., COLLET, H., GOVAERTS, A. (1974) Cell mediated immunity in diabetes mellitus.
Clinical and Experimental Immunology, **18**, 461-467.

DENBOROUGH, M.A., DOWNING, H.J., MCCREA, M.G. (1967) The ABO system in milk and saliva.
Australian Annals of Medicine, **16**, 320-325.

DOLBY, A.E., WALKER, D.M., MATTHEWS, N. (1981) Candidosis. In: Introduction to Oral Immunity, pp 54-64, Edward Arnold Ltd., London.

DOLL, R., DRANE, H., NEWELL, A.C. (1961) Secretion of blood group substances in duodenal, gastric and stomal ulcer, gastric carcinoma and diabetes mellitus.
Gut, **2**, 352-358.

DOMER, J.E., HECTOR, R.F. (1987) Enhanced immune response in mice treated with penicillin-tetracycline or trimethoprim-sulfamethoxazole when colonized intragastrically with Candida albicans.
Antimicrobial Agents Chemotherapy, **31**, 691-697.

DOUROV, N., COREMANS-PELSENEER, J. (1987) Experimental chronic lingual candidosis induced in streptozotocin diabetic rats.
Mykosen, **30**, 175-183.

DREIZEN, S., MCCREDIE, K.B., KEATING, M.J., BODEY, G.P. (1982) Oral infections associated with chemotherapy.
Postgraduate Medicine, **71**, 133-146.

EPSTEIN, J.B. (1990) Antifungal therapy in oropharyngeal mycotic infections.
Oral Surgery, Oral Medicine, Oral Pathology, **69**, 32-41.

EPSTEIN, J.B., KIMURA, L.H., MENARD, T.W., TRUELOVE, E.L., 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., KOMIYAMA, K., DUNCAN, D. (1986) Oral topical steroid and secondary oral candidiasis.
Journal of Oral Medicine, **41**, 223-227.

ESMANN, V. (1963) Effect of insulin on human leucocytes.
Diabetes, **12**, 545-549.

FARMAN, A.G. (1976) Atrophic lesions of the tongue: A prevalence study among 175 diabetic patients.
Journal of Oral Pathology, **5**, 255-264.

FARMAN, A.G. (1977) Atrophic lesions of the tongue among diabetic patients: Their incidence and regression.
Journal of Oral Pathology, **6**, 396-400.

FARMAN, A.G., NUTT, G. (1976) Oral Candida, debilitating diseases and atrophic lesions of the tongue.
Journal Biologie Buccale, **4**, 203-216.

FERGUSON, D.B. (1975) Salivary gland and saliva. In: Applied Physiology of the Mouth, ed. Lavelle, C.L.B., pp 145-179. John Wright and Sons Ltd. Bristol.

FERRARI, F.A., PAGANI, A., MARCONI, M., STEFANONI, R., SICCARDI, A.G. (1980) Inhibition of candidacidal activity of human neutrophil leukocytes by aminoglycoside antibiotics.
Antimicrobial Agents and Chemotherapy, **17**, 87-88.

FERRETTI, G.A., HANSEN, I.A., WHITTENBURG, K., BROWN, A.T., LILLICH, T.T., ASH, R.C. (1987) Therapeutic use of chlorhexidine in bone marrow transplant patients: Case studies.
Oral Surgery, Oral Medicine, Oral Pathology, **63**, 683-687.

FISHER, B.M., LAMEY, P-J., SAMARANAYAKE, L.P., MACFARLANE, T.W., FRIER, B.M. (1987) Carriage of Candida species in the oral cavity in diabetic patients: Relationship to glycaemic control.
Journal of Oral Pathology, **16**, 282-284.

FLETCHER, J., MATHER, J., LEWIS, M.J., WHITING, G. (1975) Mouth lesions in iron deficiency anaemia: Relationship to Candida albicans in saliva and to impairment of lymphocyte transformation.
Journal of Infectious Diseases, **131**, 44-50.

FOLB, P.I., TROUNCE, J.R. (1970) Immunological aspects of Candida infection complicating steroid and immunosuppressive drug therapy.
Lancet, **2**, 1112-1114.

2-
GASIC, G.J., BERWICK, L., SORENTINO, M. (1968) Positive and negative colloidal ion as cell surface electron stains.
Laboratory Investigation, **18**, 63-71.

GHANNOUM, M., ABU ELTEEN, K. (1986) Correlative relationship between proteinase production, adherence and pathogenicity of various strains of Candida albicans.
Journal of Medical and Veterinary Mycology, **24**, 407-413.

GHANNOUM, M.A., ABU ELTEEN, K. (1987) Effect of growth of Candida species in the presence of various glucocorticoids on the adherence to human buccal epithelial cells.
Mycopathologia, **98**, 171-178.

GHANNOUM, M.A., BURNS, G.R., ABU ELTEEN, K., RADWAN, S.S. (1986) Experimental evidence of the role of lipids in adherence of Candida species to human buccal epithelial cells.
Infection and Immunity, **54**, 189-193.

GIBBONS, R.J., VAN HOUTE, J. (1975) Bacterial adherence in oral microbial ecology.
Annuals Review of Microbiology, **29,** 19-44.

GILMORE, B.J., RETSINAS, E.M., LORENZ, J.S., HOSTETTER, M.K. (1988) An iC3b receptors on Candida albicans: Structure, function and correlates for pathogenicity.
Journal of Infectious Diseases, **157,** 38-46.

GLASS, R.L. (1951) Occurrence of yeasts in the saliva of children.
Journal of Dental Research, **30,** 468.

GODFREY, S., HAMBLETON, G., KONIG, P. (1974) Steroid aerosols and candidiasis.
British Medical Journal, **1,** 387.

