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ORAL CANDIDIASIS IN HEALTH AND DISEASE: A QUANTITATIVE AND QUALITATIVE STUDY

by

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THESIS

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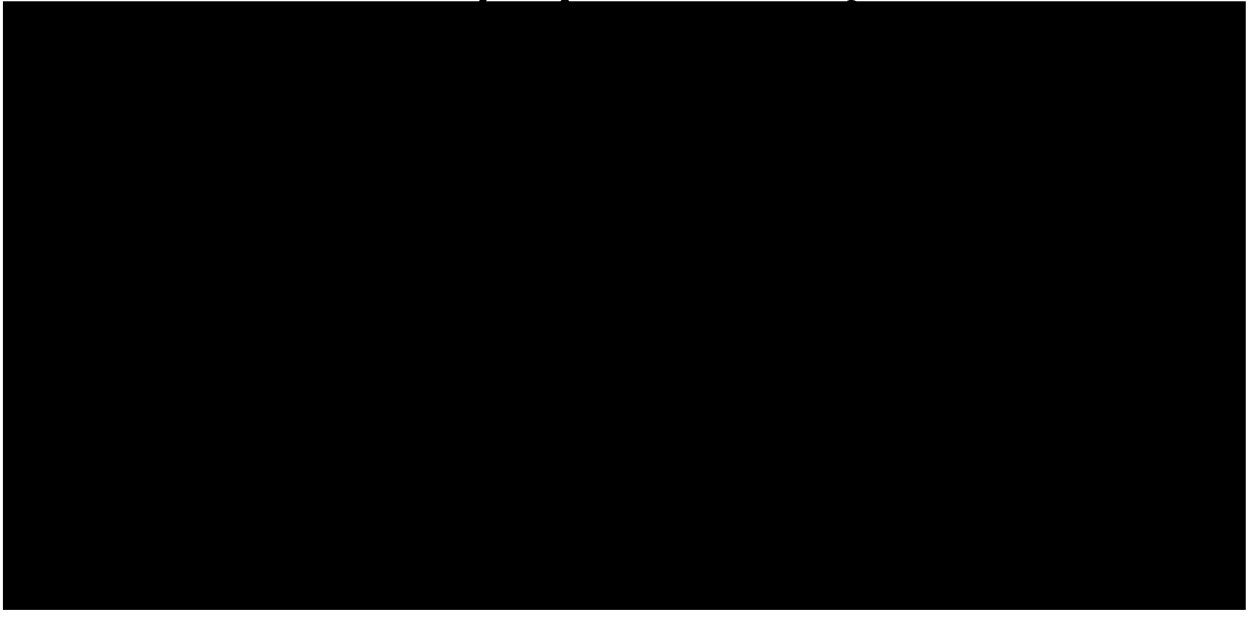

ORAL CANDIDIASIS IN HEALTH AND DISEASE:
A QUANTITATIVE AND QUALITATIVE STUDY

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DEDICATION

To our Families - Thamomsinghs and Luangjarmekorns whose love and unlimited support have made this thesis possible.

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INTRODUCTION

Oral candidiasis (oral moniliasis, oral candidosis, oral thrush) is an opportunistic infection induced by an overgrowth of yeast-like fungi which belong to the genus Candida.

Despite the increasing incidence of Candida infection in many groups of patients (Griffin et al. 1973, Anschn 1974, Louria, 1976), clear-cut criteria for the diagnosis of the infection have not yet been established.

The recognition of Candida albicans in the oral cavity plus the presence of signs and symptoms such as dryness, altered taste, burning and tingling sensation, and/or erythematous areas with or without curd-like materials, should suggest Candida infection. Unfortunately, these findings may not completely resolve the clinician's dilemma, since several studies have indicated that Candida albicans is carried as a commensal organism in the oral cavity of normal individuals (Arendorf and Walker, 1980, Tapper-Jones et al. 1981, Berdicevsky et al. 1984). Another problem for the clinician is that other disease states may present signs and symptoms similar to those of oral candidiasis. In cases of Vitamin B 12 and/or iron folate deficiency anemia, cheilosis and sore and burning tongue are common findings (Wallerstein 1981). The mucositis that often appears as a complication of irradiation and/or chemotherapeutic agents is likely to represent Candida infection. In addition, the presence of candidal organisms in leukoplakia and speckled leukoplakia, which are high risk lesions for malignancy, have been described in previous studies (Cawson 1966, Renstrup 1970, Silverman 1984 a).

Currently, there is much interest in the pathogenesis of candidiasis as related to host immunity, particularly in a number of cases in homosexuals (Gottlieb et al. 1981, Masur et al. 1981, Follansbee et al. 1982, Lozada et al. 1983) who are the highest risk group in acquired immunodeficiency syndrome (CDC 1982).

Since the presence of Candida organisms may indicate a normal flora or may represent pathogens of the oral cavity (Johnston et al. 1967, Budtz-Jörgensen et al. 1975, Arendorf and Walker 1979, Epstein et al. 1980), and because the tests for recognizing the occurrence and concentrations of the organisms are uncertain, it is hypothesized that a quantitative test or tests for the diagnosis of Candida infection can be developed which would assist the clinician in accurately recognizing the presence of Candida and in differentiating normal flora from pathogenic activity.

In order to test the hypothesis, various tests for detecting oral Candida were compared in patients and controls in an attempt to develop methods for quantitating and differentiating Candida carriers and pathologic states. As part of the thesis, the literature regarding all aspects of oral candidiasis was reviewed to elucidate the known and speculated mechanisms related to Candida density and to achieve a better understanding of the natural history, diagnosis and management.

REVIEW OF THE LITERATURE

Candida organisms are yeast-like fungi which have been classified in groups of imperfect fungi (Lodder 1970). They are essentially unicellular organisms. In their early development, the fungi produce moist colonies of creamy consistency. Candida species have the ability to produce pseudomycelia or true mycelia, but not sexual spores (ascospores) (Van Uden and Buckley 1970).

The term 'pseudomycelium' refers to chains of elongated cells forming from blastospores which elongate, but do not break off from mother cells. A true mycelium is formed by elongation and branching of a germ tube which is produced by the mother cell (Schweritz et al. 1978). True mycelia are distinguished by the absence of any constriction at the original site, and the hyphae grow by an extension of their parallel wall tube.

There are many Candida species which have been isolated from humans including Candida albicans (C. albicans) (Meckstroth et al. 1981, DeGregorio et al. 1982 a, Pellinen et al. 1983).

Hasenclever and Mitchell (1963) identified two serotypes of C. albicans, A and B, on the basis of the polysaccharide antigens on the cell wall. Subsequent epidemiological investigations of the organisms isolated from oral and extraoral specimens led to the conclusion that serotype A predominates (Hasenclever and Mitchell 1963, Stallybrass 1964, Martin and Lamb 1982).

The controversy over the relative virulence of yeast and mycelial forms of C. albicans has not been settled. Evidence has been presented suggesting

that germination and filament formation are not necessary for pathogenesis (Simonetti and Strippoli 1973, Mardon et al. 1975, Evans and Mardon 1977), and other evidence supports the opinion that the mycelial form is more virulent than the yeast form (Russell and Jones 1973, Iannini et al. 1977, Kimura and Pearsall 1980, Richardson and Smith 1981, Sandin et al. 1982). On the other hand, the blastospores have been reported to be an important state in the colonization of the host (Taschdjian and Kozinn 1957, Liljemark and Gibbons 1973, Schnell and Voight 1976, Klotz et al. 1983), and more rapidly and consistently fatal to mice than hyphae (Evans 1980). However, both hyphae and blastospores may been seen in human sources (Davenport 1970, Budtz-Jörgensen 1978, Arendorf and Walker 1979, Holmstrup and Bessermann 1983).