GOUGH, P.M., WARNOCK, D.W., RICHARDSON, D.M., MANSELL, N.J., KING, J.M. (1984) IgA and IgG antibodies to Candida albicans in the genital tract secretions in women with or without vaginal candidosis.
Sabouraudia, **22,** 265-271.

HAMILTON-MILLER, J.M.T. (1973) Chemistry and biology of the polyene macrolide antibiotics.
Bacteriological Reviews, 37, 166-196.

HARRIS, H.J. (1950) Aureomycin and chloramphenicol in brucellosis, with special reference to side effects.
Journal of American Medical Association, **142,** 161-163.

HIGGS, J.M., WELLS, R.S. (1972) Chronic mucocutaneous candidosis: Associated abnormalities of iron metabolism.
British Journal of Dermatology, **86,** 88-101.

HILL, L.V.H., TAN, M.H., PERIERA, L.H., EMBIL, J.A. (1989) Association of oral candidiasis with diabetic control.
Journal of Clinical Pathology, **42,** 502-505.

HJELJORD, L.G., ROLLA, G., BONESVOLL, P. (1973) Chlorhexidine-protein interactions.
Journal of Periodontal Research, **8 (Supplement 12),** 11-16.

HOWLETT, J.A., SQUIER, C.A. (1980) Candida albicans ultrastructure: Colonization and invasion of oral epithelium.
Infection and Immunity, **29,** 252-260.

HURLEY, R. (1966) Experimental infection with Candida albicans in modified hosts.
Journal of Pathology and Bacteriology, **92,** 57-67.

HURLEY, D.L., BALOW, J.E., FAUCI, A.S. (1975) Experimental disseminated candidiasis. Administration of glucocorticosteroid, susceptibility to infection and immunity.
Journal of Infectious Diseases, **132,** 393-398.

3_

ISENBERG, H.D., BERKMAN, J.I., CARITO, S.L. (1960) Factors leading to overt monilial disease. Retardation of growth of Candida albicans by metabolic end products of intestinal bacteria.
Antimicrobial Agents Annals, **1960,** 570-575.

JEGANATHAN, S., UFOMATA, D., HOBKIRK, J.A., IVANYI, L. (1987) Immunoglobulin A1 and A2 subclass of salivary antibodies to Candida albicans in patients with oral candidosis.
Clinical and Experimental Immunology, **70,** 316-321.

JENKINS, W.M.M., MACFARLANE, T.W., FERGUSON, M.M., MASON, D.K. (1977) Nutritional deficiency in oral candidosis.
International Journal of Oral Surgery, **6,** 204-210.

JENNINGS, K.J. (1988) Laboratory and experimental studies of prosthesis related oral candidiasis.
M.Sc. Thesis, University of Glasgow.

JOHNSTON, R.D., CHICK, E.W., JOHNSTON, N.S., JARVIS, M.A. (1967) Asymptomatic quantitative increase of Candida albicans in the oral cavity. Predisposing conditions.
Southern Medical Journal, **60,** 1244-1247.

JOYSON, D.H.M., JACOBS, A., WALKER, D.M., DOLBY, A.E. (1972) Defect of cell mediated immunity in patients with iron deficiency anaemia.
Lancet, **2,** 1058-1059.

KANDHARI, K.C., PASRICHA, J.S., DUGGAL, S.K., PANDHI, R.K. (1969) Investigation into the causation of increased susceptibility of diabetics to cutaneous infections.
Indian Journal of Medical Research, **57,** 1295-1302.

KASHIMA, M., TAKAHASHI, H., SHIMOZUMA, M., EPSTEIN, W.L., FUKUYAMA, K. (1989) Candidacidal activities of proteins partially purified from rat epidermis.
Infection and Immunity, **57,** 186-190.

KEARNS, M.J., DAVIES, P., SMITH, H. (1983) Variability of the adherence of Candida albicans strains to human buccal epithelial cells: Inconsistency of difference between strains related to virulence.
Sabouraudia, **21,** 93-98.

KEENE, J.J. (1975) Arteriosclerotic changes within the diabetic oral vasculature.
Journal of Dental Research, **54,** 77-82.

KENNEDY, M.J. (1981) Inhibition of Candida albicans by the anaerobic oral flora of mice in vitro.
Sabouraudia, **19,** 205-208.

KENNEDY, M.J. (1988) Adhesion and association mechanisms of Candida albicans.
Current Topics in Medical Mycology, **2,** 73-169.

KENNEDY, M.J., ROGERS, A.L., HANSELMEN, L.R., SOLL, D.R., YANCEY, R.J. (1988) Variation in adhesion and cell surface hydrophobicity in Candida albicans white and opaque phenotypes.
Mycopathologia, **102**, 149-156.

KENNEDY, M.J., SANDIN, R.L. (1988) Influence of growth conditions on Candida albicans adhesion, hydrophobicity and cell wall structure.
Journal of Medical and Veterinary Mycology, **26**, 79-92.

KIMURA, L.H., PEARSALL, N.N. (1978) Adherence of Candida albicans to human buccal epithelial cells.
Infection and Immunity, **21**, 64-68.

KIMURA, L.H., PEARSALL, N.N. (1980) Relationship between germination of Candida albicans and increased adherence to human buccal epithelial cells.
Infection and Immunity, **28**, 464-468.

KINANE, D.F., BLACKWELL, C.C., WINSTANLEY, F.P., WEIR, D.M. (1983) Blood group, secretor status and susceptibility to infection by Neisseria gonorrhoeae.
British Journal of Venereal Diseases, **59**, 44-64.