EPIDEMIOLOGY AND PREVALENCE OF ORAL CANDIDA

Among Candida spp., C. albicans is the one most frequently found in the mouths of both healthy (Barlow and Chattaway 1969, Budtz-Jörgensen et al. 1975, Arendorf and Walker 1980, Berdicevsky et al. 1984), and diseased populations (Tapper-Jones et al. 1980, Dahlén et al. 1982, Holmstrup and Bessermann 1983). Occasionally, C. tropicalis, C. pseudotropicalis, C. parasilosis, C. kruseii, or C. stellatoidea may be associated in the pathogeneses of oral candidiasis (Odds 1979, Hurley 1980, Sandford et al. 1980, Kolnick 1980, Bille et al. 1982).

Oral candidiasis is a form of superficial candidiasis which ranks among the most common of all infectious diseases.

It has not yet been established whether candidiasis is predominantly of endogenous or exogenous origin. Clayton and Noble (1966) studied environmental candidal isolates and found that organisms were human-related, not primarily an environmental contamination. Many authors reported

increased incidences of oral thrush in infants born to mothers with vaginal candidiasis (Woodruff and Husseltine 1938, Kozinn et al. 1957, Drake and Maibach 1973 a).

The prevalence of C. albicans in normal oral flora has been variously documented as ranging from 4 percent to 52 percent (Clayton and Noble 1966, Johnston et al. 1967, McFarlane and Mason 1974, Arendorf and Walker 1979, Tapper-Jones et al. 1981, Berdicevsky et al. 1980) in normal and from 15 percent to 100 percent in patients with various conditions (Peters et al. 1966, Mackendrich et al. 1967, Barlow and Chattaway 1969, Budtz-Jörgensen and Bertram 1970, Davenport 1970, Berdon and Seita 1971, McFarlane and Mason 1974, Epstein et al. 1980, Tapper-Jones et al. 1981, Bastiaan and Reade 1982, Dahlén et al. 1982, Silverman et al. 1984 b).

PREDISPOSING FACTORS

The pathogenesis of oral Candida infection may be related to a number of organisms (Campbell and Heseltine 1960, Epstein et al. 1980). Therefore, any local or systemic condition leading to an overgrowth of oral Candida should be considered to be a predisposing factor.

Local Factors: Intra-oral changes of temperature and moisture (Barlow and Chattaway 1969, Drake and Maibach, 1973 a, Joshi et al 1975), lowered salivary pH (Arendorf and Walker 1980, Parvinen and Larmas 1982) and lowered flow rates (Tapper-Jones et al. 1980, Parvinen and Larmas 1982) predispose to the development of candidiasis. Dentures (Budtz-Jörgensen et al. 1975, Cawson 1978, Arendorf and Walker 1979, Berdicevsky et al 1980, Watson et al. 1982) and denture-associated trauma (Nyquist 1953, Budtz-Jörgensen and Bertram 1970), or tobacco smoking (Daftary et al. 1972, Arendorf and Walker 1980, Holmstrup

and Bessermann 1983) may provide a favorable environment for oral Candida growth.

Biologic and pathologic Factors: There are a variety of systemic changes which increase susceptibility to Candida infection. These changes include:

Age and sex: The higher incidence of Candida infection in infants is probably due to an immature immunological defense and complete saturated iron binding capacity found in these newborn infants during their first week of life. However, no relationship between candidal infection and premature delivery has been documented (Kozinn et al. 1958 a,b, Shrand 1961). Most authors agree that the infections arise primarily because of maternal contamination of newborns with yeast from the birth canal (Kozinn and Taschidjian 1962, Somerville 1964, Farmen 1966). On the other hand, the Candida in aging populations is usually associated with denture wearing (Budtz-Jörgensen et al. 1975).

Idiopathic congenital or acquired debilitating diseases and disorders: Many diseases and disorders in this category have been implicated as factors in oral candidiasis, mainly endocrinopathies, immunodeficiencies and malignancies.

I. Endocrinopathies:

a) Diabetes mellitus: The mechanism by which diabetes mellitus increases the host susceptibility to candidiasis is unclear. Knight and Fletcher (1971) have proposed that high blood and tissue glucose levels favor the growth of oral Candida. This hypothesis corresponds to the high carrier rate of oral yeast found among diabetics (Barlow and Chattaway 1969, Joynson et al. 1972, Tapper-Jones et al. 1981). On the other hand, some investigators did not find such evidence in their diabetic patients (Peters et al. 1966).

b) Other endocrinopathies: Hypothyroidism (Bortolussi et al. 1981), and hypoparathyroidism (Nally 1970) are sometimes associated with the oral form of chronic mucocutaneous candidiasis. Of interest, Louria and co workers (1966) found that hyperthyroidism alone did not increase the susceptibility to C. tropicalis in experimental mice.

II. Immunodeficiencies: Clinical patterns of immune deficiency categorized as primary, implying congenital absence or dysfunction of essential immune cells; or secondary, resulting from associated disease processes or therapies.

a) Primary immunodeficiency: Patients with primary defects of humoral immunity are often susceptible to bacterial infections, while those with defects of cellular-mediated immunity are classically prone to viral and fungal diseases. Candida infections are encountered in many, but not all, patients with primary immunodeficiency syndrome.

Primary immune deficiences associated with candidiasis*

Congenital thymic aplasis

(DiGeorge syndrome - immunodeficiency with parathyroidism)

Chronic mucocutaneous candidiasis

(with or without endocrinopathy)

Cellular immunodeficiency with abnormal immunoglobulin synthesis

Myeloperoxidase deficiency

Chronic granulomatous disease

Chediak-Higashi Syndrome

Tuftsin deficiency

Hyperimmunoglobulinemia E syndrome

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*Ammann and Fudenberg (1982)

b) Secondary or acquired immunodeficiency: Acquired diseases, particularly hematologic malignancy, frequently exhibit variable impaired immune responses. Oral and gastrointestinal forms of candidiasis have been described as complications of malignant diseases (Levine et al. 1972, Katz and Cassileth, 1977, DeGregorio et al. 1982 a, b).

Oral candidiasis has been reported in 15 percent of 148 cancer patients during the terminal stage of their illness (Boggs 1961). Chen and Webster (1974)

and Silverman et al.(1984 b) reported an increased incidence of positive oral Candida cultures in their head and neck cancer patients after irradiation.

Evidence of reduced candidacidal ability by peripheral T lymphocytes in leukemic patients has been observed (Caroline et al. 1969, Rosner et al. 1970, Myerowitz et al. 1977), which may account for the higher incidence of candidal infection in these populations. Antibacterial and immunosuppressive drugs used in treatment regimens for malignancies, rather than the underlying disease itself, may predispose patients to candidal infection (Young et al, 1974, DeGregorio et al. 1982 b).

Currently, there is a high incidence of oral candidiasis in those homosexuals, who are classified in the highest risk group for acquired immunodeficiency syndrome (AIDS) (CDS 1982). The lesion takes various forms, such as acute oral candidiasis (Gottlieb et al. 1981, Lozada et al. 1982), or a plaque-like lesion (Follansbee et al. 1982). Most of the lesions respond well to antifungal therapies, but the risk of recurrence is high when antifungal treatment is discontinued.

Anemia: Iron-deficiency anemia (Fletcher et al. 1975, Odds 1979) may predispose to Candida infections. However, Jenkins and co-workers (1977) and Samaranayake and MacFarlane (1981) could not demonstrate any relationship between iron and folate deficiency with oral candidiasis in their patients.

Sjögren's syndrome: Sjögren's syndrome consists of the triad of xerostomia, keratoconjunctivitis sicca and, in most cases, associated connective tissue disorders. Recently, an association between Sjögren's syndrome and oral candidiasis has been documented (MacFarlane and Mason, 1974, Tapper-Jones et al. 1980).

Xerostomia: A correlation between xerostomia and an increased incidence of Candida organisms has been reported (Parvinen and Larmas 1981). This is probably influenced by changes in salivary flow rates, pH of the saliva and/or concentration of candidal antibodies in saliva of xerostomic patients.

Tranquilizer-type drugs often diminish salivary production, causing various degrees of xerostomia. Oral candidiasis associated with tranquilizer therapy (Kane 1963 a,b, Kane and Anderson 1964) and a high incidence of oral Candida carriage and infection in 3000 mental patients treated with tranquilizers (Pollack et al. 1964) have been reported. These tranquilizers included chlorpromazine, imipramine and phenothiazine.

Antibiotic treatments: It has been suggested that antibiotic administration, especially tetracycline, increases the liability of patients to develop candidal infection or increases the prevalence of Candida organisms (Lehner and Wards, 1970, Forsgren and Schmeling 1977, Forsgren et al. 1980, Hauser and Remington 1982). The administration of antibiotics can alter the bacterial flora of the mouth so that an increase in the yeast proportion may occur. While antibiotics may affect the numbers or prevalence of Candida organisms, some authors have claimed there must be underlying systemic factors present before the infection occurs (Kennedy et al. 1954, Torack 1957). In a recent review, various effects of antibiotics on immune responses have been cited (Hauser and Remington 1982), and these may enhance Candida density (Seelig 1966).

Corticosteroid therapy (Hurley et al. 1975, Spector et al. 1982, Zegarelli 1983): Acute pseudomembranous candidiasis developed with topical betamethasone-17-

valerate, in the presence of significant titres of IgG, IgA and IgM classes of Candida antibodies. It is possible that the steroid overcomes locally the protective action of antibodies, allowing overgrowth of Candida in patients predisposed to the infection.

It is believed that long term, high doses of glucocorticosteroids involve a significant risk factor for disseminated (Louria et al. 1962) and mucocutaneous (Forman 1966) candidiasis. However, susceptibility to the infection is not enhanced by short acting glucocorticosteroid (hydrocortisone sodium succinate). The development of immunity and delayed hypersensitivity are not adversely affected, and the increased resistance of immune animals to further challenge with C. albicans is not diminished (Hurley et al. 1975).

MANIFESTATIONS

The clinical manifestations of oral candidiasis vary considerably. Lehner (1967) has classified oral candidiasis in terms of acute and chronic forms based on clinical, histological, therapeutic and immunological criteria. They are as follows: acute pseudomembranous, acute atrophic, chronic hyperplastic and chronic atrophic candidiasis.

Acute pseudomembranous candidiasis: Acute pseudomembranous candidiasis is commonly known as oral thrush. The infection is most prevalent among infants (Taschdjian and Kozinn 1957) and elderly debilitated patients, but the lesion has also been seen in individuals as a consequence of predisposing factors to the infection. The most common factors are irradiation for head and neck cancer (Silverman 1981, Silverman et al. 1984 b), broad spectrum antibiotics (Seelig 1966, DeGregorio et al. 1982 b) and chemotherapy (Michaud et al. 1977). Oral thrush has seldom been attributed to yeast species other than C. albicans. The lesions characteristically consist of removable white patches

or discrete pseudomembranes on the mucosa, tongue and/or gingival lining. Scraping of the pseudomembrane leaves raw erythematous lesions. Oral thrush is usually not painful, even in severe erosive and ulcerative lesions. In the late stage of oral thrush, there may be fungal plaques which persist leading to acute atrophic candidiasis (Lehner 1964).

Acute atrophic candidiasis: Acute atrophic candidiasis or candida glossitis (Lehner 1967, Cawson 1978, Odds 1979, Roitt and Lehner 1980, Tyldesley 1981) is a complication of broad spectrum antibiotics. The lesion may follow oral thrush or occur in hypersensitive individuals. Most cases of 'antibiotic sensitive tongue' belong to this form of oral candidiasis (Odds 1979). The lesion presents as a soft, smooth glossitis and angular cheilitis; the atrophic red oral mucosa is usually painful.

Chronic atrophic candidiasis: Chronic atrophic candidiasis, denture stomatitis or denture sore mouth (Cawon 1978, Lehner 1967, Odds 1979, Roitt and Lehner 1980, Samaranayake and MacFarlane 1981, Tyldesley 1981) can occur with any form of intraoral prosthesis, but maxillary removable complete dentures (Budtz-Jörgensen et al. 1975, Roitt and Lehner 1980) produce the largest number of cases. The condition manifests as a diffuse inflammation of the denture bearing area with or without angular chelitis and glossitis. The inflammation described as type 1. exhibits localized and pinpoint hyperemia; type 2. exhibits diffuse erythema; type 3. is characterized by diffuse inflammation and papillary hyperplasia (Budtz-Jörgensen 1978). It is not always possible to isolate Candida organisms from the oral mucosa by smear, scraping or biopsy; however, the fungus can usually be cultured from the plaque-like material covering the tissue surface of the denture (Davenport 1970, Budtz-Jörgensen 1978, Walker et al. 1981). Patients with denture stomatitis may have mild soreness, but the majority are often free of symptoms. Angular cheilitis may be another manifestation in denture stomatitis.

Chronic hyperplastic candidiasis: Chronic hyperplastic candidiasis or Candida leukoplakia (Lehner 1967, Cawson and Lehner 1968, Cawson 1978, Odds 1979, Pindborg 1980, Roitt and Lehner 1980, Tyldesley 1981) is clinically characterized by reddening adjacent to the white patches which cannot be scraped off (speckled leukoplakia) (Pindborg 1980). Occasionally, the lesion presents as a white, firm, painless patch (homogeneous leukoplakia) (Cawson 1978).

There is a higher prevalence of Candida in speckled leukoplakias than in homogeneous leukoplakias (Renstrup 1970, Daftary et al. 1972). Epithelial atypia is sometimes associated with concurrent Candida in speckled leukoplakias (Renstrup 1970) confirming the greater risk of the organism in red and white lesions.

Chronic mucocutaneous candidiasis (CMC): Because the most common form of CMC has been seen in the oral cavity, Lehner (1966 a) included 4 types of CMC in his classification of oral candidiasis: Candida endocrinopathy syndrome, Candida granuloma, chronic diffuse and chronic hyperplastic oral candidiasis.

CMC is not a single disease entity; rather underlying causes of CMC follow a variety of inheritance patterns (Kirkpatrick et al. 1971 Sadeghi and Witkop 1979), immunodeficiency status, usually of cellular immunity (Kirkpatrick 1970, Kirkpatrick et al. 1971, Budtz-Jörgensen 1973, Rogers and Balish 1980) and associated medical conditions (Wells 1973, Fletcher et al. 1975, Sam et al. 1979, Kirkpatrick and Windhorst 1979, Odds, 1979).

Patients with CMC have a wide range susceptibility to infections. Lung infections are especially common and may be fatal (Masur et al. 1977, Humphrey and Weiner 1983). Dental enamel dysplasia has been noted in a large number of CMC cases which occur together with alopecia areata (Stanklar and

Bewsher 1973, Myllarniemi and Perheentupa 1978). Some of these patients have esophagitis and laryngitis (Dudley et al. 1980). Oral forms of CMC closely resemble the oral candidiasis previously described, except for their remarkable chronicity.