KING, R.D., LEE, J.C., MORRIS, A.L. (1980) Adherence of Candida albicans and other Candida species to mucosal epithelial cells.
Infection and Immunity, **27**, 667-674.

KIRKPATRICK, C.H. (1984) Host factors in defence against fungal infection.
American Journal of Medicine, **77**, 1-12.

KIRKPATRICK, C.H., BENNETT, J.E. (1971) Chronic mucocutaneous candidiasis. Model-building in cellular immunity.
Annals of Internal Medicine, **47**, 955-978.

KIRKPATRICK, C.H., GREEN, I., RICH, R.R., SCHADE, A.L. (1971) Inhibition of growth of Candida albicans by iron unsaturated lactoferrin: Relation to host defense mechanisms in chronic mucocutaneous candidiasis.
Journal of Infectious Diseases, **124**, 539-544.

KLEIN, R.S., HARRIS, C.A., SMALL, C.B., MOLL, B., LESSER, M.L., FRIEDLAND, G.H. (1984) Oral candidiasis in high risk patients as the initial manifestation of acquired immunodeficiency syndrome.
New England Journal of Medicine, **311**, 354-358.

KNIGHT, L., FLETCHER, J. (1971) Growth of Candida albicans in saliva: Stimulation by glucose associated with antibiotics, corticosteroids and diabetes mellitus.
Journal of Infectious Diseases, **123**, 371-377.

KOLTZ, S.A., DRUTZ, D.J., ZAJIC, J.E. (1985) Factors governing adherence of Candida species to plastic surfaces.
Infection and Immunity, **50**, 97-101.

KORTING, H.C., OLLERT, M., GEORGII, A., FROSCHL, M. (1988) In vitro susceptibility and biotype of Candida albicans isolates from the oral cavities of patients infected with human immunodeficiency virus. Journal of Clinical Microbiology, **26,** 2626-2631.

KOSTIALA, I. (1986) Acute fungal stomatitis in compromised host. Causative agents, serological findings and topical treatment. Proceedings of the Finnish Dental Society, **82 (Supplement 7-8),** 1-96.

KUMAR, S., MATHUR, D.R., CHAUDHARY, S.K., CHAWLA, S.N. (1982) A comparative study of germ tube formation by Candida albicans in serum and saliva of healthy individuals and diabetic patients. Journal of Postgraduate Medicine, **28,** 84-87.

LAMB, D.J., MARTIN, M.V. (1983) An in vitro and in vivo study of the effect of incorporation of chlorhexidine into autopolymerizing acrylic resin plates upon the growth of Candida albicans. Biomaterials, **4,** 205-209.

LAMEY, P-J., DARWAZEH, A., FISHER, B.M., SAMARANAYAKE, L.P., MACFARLANE, T.W., FRIER, B.M. (1988) Secretor status, candidal carriage and candidal infection in patients with diabetes mellitus. Journal of Oral Pathology, **17,** 354-357.

LANGSLET, A., OLSEN, I., LIE, S.O., LOKKEN, P. (1974) Chlorhexidine treatment of oral candidiasis in seriously diseased children. Acta Pediatrica Scandinavica, **63,** 809-811.

LARKIN, J.G., FRIER, B.M., IRELAND, J.T. (1985) Diabetes mellitus and infection. Postgraduate Medical Journal, **61,** 233-237.

LEE, J., KING, R.D. (1983) Characterization of Candida albicans adherence to human vaginal epithelial cells in vitro. Infection and Immunity, **41,** 1024-1030.

LEHNER, T. (1965) Immunofluorescent investigation of Candida albicans antibodies in human saliva. Archives of Oral Biology, **10,** 975-980.

LEHNER, T., WARD, R.G. (1970) Iatrogenic oral candidosis. British Journal of Dermatology, **83,** 161-166.

LEHNER, T., WILTON, J.M.A., IVANYI, L. (1972) Immunodeficiency in chronic mucocutaneous candidosis. Immunology, **22,** 775-787.

4&5
LEHRER, N., SEGAL, E., CIHLAR, R.L., CALDERONE, R.A. (1986) Pathogenesis of vaginal candidosis: Studies with a mutant which has reduced ability to adhere in vitro. Journal of Medical and Veterinary Mycology, **24,** 127-131.

LILJEMARK, W.F., GIBBONS, R.J. (1973) Suppression of Candida albicans by human oral streptococci in gnotobiotic mice. Infection and Immunity, **8,** 846-849.

LINCOLN, P.J., DODD, B.E. (1972) Variations in secretor and Lewis type frequencies within the British Isles.
Journal of Medical Genetics, **9**, 43-45.

LUDLAM, G.B., HENDERSON, J.L. (1942) Neonatal thrush in a maternity hospital.
Lancet, **1**, 64-70.

MACAFEE, A.L. (1964) Blood group and diabetes mellitus.
Journal of Clinical Pathology, **17**, 39-41.

MCCOURTIE, J., DOUGLAS, L.J. (1981) Relationship between cell surface composition of Candida albicans and adherence to acrylic after growth in different carbon sources.
Infection and Immunity, **32**, 1234-1241.

MCCOURTIE, J., DOUGLAS, J. (1984) Relationship between cell surface composition, adherence and virulence of Candida albicans.
Infection and Immunity, **45**, 6-12.

MCCOURTIE, J., MACFARLANE, T.W., SAMARANAYAKE, L.P. (1985) Effect of chlorhexidine gluconate on the adherence of Candida species to denture acrylic.
Journal of Medical Microbiology, **20**, 97-104.

MCCOURTIE, J., MACFARLANE, T.W., SAMARANAYAKE, L.P. (1986a) Effect of saliva and serum on the adherence of Candida species to chlorhexidine treated denture acrylic.
Journal of Medical Microbiology, **21**, 209-213.

MCCOURTIE, J., MACFARLANE, T.W., SAMARANAYAKE, L.P. (1986b) A comparison of the effect of chlorhexidine gluconate, amphotericin B and nystatin on the adherence of Candida species to denture acrylic.
Journal of Antimicrobial Chemotherapy, **17**, 575-583.

MACFARLANE, T.W., HELNARSKA, S.J. (1976) The microbiology of angular cheilitis.
British Dental Journal, **140**, 403-406.

MACFARLANE, T.W., MASON, D.K. (1974) Changes in the oral flora in Sjogren's syndrome.
Journal of Clinical Pathology, **27**, 416-419.

MCKENDRICK, A.J.W. (1968) Denture stomatitis and angular cheilitis in patients receiving long term tetracycline therapy.
British Dental Journal, **124**, 412-417.

MCKENDRICK, A.J.W., WILSON, M.I., MAIN, D.M.G. (1967) Oral Candida and long term tetracycline therapy.
Archives of Oral Biology, **12**, 281-290.

MACKENZIE, D.W.R. (1962) Serum tube identification of Candida albicans.
Journal of Clinical Pathology, **15**, 563-565.

MACURA, A.B. (1988) The influence of some antifungal drugs on in vitro adherence of Candida albicans to human buccal epithelial cells. Mycoses, **31**, 371-376.

MAHMOUD, A.A.F., RODMAN, H.M., MANDEL, M.A., WARREN, K.S. (1975) Induced and spontaneous diabetes mellitus and suppression of cell mediated immunological response. Journal of Clinical Investigation, **57**, 362-367.

MAKRIDES, H.C., MACFARLANE, T.W. (1982) The effect of commensal bacteria on the adherence of Candida albicans to epithelial cells in vitro. Microbios Letters, **21**, 55-61.

MAKRIDES, H.C., MACFARLANE, T.W. (1983) An investigation of the factors involved in increased adherence of Candida albicans to epithelial cells mediated by E. coli. Microbios, **38**, 177-185.

MARQUIS, G., MONTPLAISIR, S., GARZON, S., STRYKOWSKI, H., AUGER, P. (1982) Fungitoxicity of muraminidase. Laboratory Investigation, **46**, 627-636.

MARTIN, M.V., AL-TIKRITI, U., BRAMLEY, P.A. (1981) Yeast flora of the mouth and skin during and after irradiation for oral and laryngeal cancer. Journal of Medical Microbiology, **14**, 457-467.

MARTIN, H.E., HOWE, M.E. (1983) Glossitis rhombica mediana. Annals of Surgery, **107**, 39-49.

MAY, S.J., BLACKWELL, C.C., WEIR, D.M. (1986) Non-secretion of blood group antigens and susceptibility to Candida albicans. Journal of Dental Research, **65**, 503.

MAY, S.J., BLACKWELL, C.C., WEIR, D.M. (1989) Lewis^a blood group antigen of non-secretors: A receptor for Candida blastospores. FEMS Microbiology Immunology, **47**, 407-409.

MEHNERT, B., MEHNERT, H. (1958) Yeast in urine and saliva of diabetic and non-diabetic patients. Diabetes, **7**, 293-297.

MILLER, M.E., BAKER, L. (1972) Leukocyte functions in juvenile diabetes mellitus: Humoral and cellular aspects. Journal of Paediatrics, **81**, 979-982.

MILNE, L.J.R., CROMPTON, G.K. (1974) Beclomethasone dipropionate and oropharyngeal candidiasis. British Medical Journal, **21**, 797-798.

MINAGI, S., MIYAKE, Y., AKAGAWA, Y., TSURU, H., SUGINAKA, H. (1986) Effect of saliva on candidal adherence to polymethyl methacrylate resin. Horishima Journal of Medical Science, **35**, 93-97.

MIYAKE, Y., FUJITA, Y., MINAGI, S., SUGINAKA, H. (1986) Surface hydrophobicity and adherence of Candida to acrylic surfaces. Microbios, **46**, 7-14.

MOLENAAR, D.M., PALUMBO, P.J., WILSON, W.R., RITTS, R.E. (1976) Leukocyte chemotaxis in diabetic patients and their non-diabetic first degree relatives. Diabetes, **25 (Supplement 2)**, 880-883.

MONTES, L.F., KRUMDIECK, C., CORNWELL, P.E. (1973) Hypovitaminosis A in patients with mucocutaneous candidiasis. Journal of Infectious Diseases, **128**, 227-230.

MORAN, J., ADDY, M. (1985) The pattern of adsorption of cationic antiseptics to polymethylmethacrylate. Journal of Oral Rehabilitation, **12**, 81-90.

MOWAT, A.G., BAUM, J. (1971) Chemotaxis of polymorphonuclear leukocytes from patients with diabetes mellitus. New England Journal of Medicine, **284**, 621-627.

MURATSU, K., MORIOKA, T. (1985) Levels of salivary lysozyme, lactoperoxidase and lactoferrin in diabetic hamsters. Infection and Immunity, **48**, 389-394.

NATHAN, D.M., SINGER, D.E., HURXTHAL, K., GOODSON, J.D. (1984) The clinical information value of the glycosylated haemoglobin assay. New England Journal of Medicine, **310**, 341-346.

NEWTON, A.V. (1962) Denture sore mouth. A possible aetiology. British Dental Journal, **112**, 868-874.

NIKAWA, H., SADAMORI, S., HAMADA, T., SATOU, N., OKUDA, K. (1989) Non-specific adherence of Candida species to surface-modified glass. Journal of Medical and Veterinary Mycology, **27**, 269-271.