Histopathologic Findings

Acute stages: During the acute stages of oral candidiasis, lesions consist of necrotic material, food debris, leukocytes and bacteria on the superficial layer of nucleated epithelial cells. Candida hyphae penetrate to the base of stratum corneum while an inflammatory cellular reaction is present in the subepithelium (Lehner 1967).

Chronic stages: Biopsies of denture stomatitis lesions have consistently failed to demonstate Candida invasion (Budtz-Jörgensen et al. 1975). Budtz-Jörgensen found only epithelial atrophy and thinning or total absence of the superficial parakeratotic layer in such lesions. This appearance is contrary to that of Candida leukoplakia. Parakeratosis and/or epithelial hyperplasia with hyphae invasion, restricted to the epithelial layer, have always been observed in Candida leukoplakia lesions (Cawson 1965, Cawson and Lehner 1968, Cawson 1973). The plaque lesions are firm and cannot be rubbed off and hence resemble leukoplakia of different origin. The nodular Candida leukoplakia (speckled leukoplakia), in which epithelial cellular atypias are most often seen (Pindborg 1980), also exhibits marked variations in the thickness of the epithelium with elongated rete ridges. A marked migration of inflammatory cells, mainly polymorphonuclear leukocytes, and microabscesses have been observed in lesions. These changes have been also observed in experimental C. albicans infection of rat tongue (Jones and Russell 1973,1974), chick chorioallantoic membrane (Cawson 1973) and monkeys' palates (Budtz-Jörgensen 1971).

DIAGNOSIS

There are several methods used as diagnostic tools for oral candidiasis. The methods include direct epithelial smear (Lehner 1966, Budtz-Jörgensen 1974, Jenkins et al 1977, Arendorf and Walker 1979), various culture techniques (Peters et al 1966, Barlow and Chattaway 1969, Davenport 1970, Budtz-Jörgensen and Bertram 1970, Arendorf and Walker et al 1980, Epstein et al 1980, Berdicevsky et al 1980, 1984, Tapper-Jones et al 1981, Bastiaan and Reade 1982, Silverman et al 1984 b), fluorescent antibody stains (Lehner 1966 b), gas-liquid chromatography of patient sera (Miller et al. 1974, Roboz et al. 1980, Marier et al. 1982), enzyme-linked immunosorbant assay (Araj et al. 1982) and tissue biopsy (Renstrup 1970, Holmstrup and Dabelsteen 1974)

The disparities in detection methods for Candida have been reported ranging from 4 to 100 percent. This disparity may reflect not only predisposing factors but also differences in sampling sites and in patient selection. The identification of Candida organisms has been made on the basis of gross and microscopic morphology, biology, serology as well as pathogenicity in laboratory animals.

Isolation of Candida from clinical materials: Because C. albicans and other yeasts are part of the normal oral flora, isolation of organisms is essential to their differentiation. When the primary isolated colony is observed, rapid tests for properties specific to C. albicans are performed and if these are negative, colony isolates are further treated for their speciation.

For isolation, the specimen should be conveyed to the laboratory as quickly as possible to prevent dessication. Many laboratories recommend a transport medium such as the Modified Stuart transport medium (Amies 1967) for oral swabs.

Many media are available for the cultivation of Candida, including the peptone-glucose or peptone maltose sugars. The modern versions of these media are popular for the isolation of Candida due to their property of suppressing many bacterial growths. Adding chloramphenicol into the media will yield more inhibition. The isolation media should be incubated for at least three days at 37° C before a specimen is reported as negative for yeasts.

Presumptive positive culture for C. albicans: A representative yeast colony (or colonies) should be microscopically examined for size, shape of blastospores, presence or absence of pseudomycelium and capsules or ascoscopes. Colony characteristics, however, are of little value in yeast identification. The germ tube test and the chlaymdospore are two rapid and specific tests available for C. albicans (Beheshti et al. 1975, Joshi 1975).

a) Germ tube test The germ tube test demonstrates the initiation of hyphae or hyphae-like growth, arising directly from the yeast cell. The parallel walls of the germ tube are not constricted (Joshi et al, 1973, Odds 1979). They are considered as an intermediate growth phase.

In addition to C. albicans, C. stellatoidea (Joshi et al. 1973, Katsura and Uesaka 1974, Huppert et al. 1975) and a few strains of C. tropicalis (Land et al 1979) can also form germ tubes.

Germ tubes are formed in human serum (Taschdjian et al. 1960) and tryptic and tryptic soy broths (Joshi et al. 1973). Neither blood group antibodies associated with human serum, nor the age of the serum have any effect on the germ tube formation. On the other hand, many circumstances can influence germ tube formation including bacteria-contaminated serum (Auger and Joly 1975), size of the inoculum (Mackenzie 1962, Joshi et al 1973), concentrations of simple carbohydrates and temperature of incubation (Joshi and Gavia 1974). The germ tubes develop at 32-42 ° C and their formation is more favored by a young culture inoculum than an old one.

b) chlamydospore formation: The production of chlamydospores by C. albicans has been performed on corn meal agar (Taber and Wiley 1962) adding a surfactant - Tween 80, (Sukroongreung 1971). The optimal production of chlamydospores requires a partial anerobiosis and a controlled inoculation temperature at 25 ^{o} C (Kapica et al. 1969).

Physiological properties of C. albicans: The ability to assimilate and ferment individual carbon and nitrogen sources has been used in the final stage of the identification of Candida species. The method is based on the fundamental principles of auxanography (Beijerinek 1889) and a liquid assimilation test (Wickerham and Burton 1948). The fermentative yeast produces CO_2 and alcohol in appropriate indicator media (Lodder 1970, Haley 1971, Boyd 1975). Recently, manual commercial systems such as Yeast-Tek (UYT) (Bowman and Ahearn 1976, Cooper et al. 1978) and APT 20 C (Buesching et al. 1979, Land et al. 1979) have been developed for the identification of medically important yeasts, including C. albicans.

Other culture media have been used in the isolation and identification of C. albicans. One is a bismuth polysulphite medium, which is reduced to a brown-black sulphide solely by C. albicans (Nickerson 1953). Triphenyl tetrazolium-chloride-containing medium (Pagano et al. 1958) has chloride-reducing properties due to C. albicans appearing white while other species acquire a pink hue.

Odds and Abbott (1980,1983) have modified the test for differentiation of Candida spp. and strains using basal growth and multipoint inoculation procedures. They reported that only C. albicans isolates grow on a media whose pH is lowered nominally to 1.55. Their tests, however, have been regarded as adjuncts to identification, not as substitutes for classical methods.

Recently, Polonelli et al. (1983) have employed factors affecting the killer phenomenon of C. albicans to differentiate its isolates. This method might be easily applied to epidemiological investigation of infection due to C. albicans.

HOST DEFENSE MECHANISMS AGAINST *CANDIDA*

Generally, host defenses against infections can be defined as the combined protective effect of anatomic barriers, baseline cellular phagocytosis, digestion by phagocytic cells and effector mechanisms (Drutz and Mills 1982). The mechanisms can be local or systemic, nonspecific or specific, and humoral or cellular, depending upon the pathogenic organism, organ involvement, and associated factors.

Oral candidiasis, as mentioned earlier, may be only a superficial infection confined to the oral cavity or an oral manifestation of CMC. Once the organism colonizes on the mucosa, it can invade locally and become a systemic infection.

Host Defenses Against *Candida* at Oral Mucosae

Candida species are commensals in the oral cavity of normal individuals who have no overt disease. The organisms are able to adhere to human oral epithelial cells in vitro (Kimura and Pearsall, 1980, King et al. 1980) and their colonization may be the further step contributing to their pathogenicity (Lilijmark and Gibbons 1973, Gibbons 1977).