NOLAN, C.N., BEATY, H.N., BAGDADE, J.D. (1978) Further characterization of the impaired bactericidal function of granulocytes in patients with poorly controlled diabetes. Diabetes, **27**, 889-894.

NUGENT, K.M., COUCHOT, K.R. (1986) Effects of sublethal concentrations of amphotericin B on Candida albicans. Journal of Infectious Diseases, **154**, 665-669.

NYQUIST, G. (1952) A study of denture sore mouth. An investigation of traumatic, allergic and toxic lesions of the oral mucosa arising from the use of full dentures. Acta Odontologica Scandinavica, **10 (Supplement 9)**, 11-154.

ODDS, F.C. (1988) Candida and candidosis. A review and bibliography. 2nd edition. Baillier Tindall, London, Philadelphia, Toronto, Sedney, Tokyo.

6-

ODDS, F.C., EVANS, E.G.V. (1980) Distribution of pathogenic yeasts and humoral antibodies to Candida among hospitalized patients.
Journal of Clinical Pathology, **33**, 750-756.

ODDS, F.C., EVANS, E.G.V., TAYLOR, M.A.R. (1978) Prevalence of pathogenic yeasts and humoral antibodies to Candida in diabetic patients.
Journal of Clinical Pathology, **31**, 840-844.

ODDS, F.C., WEBSTER, C.E. (1988) Effects of azole antifungals in vitro on host parasite interactions relevant to Candida infections.
Journal of Antimicrobial Chemotherapy, **22**, 473-481.

OLSEN, I. (1975a) Denture stomatitis. Effects of chlorhexidine and amphotericin B on the mycotic flora.
Acta Odontologica Scandinavica, **33**, 41-46.

OLSEN, I. (1975b) Denture stomatitis. The clinical effects of chlorhexidine and amphotericin B.
Acta Odontologica Scandinavica, **33**, 47-52.

OLSEN, I., BIRKELAND, J.M. (1976) Initiation and aggravation of denture stomatitis by sucrose rinse.
Scandinavian Journal of Dental Research, **84**, 94-97.

OLSEN, I., HAANAES, H.R. (1977) Experimental palatal candidosis and saliva flow in monkeys.
Scandinavian Journal of Dental Research, **85**, 135-141.

PALLISTER, C.J., WARNOCK, D.W. (1989) Effect of antimicrobial and antineoplastic drugs alone and in combination on the phagocytic and candidacidal function of human polymorphonuclear leucocytes.
Journal of Antimicrobial Chemotherapy, **23**, 87-94.

PARK, K.K., KATZ, S., STOOKEY, K. (1984) Chlorhexidine uptake by saliva-coated and uncoated hydroxyapatite and salivary sediment.
Journal of Oral Medicine, **39**, 126-130.

PARVINEN, T. (1984) Stimulated salivary flow rate, pH and Lactobacillus and yeast concentrations in persons with different type of dentition.
Scandinavian Journal of Dental Research, **92**, 412-418.

PARVINEN, T., LARMAS, M. (1981) The relation of stimulated salivary flow rate and pH to Lactobacillus and yeast concentration in saliva.
Journal of Dental Research, **60**, 1929-1935.

PERIERA, M., DODD, B.E., MARCHANT, J.V. (1969) The detection of A,B and H group specific substances in stains from body fluids by absorption-elution and mixed agglutination technique.
Medicine, Science and the Law, **9**, 116-121.

PEREIRA, M.A.A., SANNOMIYA, P., LEME, J.G. (1987) Inhibition of leukocyte chemotaxis by factors in alloxan induced diabetic rat plasma.
Diabetes, **36**, 1307-1314.

PETERS, R.B., BAHN, A.N., BARENS, G. (1966) Candida albicans in the oral cavities of diabetics.
Journal of Dental Research, **45**, 771-777.

PETERS, W.H., GOHLER, W. (1986) ABH secretion and Lewis red cell groups in diabetic and normal subjects from Ethiopia.
Experimental and Clinical Endocrinology, **88**, 64-70.

PHELAN, J.A., LEVIN, S.M. (1986) Prevalence study of denture stomatitis in subjects with diabetes mellitus or elevated plasma glucose level.
Oral Surgery, Oral Medicine, Oral Pathology, **62**, 303-305.

PHILLIPS, A.W., BALISH, E. (1966) Growth and invasion of Candida albicans in the germ free and conventional mouse after oral challange.
Applied Microbiology, **14**, 737-741.

POHJAMO, L., KNUUTTILA, M., TERVONEN, T., HAUKIPURO, K. (1988) Caries prevalence related to the control of diabetes.
Proceedings of the Finnish Dental Society, **84**, 247-251.

POLLOCK, J.J., DENEPITIYA, L., MACKAY, B.J., IACONO, V.J. (1984) Fungistatic and fungicidal activity of human parotid salivary histidine-rich polypeptides on Candida albicans.
Infection and Immunity, **44**, 702-707.

PORTER, S.R., SCULLY, C. (1986) Candidiasis endocrinopathy syndrome.
Oral Surgery, Oral Medicine, Oral Pathology, **61**, 573-578.

PRADHAN, A.C., CHAWLA, T.N., SAMUEL, K.C., PRADHAN, S. (1971) The relationship between periodontal disease and blood groups and secretor status.
Journal of Periodontal Research, **6**, 294-300.

QVIST, R., LARKINS, R.G. (1981) Decreased stimulated glucose oxidation by polymorphonuclear leukocytes from insulin treated diabetic subjects.
Diabetes, **30**, 256-260.

RAGAB, A.H., HAZLETT, B., COWAN, D.H. (1972) Response of peripheral blood lymphocytes from patients with diabetes mellitus to phytohemagglutinin and Candida albicans antigen.
Diabetes, **21**, 906-907.

RAY, T.L., DIGRE, K.B., PAYNE, C.D. (1984) Adherence of Candida species to human epidermal corneocytes and buccal mucosal cells: Correlation with cutaneous pathogenicity.
Journal of Investigative Dermatology, **83**, 37-41.

RAYFIELD, E.J., AULT, M.J., KEUSCH, G.T., BROTHERS, M.J., NECHEMIAS, C., SMITH, H. (1982) Infection and diabetes: The case of glucose control.
American Journal of Medicine, **72**, 439-450.

REINHOLDT, J., KROGH, P., HOLMSTRUP, P. (1987) Degradation of IgA1, IgA2 and s-IgA by Candida and Torulopsis species.
Acta Pathologica Microbiologica Immunologica Scandinavica Section C, **95**, 265-279.

RENNIE, J.S., HUTCHEON, A.W., MACFARLANE, T.W., MACDONALD, D.G. (1983) The role of iron deficiency in experimentally-induced oral candidosis in rat.
Journal of Medical Microbiology, **16**, 363-369.

RENNIE, J.S., MACDONALD, D.G. (1982) Quantitative histological analysis of the epithelium of the ventral surface of hamster tongue in experimental iron deficiency.
Archives of Oral Biology, **27**, 393-397.

RENNIE, J.S., MACDONALD, D.G., DAGG, J.H. (1984) Iron and the oral epithelium. A review.
Journal of the Royal Society of Medicine, **77**, 602-607.

REPINE, J.E., CLAWSON, C.C., GOETZ, F.C. (1980) Bactericidal function of neutrophils from patients with acute bacterial infections and from diabetics.
Journal of Infectious Diseases, **142**, 869-875.

ROBINSON, J.E. (1976) Characteristics of irradiated soft and hard tissues.
Journal of Prosthetic Dentistry, **35**, 549-552.

ROGERS, T.J., BALISH, E. (1980) Immunity to Candida albicans.
Microbiology Review, **44**, 660-682.

ROITT, I.M., LEHNER, T. (1983) Oral immunity. In: Immunity of oral diseases, 2nd. edition, pp 279-304. Blackwell scientific publications, Oxford, London, Edinburgh, Bristol, Melbourne.

ROMANOW, I. (1964) The relationship of moniliasis to the presence of antibiotics in periodontal packs.
Periodontics, **2**, 298-300.

ROSE, J.A. (1968) Aetiology of angular cheilosis: Iron metabolism.
British Dental Journal, **125**, 67-73.

ROSNER, F., VALMONT, I., KOZINN, P.J., CAROLINE, L. (1970) Leukocyte function in patients with leukemia.
Cancer, **25**, 835-842.

ROSSIE, K.M., TAYLOR, J., BECK, F.M., HODGSON, S., BLOZIS, G. (1987) Influence of radiation therapy on oral Candida albicans colonization. A quantitative assessment.
Oral Surgery, Oral Medicine, Oral Pathology, **64**, 698-700.

ROTH, F.J., GOLDSTEIN, M.I. (1961) Inhibition of growth of pathogenic yeasts by human serum.
Journal of Investigative Dermatology, **36,** 383-387.

SAHAY, J.N., CHATTERJEE, S.S., STANBRIDGE, T.N. (1979) Inhaled corticosteroid aerosols and candidiasis.
British Journal of Diseases of Chest, **73,** 164-168.

SAIKI, O., NEGORO, S., TSUYUGUCHI, I., YAMAMURA, Y. (1980) Depressed immunological defense mechanisms in mice with experimentally induced diabetes.
Infection and Immunity, **28,** 127-131.

SALEM, A.M., ADAMS, D., NEWMAN, H.N., RAWLE, L.W. (1987) Antimicrobial properties of 2 aliphatic amines and chlorhexidine in vitro and in saliva.
Journal of Clinical Periodontology, **14,** 44-47.

SAMARANAYAKE, L.P. (1986) Nutritional factors and oral candidosis.
Journal of Oral Pathology, **15,** 61-65.

SAMARANAYAKE, L.P., CALMAN, K.C., FERGUSON, M.M., KAY, S.B., MACFARLANE, T.W., MAIN, B., WELSH, J., WILLOX, J. (1984) The oral carriage of yeasts and coliforms in patients on cytotoxic therapy.
Journal of Oral Pathology, **13,** 390-393.

SAMARANAYAKE, L.P., HUGHES, A., MACFARLANE, T.W. (1984) The proteolytic potential of Candida albicans in human saliva supplemented with glucose.
Journal of Medical Microbiology, **17,** 13-22.

SAMARANAYAKE, L.P., HUGHES, A., WEETMAN, D.A., MACFARLANE, T.W. (1986a) Growth and acid production of Candida species in human saliva supplemented with glucose.
Journal of Oral Pathology, **15,** 251-254.

SAMARANAYAKE, L.P., MCCOURTIE, J., 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., MACFARLANE, T.W. (1980) An in vitro study of the adherence of Candida albicans to acrylic surfaces.
Archives of Oral Biology, **25,** 603-609.

SAMARANAYAKE, L.P., MACFARLANE, T.W. (1981a) A retrospective study of patients with recurrent chronic atrophic candidosis.
Oral Surgery, Oral Medicine, Oral Pathology, **52,** 150-153.