C. albicans grows either in a budding yeast form or a mycelial form, and both forms are present in invasive lesions. However, the transition from the budding to the mycelial form can be induced only after the budding cells have entered the stationary (starving) phase (Chaffin and Sogin 1976, Soll and Bedell 1978, Soll and Herman 1983).

The adherence of *C. albicans* to oral epithelial cells is greater than that of other candidal species (King et al. 1980), and the process is enhanced in saliva as compared to adherence in phosphate buffered saline (Kimura and

Pearsall 1978). Adherence is also markedly influenced by temperature, pH (Kimura and Pearsall 1978, 1980, Samaranayake and MacFarlane 1982) and serum and salivary components (Kimura and Pearsall 1980). Glycoproteins on the cell wall of Candida are also responsible for its adherence. The glycoproteins may be concentrated on the germ tube walls (Lee and King 1983), since mucosal surfaces colonized by C. albicans are populated by organisms in the budding yeast phase (Taschgjian and Kozinn 1957, Barlow and Chattaway 1969).

Once the yeast attaches to the oral mucosa, secretory immunoglobulin A (sIg A), a substance in external secretions produced by submucosal plasma cells, is the other factor influencing the outcome of the mucosal invasion. SIgA isolated from saliva (Epstein et al. 1981) as well as that isolated from breast milk (Vudhichamnong et al. 1982) can inhibit the adherence of C. albicans to human oral epithelial cells in vitro.

Phagocytosis and Digestion by Phagocytic Cells

Engulfment and destruction of Candida organisms by tissue macrophages, circulating monocytes or polymorphonuclear leukocytes (PMNs) are other mechanisms in host defense against infections.

PMNs and mononuclear phagocytes possess special receptors for the portion of IgG molecules which activate their phagocytosis, assisting ingestion of microorganisms with IgG or activated C3 on their surfaces. It is, therefore, difficult to separate the phagocytosis system of the innate defense from the phagocytosis that is mediated by acquired immune mechanisms, since products of lymphocytes (e.g. antibodies, lymphokines) can influence any or all of the phagocytes.

Lehrer and Cline (1969) were the first to demonstrate the phagocytosis of Candida in vitro by PMNs and monocytes from humans. The most effective system appears to be a myeloperoxidase-hydrogen peroxide-halide system (Lehrer and Cline 1969, Klebanoff 1970, Hilger and Danlay 1980), but the phagocytosis can be a nonoxidative fungicidal mechanism (Brune et al. 1973, Lehrer et al. 1975) that may involve hydrolytic enzymes (Lehrer et al. 1975).

This evidence has been confirmed by an incidence of systemic candidiasis in patients with myeloperoxidase deficiency or chronic granulomatous diseases (Lehrer and Cline 1969, Lehrer 1975). PMNs from the MPO-deficient patients possess normal phagocytic activity, but their capacity to kill ingested Candida cells is much below normal. On the other hand, PMNs have the ability to kill C. parapsilosis and C. pseudotropicalis by a mechanism that is completely independent of the MPO, iodination and the hydrogen peroxide generated by the endogenous metabolism of the cells.

Murine PMNs respond to live blastospores of C. albicans more vigorously than to killed blastospores (Hilger and Danlay 1980), and the live blastospores can alter or inhibit the release of hydrogen peroxide by PMNs. The mechanism for this may be the strong chemotactic components present on Candida cell surfaces (Denning and Davis 1973). Recently, Fischer and co-workers (1982) have found carbohydrate antigens from C. albicans, essentially mannan, persisting in the serum of some patients with CMC. The antibodies to those antigens have been shown to be T-cell dependent and in the IgM and IgG classes (Durandy et al. 1983), and the titer 1:16 has been demonstrated as a significant level distinguishing the infected from carrier states (Lehner 1965, 1966 b).

The evidence that PMNs are important for defense against candidiasis has been confirmed by a susceptibility to Candida in patients with Chediak - Higashi syndrome (Anderson 1971) and in mice with Chediak - Higashi syndrome

(Elin et al 1974), a disease in which PMNs are impaired. Although susceptible to Candida, mice with Chediak - Higashi syndrome have circulating levels of immunoglobulins equal to or higher than littermate normal mice (Elin et al. 1974).

C. albicans may resist phagocytosis by its ability to grow out of PMNs and macrophages. The relative ability of Candida to escape host phagocytosis correlates with its virulence and with its ability to produce a filamentous form. Davis and Denning (1973) have shown that C. albicans hypahae longer than 200 um tend to survive phagocytosis. Louria and Brayton (1964) found that mouse PMNs were able to ingest up to seven C. albicans cells per phagocytic cell within 30 min, but up to 64 percent of the phagocytes had pseudomycelia penetrating their cell membranes after 4 hr of incubation.

When Candida is in sites other than the bloodstream, the response of phagocytes is less. Young (1958) found that in intraperitoneal Candida inoculation, monocytes were the first cells attached to the infection site. PMNs appeared 4 hrs later. The evidence may correspond to the recent studies of many groups on the role of cell-mediated cytotoxicity in natural resistance to foreign cells, tumors, and infections (Landolfo et al. 1978, Murphy and McDaniel 1982, Bistoni et al. 1983). The mediating cells are presumably of the macrophage lineage that are distinct from natural killer cells (Baccarini et al. 1983). On the other hand, Goldberg et al. (1971) reported that patients with CMC had negative delayed skin reactions and lymphocyte transformation to Candida in the presence of a positive macrophage migration inhibition test.

Normal human monocytes are able to kill C. albicans only through oxidative pathways which produce chemiluminescence. The survival of C. albicans in such cells may reflect a defect in monocytes in releasing of oxidative radicals into phagosomes containing C. albicans, or neutralization of

the oxidative products by the C. albicans before the cheiluminescence can occur (Bortolussi et al. 1981).

Serum complement levels are commonly associated with the acquired antibody-mediated immune response, but they are also important factors in the arsenal of innate factors present in normal serum. Morelli and Rosenberg (1971) showed that the complement system enhanced the rate of phagocytosis but not the rate of intra-cellular killing of Candida organisms. Larcher (1982) found a significant correlation among the severity of defects in yeast opsonisation, C_4 and factor B activity at the presentation and the subsequent development of the infection in 15 children with fulminant hepatic failure.

Cell-mediated Immunity

Studies of CMC in humans indicate that the classical thymus-dependent cell (T-cell) mediated immunity plays a major role in resistance against Candida infections.

Antibody-mediated immunity in most patients with CMC is totally intact, resulting in increased anti-Candida antibody titers (Kirkpatrick et al. 1971, Provost et al. 1973). However, many patients with CMC have demonstrated positive Schick tests, despite adequate diptheria toxoid immunization (Provost et al. 1973). Rogers (1976) and Cutler (1976) and their co-workers have found that congenitally athymic mice are more resistant than normal littermates to disseminated candidiasis. Athymic mice reconstituted with a thymus gland are as susceptible as normal mice to C. albicans infection (Rogers et al. 1976).

Most patients with CMC demonstrate significantly depressed cellular immune responses, including an anergy to a battery of skin-test antigens, particularly to Candida antigen, and an inability of lymphocytes to produce

migration inhibitory factor responding to Candida antigen in vitro, or mount a proliferative response to various T-cell antigens (Provost et al. 1973, Valdimarsson et al. 1973).

A major role for T-cell dependent processes in immunity to Candida has been supported by Corbel and Eades (1976) on the basis of their finding that New Zealand Black (NZB) mice are more susceptible than mice of the CBA strain to lethal Candida infection. The NZB mice are known to develop deficiencies in cell-mediated immunity and in the C5 component of the complement system. The authors suggested that the increased susceptibility of NZB mice to Candida from defective T-lymphocyte function and is not related to lack of C5. However, a similar increase in mortality during candidiasis in mice with C5 deficiency without any known T-cell defect has been shown by Morelli and Rosenberg (1971). Pearsall et al. (1978) suggested that adequate T-cell function might be required for the memory response.