SAMARANAYAKE, L.P., MACFARLANE, T.W. (1981b) 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., MACFARLANE, T.W. (1982a) Factors affecting the 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., MACFARLANE, T.W. (1982b) 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., MACFARLANE, T.W., LAMEY, P-J., FERGUSON, M.M. (1986b) A comparison of oral rinse and imprint sampling techniques for the detection of yeasts, Coliforms and Staphylococcus aureus carriage in the oral cavity.
Journal of Oral Pathology, **15**, 386-388.

SAMARANAYAKE, L.P., MCLAUGHLIN, L., MACFARLANE, T.W. (1988) Adherence of Candida species to fibrin clots in vitro.
Mycopathologia, **102**, 135-138.

SAMARANAYAKE, L.P., RAESIDE, J., MACFARLANE, T.W. (1984) Factors affecting the phospholipase activity of Candida species in vitro.
Journal of Medical and Veterinary Mycology, **22**, 201-204.

SANDIN, R.L., ROGERS, A.L., BENEKE, E.S., FERNANDEZ, M.I. (1987a) Influence of mucosal cell origin on the in vitro adherence of Candida albicans: Are mucosal cells from different sources equivalent ?
Mycopathologia, **98**, 111-119.

SANDIN, R.L., ROGERS, A.L., FERNANDEZ, M.I., BENEKE, E.S. (1987b) Variations in affinity to Candida albicans in vitro among human buccal epithelial cells.
Journal of Medical Microbiology, **24**, 151-155.

SANDIN, R.L., ROGERS, A.L., PATTERSON, R.J., BENEKE, E.S. (1982) Evidence of mannose mediated adherence of Candida albicans to human buccal cells.
Infection and Immunity, **35**, 79-85.

SCHUMACHER, H.R., GINNS, D.A., WARREN, W.J. (1964) Fungus infection complicating leukemia.
American Journal of Medical Science, **247**, 313-323.

SCULLY, C., CAWSON, R.A., PORTER, S.R. (1986) Acquired immune deficiency syndrome: Review.
British Dental Journal, **161**, 53-60.

SEELIG, M.S. (1966) Mechanisms by which antibiotics increase the incidence and severity of candidiasis and alter the immunological defenses.
Bacteriological Review, **30**, 442-454.

SEGAL, E., LEHRER, N., 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., SCHECHTER, A. (1984) Correlative relationship between adherence of Candida albicans to human vaginal cells in vitro and candidal vaginitis.
Sabouraudia, **22**, 191-200.

SENALDI, G., MILLWARD, B.A., HUSSAIN, M.J.; PYKE, D.A., LESLIE, R.D.G., VERGANI, D. (1988) Low serum haemolytic function of the fourth complement (C4) in insulin dependent diabetes.
Journal of Clinical Pathology, **41**, 1114-1116.

SHIBL, A.M. (1985) Effect of antibiotics on adherence of microorganisms to epithelial cell surfaces.
Reviews of Infectious Diseases, **7**, 51-56.

SHIPMAN, B. (1979) Clinical evaluation of oral Candida in cancer chemotherapy patients.
Journal of Prosthetic Dentistry, **41**, 63-67.

SHUTTLEWORTH, C.W., GIBBS, F.J. (1960) The aetiological significance of Candida albicans in chronic angular cheilitis and its treatment with nystatin.
British Dental Journal, **108**, 354-356.

SKERL, K.G., CALDERONE, R.A., SEGAL, E., SREEVATSAN, T., SCHELD, M.W. (1984) In vitro binding of Candida albicans yeast cells to human fibronectin.
Canadian Journal of Microbiology, **30**, 221-227.

SOBEL, J.D., MYERS, P.G., KAYE, D., 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., 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.

SONCK, C.E., SOMERSALO, O. (1963) The yeast flora of the anogenital region in diabetic girls.
Archives of Dermatology, **88**, 846-852.

STEDHAM, M.A., KELLEY, D.C., COLES, E.H. (1966) Modified Pagano-Levin medium to isolate Candida species.
Applied Microbiology, **14**, 525-528.

TAN, J.S., ANDERSON, J.L., WATANAKUNAKORN, C., PHAIR, J.P. (1975) Neutrophil dysfunction in diabetes mellitus.
Journal of Laboratory and Clinical Medicine, **85**, 26-33.

TAPPER-JONES, L.M., ALDRED, M.J., WALKER, D.M., HAYES, T.M. (1981) Candidal infections and population of Candida albicans in mouths of diabetics.
Journal of Clinical Pathology, **34**, 706-711.

TENOVUO, J., LEHTONEN, O.P., VIIKARI, J., LARJAVA, H., VILJI, P., TUOHIMAA, P. (1986) Immunoglobulin and innate antimicrobial factors in whole saliva of patients of insulin-dependent diabetes mellitus. Journal of Dental Research, **65**, 62-66.

THOM, S.M., BLACKWELL, C.C., MACCALLUM, C.J., WEIR, D.M., BRETTLE, R.P., KINANE, D.F., WRAY, D. (1989) Non-secretion of blood group antigens and susceptibility to infection by Candida species. FEMS Microbiology Immunology, **47**, 401-405.

THOMPSON, P.J., WINGFIELD, H.J., COSGROVE, R.F., HUGHES, B.O., TURNER-WARWICK, M.E. (1986) Assessment of oral candidiasis in patients with respiratory disease and efficacy of a new nystatin formulation. British Medical Journal, **292**, 1699-1700.

THORSTENSSON, H., FALK, H., HUGOSON, A., OLSSON, J. (1989) Some salivary factors in insulin-dependent diabetics. Acta Odontologica Scandinavica, **47**, 175-183.

TOBGI, R.S., SAMARANAYAKE, L.P., MACFARLANE, T.W. (1987) Adhesion of Candida albicans to buccal epithelial cells exposed to chlorhexidine gluconate. Journal of Medical and Veterinary Mycology, **25**, 335-338.

TOBGI, R.S., SAMARANAYAKE, L.P., MACFARLANE, T.W. (1988) In vitro susceptibility of Candida species to lysozyme. Oral Microbiology and Immunology, **3**, 35-39.

TOMASZEWSKI, T. (1951) Side effects of chloramphenicol and aureomycin with special reference to oral lesions. British Medical Journal, **1**, 388-392.

TRONCHIN, G., POULAIN, D., VERNES, A. (1984) Cytochemical and ultrastuctural studies of Candida albicans. Evidence for modification of the cell wall coat during adherence to human buccal epithelial cells. Archives of Microbiology, **139**, 211-224

TURRELL, A.J. (1966) Aetiology of inflammed upper denture bearing tissues. British Dental Journal, **120**, 542-546.

VANDENBUSSCHE, M., SWINNE, D. (1984) Yeast oral carriage in denture wearers. Mykosen, **27**, 431-435.

VELICHKO, E.V., KARAEV, Z.O. (1988) Antibiotic effect on Candida adhesion to epitheliocytes. Antibiotiki Khemotherapia, **32**, 360-362.

VELIKOV, V.K., SHUBINA, O.I., SALTYKOV, B.B., PERSHIN, B.B., KUZMIN, S.N., FROLOVA, A.I., OVSYANNIKOVA, N.G., TARASOVA, V.K., SALTYKOV, A.B. (1985) Humoral factors of general and local immunity in diabetes mellitus. Terapevticheskii Arkhiv, **57**, 20-22.

VERRAN, J., MOTTERAM, K.L. (1987) The effect of adherent oral streptococci on the subsequent adherence of Candida albicans to acrylic in vitro.
Journal of Dentistry, **15**, 73-76.

VUDDHAKUL, V., MCCORMACK, J.G., SEOM, W.K., SMITH, S.E., THONG, Y.H. (1988) Inhibition of adherence of Candida albicans by conventional and experimental antifungal drugs.
Journal of Antimicrobial Chemotherapy, **21**, 755-763.

VUDHICHAMNONG, K., WALKER, D.M., 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.

WAIN, W.H., PRICE, M.F., CAWSON, R.A. (1976) Factors affecting plaque formation by Candida albicans infecting the chick-allantoic membrane.
Sabouraudia, **14**, 149-151.

WAISSBLUTH, J.G., LANGMAN, J.S. (1971) ABO blood group, secretor status, salivary protein, and serum and salivary immunoglobulin concentrations.
Gut, **12**, 646-649.

WALKER, P.M. (1986) Infectious diseases with oral manifestations. In: Immunological Aspects of Oral Diseases ed. Ivanyi, L., pp 101-123, MTP Press Ltd., Lancaster, Boston, The Hague, Dordrecht.

WARD, P.A. (1966) The chemosuppression of chemotaxis.
Journal of Experimental Medicine, **124**, 209-226.

WEINSTEIN, I.W., DUKE, L.B., PETERS, R.S., BAHN, A.N. (1960) Candida albicans in the saliva of diabetics.
Journal of Dental Research, **39**, 650.

WEIR, D.M., BLACKWELL, C.C., MCLEAN, C.A. (1981) Impaired bacterial binding to peritoneal exudate cells from mice with alloxan induced diabetes.
Journal of Clinical and Laboratory Immunology, **5**, 37-40.

WHEAT, L.J. (1980) Infection and diabetes mellitus.
Diabetes Care, **3**, 187-197.

WILSON, R.M. (1986) Neutrophil function in diabetes.
Diabetic Medicine, **3**, 509-512.

WILSON, R.M., REEVES, W.G. (1986) Neutrophil phagocytosis and killing in insulin dependent diabetes.
Clinical and Experimental Immunology, **63**, 478-484.

WILSON, R.M., TOMLINSON, D.R., REEVES, W.G. (1987) Neutrophil sorbitol production impairs oxidative killing in diabetes.
Diabetic Medicine, **4**, 37-40.

YAMANE, N., SAITOH, Y. (1985) Isolation and detection of multiple yeasts from a single clinical sample by use of Pagano-Levin medium. Journal of Clinical Microbiology, **21**, 276-277.

YOUNG, G., KRASNER, R.I., YUDOFSKY, P.L. (1956) Interactions of oral strains of Candida albicans and lactobacilli. Journal of Bacteriology, **72**, 525-529.

YOUNG, G., RESCA, H.G., SULLIVAN, M.T. (1951) The yeasts of the normal mouth and their relation to salivary activity. Journal of Dental Research, **30**, 426-430.

Additional refernces:

(1) CHALKLEY, H.W. (1943) Methods for the quantitative morphologic analysis of tissues. Journal of the National Cancer Institute, **4**, 47-53.

(2) FUKUI, Y., FUKUI, K., MORIYAMA, T. (1981) Source of neuraminidase in human whole saliva. Infection and Immunity, **8**, 329-334.

(3) HURTREL, B., LAGRANGE, H., MICHEL, C. (1980) Systemic candidiasis in mice. Annals of Immunology, **131**, 105-118.

(4) LEHRER, R.I. (1966) Classification and clinico-pathological features of Candida infections in the mouth. In: Symposium on Candida Infections ed. Winne, H and Hurley, R. pp 119-137, Edinburgh, Livingstone.

(5) LEHRER, R.I. (1978) Host defence mechanisms against disseminated candidiasis. Annals of Internal Medicine, **89**, 91-94.

(6) ODDS, F.C., ABBOTT, A.B. (1984) A simple system for the presumptive identification of Candida albicans and differentiation of strains within the species. Sabouraudia, **18**, 301-318.