Reports of candidiasis among patients with Pneumocystis carinii pneumonia and Kaposi's sarcoma, which are associated with acquired cellular immunodeficiency (Gottlieb et al. 1981, Masur et al. 1981), also suggest the importance of the cellular immune response to candidiasis.

There is some evidence indicating that at least part of the proposed anti-Candida effect of T-cell immunity may be via the elicitation of an inhibitory factor from sensitized lymphocytes. Pearsall et al. (1978) have discovered a lymphokine which has the capacity to reduce the number of C. albicans cells in vitro, while culturing sensitized lymphocytes. However, there is some suspicion about their use of nystatin in that experiment.

TREATMENT

Drugs used in the treatment of oral candidiasis are mainly available as mouthwash, tablet and suspension. These include the following:

Hydrogen peroxide (H_2O_2 solution): 3% H_2O_2 solution is an effective anti-bacterial and antifungal mouthwash. The effect of the mouthwash depends on both liberated oxygen and its own hydrogen peroxide molecule (Combes 1937). Dickstein (1964) has reported success in using gly-oxide (10% carbamide peroxide glycerol solution) for the treatment of oral candidiasis in 25 newborn infants. Five percent xylitol has been proved to reduce the evidence of oral candidiasis in the study of Makinen and co-workers (1975).

Other mouth-washes such as chlorhexidine have sometimes been used in the treatment of oral candidiasis (Budtz-Jörgensen and Loe, 1972, Sharon et al. 1977) as a denture disinfectant whereas other specific antifungal drugs are also required to eradicate Candida organisms.

Nystatin (Mycostatin, Nystan, Fungicidin, Polyfungin A) and amphotericin B: Both nystatin and amphotericin B are drugs in the polyene group. The biological effects of these drugs on yeasts depend upon their binding cell membrane sterols, resulting in an increase in permeability of the cell membrane. The leakage of intracellular contents of yeast cells is the primary effect of these drugs, and cell death is a secondary consequence (Gale 1974, Hammond et al. 1974). Nystatin and amphotericin B bind more to ergosterol (the primary sterole in fungal membrane) than to the cholesterol found in membranes of the host cells. Drugs are only effective on actively growing fungal cells.

Both of these antifungal polyenes are not absorbed from the gut. Amphotericin B can be very toxic to humans, causing kidney damage, uremia and hypokalemia; therefore, nystatin troches 100,000 U are more widely used for oral candidiasis.

Imidazole derivatives: A number of substituted imidazole preparations are now available as broad-spectrum antifungal medicines, including clotrimazole, and ketoconazole. These compounds appear to alter membrane permeability of susceptible yeast and fungal cells leading to damage to the structure and organization of cell organelles (Cope 1980, Borgers 1980, Sud and Feingold 1981, Borgers et al. 1983). The potency of these imidazoles is based upon their ability to prevent further development of yeast to invasive mycelial forms (Borgers et al. 1979, Aerts et al. 1980, Van Cutsem et al. 1980).

Clotrimazole is poorly absorbed from the digestive tract and is not always well tolerated by patients, while ketoconazole, which can be given orally, is well absorbed. The side effects of systemic clotrimazole have been reported as nausea, vomiting, anorexia, and fatigue (Goldstein and Hoeprich 1972, Hirsch and Dedes 1974). There are some failures of ketoconazole in fungal treatment, related to low serum levels (Hay 1983). This had led to the suggestion that it should be given in an acid medium. Adverse reactions to ketoconazole are uncommon and the drug is relatively well tolerated, especially at a dosage of 200 mg daily (Horsburgh and Kirkpatrick 1983). However, the occurrence of an apparent drug-related hepatitis in patients (Jansen and Symoens 1983) suggests that all patients treated with ketoconazole should have periodic monitoring of their liver function.

Chart A

Prevalence of Candida spp. or Candida Albicans
Isolated From The Oral Cavities of Normal Individuals

References (yr)	Sampling Techniques	Nature of Subjects	No. Studied	Candida Albicans or Candid %
Marples & DiMunna (1952)	a) oral swab	adults & children	381	18.3
	b) mouth wash	" "	307	41.0
Clayton & Noble (1966)	oral swab	a) medical students	23	8.6
		b) children	503	5.4
Johnston et al. (1967)	mouth wash	dental students	23	4.0
Arendorf & Walker (1979)	a) saliva	adults	60	29.6
	b) impression			13.0
	c) imprint			44.4
	d) smear			48.1
Berdicevsky (1980)	saliva	adults	30	52.0
Tapper-Jones et al. (1981)	imprint	adults	50	42.0

Chart B

Prevalence of Candida or Candida Albicans
Isolated From The Oral Cavities of Patients

References (yr)	Sampling Techniques	Nature of Subjects	No. Studied	Candida Albicans or Candid %
Peters et al. (1966)	oral swab	diabetic	400	40.8
Johnston et al. (1967)	mouth wash	a) hospitalized b) dental	81 104	42.0 15.4
Mackendrick et al. (1967)	mouth wash	chronic bronchitis	96	52.1
Barlow & Chattaway (1969)	oral swab	patients	128	39.1
Budtz-Jörgensen (1970)	impression	denture stomatitis	58	82.8
McFarlane & Mason (1974)	oral swab	Sjögren's syndrome	10	100
Epstein et al. (1980)	saliva	oral lesions	67	68.7
Berdicevsky et al. (1980)	saliva	denture wearers	26	88
Tapper-Jones et al. (1981)	imprint	diabetic	50	60
Dahlén et al. (1982)	oral swab /scraping	oral lesions	271	39.5

Chart C

Titers of Candida Organisms Isolated from the Oral Cavities of Individuals by Different Sampling Techniques

A) Saliva Sampling Technique

References (yr)	Subjects	No. Studied	Candida titers (means)
Fletcher et al. (1975)	chr. anemia with oral lesion	16	3.2×10^4 cfu/ml*
	candida carriers	13	8.8×10 cfu/ml
Epstein et al. (1980)	acute candidiasis	10	3.55×10^3 cfu/ml
	chronic candidiasis	18	1.51×10^3 cfu/ml
	carriers	18	2.44×10^2 cfu/ml
Berdicevsky et al. (1981)	denture wearers	23	2.7×10^4 cfu/ml
	dentate (carriers)	16	$1. \times 10^3$ cfu/ml
Epstein et al. (1981)	acute candidiasis	10	$4. \times 10^3$ cfu/ml
	chronic candidiasis	13	1.7×10^3 cfu/ml
	carriers	13	1.5×10^2 cfu/ml
Watson et al. (1982)	denture stomatitis	54	1.7×10^4 cfu/ml

* cfu/ml = colony forming unit per millileter of saliva

Chart D

Titers of *Candida* Organisms Isolated from the Oral Cavities of Individuals by Different Sampling Techniques

B) Imprint Sampling Technique *

References (yr)	Subjects	No. Studied	*Candida* titers (means)** cfu/cm^3				
			UP	AP	PT	Rb	Lb
Arendorf & Walker (1979)	carriers (dentate)	60	0	4	9	2	2
	denture wearers	60	35	15	20	12	12
	denture stomatitis	60	65	35	45	25	22
Tapper-Jones et al. (1980)	Sjögren's syndrome pts	16	17	18.5	26	19	18
Tapper-Jones et al. (1981)	healthy carriers	21	26	6	11	4	3
	diabetic carriers	30	45	19	35	26	24
Watson et al. (1982)	denture stomatitis	54	72	7	15	3	5

UP = upper fitting surface denture
AP = anterior palate
PT = posterior tongue
Rb = right buccal cheek
Lb = left buccal cheek

ND = not done
cfu = colony forming unit

* Imprint technique (Arendorf & Walker 1979)
** coding only 5 out of 12 sites from original sources

Chart E

Titers of *Candida* Organisms Isolated from the Oral Cavities of Individuals by Different Sampling Techniques

C) Mouth Wash Technique

References (yr)	Subjects	No. Studied	Candida titers (means)
McKendrick (1967)	healthy individuals	20	7.71×10^2 cfu/ml
	chronic bronchitis & antibiotics	53	5.20×10^2 cfu/ml mouth wash
McKendrick (1968)	healthy mouth	85	4.27×10^2 cfu/ml
	denture stomatitis	11	1.36×10^3 cfu/ml mouth wash
Renner et al. (1979)	dental assistants	20	1×10^2 cfu/ml broth/swab
	dental stomatitis	25	1×10^4 cfu/ml broth/swab

cfu = colony-forming units

MATERIALS AND METHODS

STUDY POPULATION

As a condition of the study, all subjects were positive for oral Candida by laboratory testing, exclusive of signs and/or symptoms. As control subjects, 10 healthy adults were recruited from staff and student volunteers from the University of California, San Francisco School of Dentistry. All had healthy oral mucosa, free from any signs and symptoms of oral candidiasis. Invididuals who had a history of medical conditions or were taking medication likely to influence Candida populations were excluded. This control group consisted of 3 males and 7 females, ranging in age from 23 to 54 years.

The study group consisted of 48 patients with signs and/or symptoms of oral candidiasis. This group consisted of 30 males and 18 females, ranging in age from 20 to 79 years. All members of this group had clinical examinations at the Oral Medicine Clinic, UCSF. The majority, in addition to suspected oral candidiasis, had other disease conditions. Medical, dental and social histories were recorded for each patient; the patient was questioned as to history of treatment for oral candidiasis with or without underlying conditions. The signs and symptoms such as dry mouth, burning or tingling, soreness, red and white changes, were recorded for each patient. Red lesions included localized and/or generalized diffuse erythematous patterns. The white changes were characterized by any whitish-appearing change in the oral cavity. After complete evaluation of all findings, patients were classified into different groups (Table I).

CLINICAL PROCEDURES

Investigation for *Candida* Organisms

The prevalence of *Candida* spp. in each participant's mouth was estimated by a series of techniques. These consisted of:

1. Gram staining
2. Swab technique
3. Imprint technique
4. Salivary technique
5. Mouthwash technique

Whenever practical, the above techniques were carried out in normal controls. They were also done prior to, during and after treatment. All samples were immediately transported to the Clinical Laboratory of the Division of Oral Medicine, School of Dentistry, University of California.

Gram staining: Epithelial smears were obtained by scraping the oral mucosa with a thin cotton-tipped swab from a lesion or an area suspected to be candidiasis. The material was spread thinly on a glass slide marked with the name of the patient, and allowed to air dry.

Oral swab technique: Specimens using this technique were taken for two purposes, one for a routine culture and the other for a quantitative culture study.

The cultures were taken by firmly rubbing two sterile cotton-tipped swabs on the oral mucosal surfaces of each subject. The swabs were also rubbed on the dorsal surface of the tongue and the palate as well as on the lesions. As the swabs were taken, one was placed in Amies medium, which is a

solution of buffers and charcoal. The other swab was dropped into a tube containing 10 ml. sterile physiological saline solution (SPS).

Imprint technique : For the imprint culture, we used a modification of a technique described by Arendorf and Walker (1980). Sterile plastic foam pads (1 x 1 cm) were dipped in SPS solution to make them more adherent, then placed against the surfaces for 60 sec. on the following sites:

a) right buccal mucosa, close to the right commissure of the lips

b) midline of the hard palate

c) mid-posterior dorsal tongue, anterior to the circumvalate papillae

d) lesion (in patient) or left buccal mucosa (in control)

After removal, the pads were then pressed firmly on a mycobiotic agar plate (Difco Laboratories, Detroit, Michigan) and left in place about 2-4 hr at room temperature (Fig 5b).

Salivary technique : Each subject was asked to spit the mixed unstimulated saliva during a 3-minute period into a sterile graduated container. The 3-minute period was used to estimate the flow rate of unstimulated saliva in each patient.

Mouthwash technique: The subject was first instructed to swallow his or her own saliva and was then given 10 ml of sterile physiological saline solution with which to rinse the mouth for 60 sec. At the end of this time the patient was told to allow the mouth-suspension to drain out between parted lips into the original tube.

Delayed Hypersensitivity Skin Testing

Each subject was tested with a panel of four skin test antigens in an intermediate test strength. They were as follows:

a) Candida (dermatophyton 0) 1:100

b) PPD (purified protein derivative of tuberculin) 5u/0.1 ml.

c) mumps (2 complement fixing units/0.1 ml)

d) trichophyton (dermatophyton 1:30)

Each sterile tuberculin syringe equipped with a 0.5-inch, 27-gauge needle, was labelled with the name of the filled antigen. The injection sites were selected on forearms and cleaned with a sterile alcohol swab. 0.1 ml of each antigen was injected intracutaneously. This resulted in a bleb about 5-10 mm in diameter. The injection sites were circled with an indelible marker and labelled; the patient was advised not to wash off the circles until the tests were read (Fig. 11a).

Antigens were always injected in the same order. The reading and recording were performed at 48 hr. after each injection. The diameters of erythema and of induration were measured in two directions and were each recorded in millimeters.

Second-strength antigens of Candida (1:10) were applied in cases in which the initial reading was negative.

Blood Examination

Blood glucose levels and complete blood counts; which included hemoglobin, packed cell-volume and differential white blood cell counts, were performed on available patients.

LABORATORY PROCEDURES

Gram-Staining

The smear was heat-fixed by passing the slide through a flame several times. Care was taken not to overheat the slide; the temperature was just tolerable to skin on the back of the hand. The fixed smear was covered with a few drops of crystal violet for 30 sec, then washed quickly with water. It was then covered with a few drops of Gram's iodine for 30 sec, and again washed off with water. Next, the stain was decolorized with acetone-alcohol by allowing 4-5 drops to run over the slide until only faint color came off. This took about 10-20 sec, after which the slide was rinsed with water. The smear was then covered with safranin (red counterstain) for 20 sec, washed with water; and blotted dry.

The Gram-stained slides were examined microscopically using oil immersion basis.

Microbiology

As soon as cultures arrived in the laboratory, they were refrigerated up to the time of inoculation (not longer than 4 hr), while imprint-culture plates were stored at room temperature.

Routine culture: The cotton-tipped oral swab that had been placed in Amies medium was removed, using sterile technique (glove and tweezer). The swab was plated onto a petri dish of Sabouraud's dextrose Emmons and a mycobiotic agar plate. Both plates were incubated at room temperature (25 ^{o} C) for 2-7 days, after which time the colonies were identified.

Salivary specimens: The amount of saliva was translated into ml/min, estimating a flow rate of whole unstimulated saliva in each subject. The specimen was well mixed by rinsing in and out a 1 ml graduated sterile pipette. After mixing, the saliva was diluted up to 10^{-2} concentration using

0.9% sterile normal saline. Each 0.1 ml of neat saliva and of 10^{-2} dilution was duplicately spread onto mycobiotic agar plates.

Inoculation of swab and mouthwash suspensions: The oral swab which had been immersed in a tube of sterile physiological saline was squeezed out and removed from the tube. Then 0.1 ml of each thoroughly mixed suspension, obtained by the swab and the mouthwash technique was duplicately spread onto the mycobiotic agar plates.

At this time, the foam culture pads obtained by the imprint technique were removed from the mycobiotic agar plate on which they had been placed.

Incubation: All culture plates were incubated at 37°C for 48 hrs, and the number of surface colonies per ml of specimen was counted (Fig 6). Plates without fungal growth at 48 hrs. were incubated for up to 1 week before being discarded as negative findings.

Identification of Candida Organisms

The medium and culture conditions employed selected for yeast growth, and usually all the colonies were similar in appearance; therefore, only one or a few colonies were identified.

Candida spp. were identified on the basis of their gross morphologies, germ tube and chlamydospore formations (Fig 7). If the germ tube formation gave negative , carbon and nitrate assimilation tests and/or Uni-Yeast-Tek system (Flow Laboratories Inc.) were performed.

STATISTICAL ANALYSIS

Evaluation of data was done by using the following tests:

1. Analysis of variance using the Scheffé procedure
2. Kruskal-Wallis test
3. Fisher's exact test
4. Omnibus test and comparisons using the Median test
5. Pairwise comparisons between groups using the Bonferroni multiple comparison

FINDINGS

The findings will be reported in 2 parts. The first part will analyze subject characteristics and the second part will report on the colony count results.

SUBJECT CHARACTERISTICS

Table 1 shows 58 participants in the present study grouped according to their identified underlying states (see Materials and Methods).

Since some groups contained too few patients for statistical analysis, data from only 4 groups were analyzed statistically. These groups were:

1. Healthy heterosexual controls (n=10). None of the subjects in this group presented with signs and symptoms suggestive of candidal infection.

2. Homosexual men without lymphadenopathy (n=10). Six of these homosexuals presented with red and white changes in the oral cavity and past histories of multiple fungal infections. but none were taking medication for the infection at the time of the study. Red oral mucosae were reported in three of this subgroup. One patient exhibited removable curd like materials referred to as acute oral candidiasis. Four patients had xerostomia.

3. Irradiated head and neck cancer patients (n=10).

Malignancies found in this group were diagnosed as leukemias, lymphomas, adenocarcinomas, mucoepidermoid carcinoma, squamous cell carcinomas, and a metastatic tumor of unknown primary lesion. Treatments for these patients were radiation alone or in combination with chemotherapy or surgery or both. The oral cavity and salivary glands were included in the radiation field in every patient. Total radiation doses ranged between 2000 and 8000 rads.

Six of these 10 irradiated patients had completed their irradiation from one month to 8 years prior to the time of the study. Four of these six experienced recurrent episodes of chronic oral candidiasis, but only one of them was receiving antifungal treatment at the time of the study. Four patients were undergoing irradiation at the time of the study; two were being treated with chemotherapy. The latter two developed removable curd-like material associated with diffuse erythematous areas. One of these two patients was taking antifungal medicine prescribed by her oncologist.

4. The patients with red and white oral lesions (n=14). Eight of these patients were diagnosed as having vesiculoerosive diseases. Three of the eight patients were taking prednisone tablets daily in dosages ranging from 20 to 60 mg. The remaining five patients had not yet started corticosteroid treatment, but two of them reported a history of using mycostatin suspension for their oral lesions, based on positive findings of C. albicans in the mouth.

The mean ages of these four groups fell into 2 groups: the heterosexual controls and the homosexuals were in the fourth decade; and the irradiated and red and white lesion group were in the sixth decade. Analysis of variance indicated that there was a significant difference in the mean age among these selected groups ($P < 0.0001$). Further comparisons between the groups using the Scheffé procedure indicated significant differences between the healthy control group and both the irradiated patients and the red and white lesion group. No signficance in age was found between the heterosexual controls and the homosexuals.

Means of whole unstimulated salivary flow rates of the selected patient groups ranged from 0.07 to 0.59 ml/min while the mean of the control group was 0.50 ml/min. In three of 10 irradiated patients, the saliva could not be

collected in the three minute collection period.

Using the analysis of variance, there was a signficant difference in the unstimulated salivary flow rates among 4 selected groups (P=0.027); however, no differences between the salivary flow rates of each patient group and the control group were observed when the groups were analyzed using median test ($P > 0.05$).

Table II shows the tobacco smoking and denture wearing found among patients in the four groups studied.

The majority of the homosexual men who participated in the study reported frequent or occasional use of recreational drugs in addition to tobacco usage. Recreational drugs mentioned were marijuana, cocaine, heroin, and amyl nitrate. None of the heterosexual controls reported use of recreational drugs.

The frequency of positive responses to the skin test antigens in the heterosexual controls and the homosexuals who had no lymphadenopathy has been analyzed by means of the Fisher's exact test (Table III). A positive response was considered to be induration of 5 mm or more at the injection site at 48 hrs.

Seven of the 10 patients in the homosexual group showed no reactivity to any of the four skin test antigens, whereas 8 of the 9 controls responded to at least one or more antigens.

The reactivity to individual antigens was lower in the homosexual patients compared to the heterosexual controls (Table IIIa). This was statistically significant for the mumps antigen ($P = 0.001$).

The fasting blood sugar and white blood counts taken from the healthy controls, homosexuals and the patients with red and white oral lesions were

analyzed by means of analysis of variance. The test suggested that there was a significant difference in fasting blood sugar among these 3 groups ($P < 0.05$). The Pairwise comparisons between groups using the Bonferroni multiple comparison procedure indicated that the significance was between the homosexual and the red and white oral lesion patients at the 0.05 level. No such signficance was found between other groups.

As compared with bood counts obtained from the controls, those obtained from the patients were not significantly different with the exception of the white blood counts ($P < 0.05$). The significance was in both the control and red and white lesion patients ($P < 0.05$) and in the red and white lesion patients and homosexual group ($P < 0.05$).

THE DETECTION AND CONCENTRATIONS OF CANDIDA ORGANISMS

Table IV shows positive findings of oral Candida isolated from the 48 patients with oral diseases and from the 10 healthy controls.

All of the controls and patients had Candida organisms on at least one of the tests used in the study. To determine if Candida were present in the suspected candidal lesion, seven biopsy specimens were obtained and stained with Periodic-acid-Schiff reagent. Only four of these seven patients were found to be positive for C. albicans.

Four species of Candida were detected in this study. C. albicans was the main organism found in 56 subjects with and without oral signs and symptoms. The other species present were C. lusitanae, C. pseudotropicalis, and C. glabrata which were detected in 4 patients.

Of interest, C. glabrata was not identified on the first visit. Blastospores of C. glabrata were detected using the gram-staining technique.

The surface colonies of this fungus grew only on routine culture plates which were incubated at room temperature (20°C). No colony-forming units of C. glabrata were found on any culture plates incubated at 37°C.

Tables VI, VI and VII show the density of C. albicans in the groups of patients and healthy controls.

C. albicans counts isolated from the oral cavities of all patients were higher than those of healthy controls in all sampling techniques (Table V, VI).

The highest Candida isolation was detected using the undiluted salivary technique. The count (2167 cfu/ml) was approximately two times as high using the mouthwash technique (865 cfu/ml) and six times as high when the swab technique was used (341 cfu/ml) in the same group of patients. However, dilution of the same saliva (10^{-2} conc.) did not yield the same obtained from the undiluted saliva.

The efficacy of the five techniques in detecting Candida organisms was evaluated. Statistical analysis indicated significances in numbers of C. albicans isolated among four selected groups (control, irradiated, homosexuals without lympadenopathy syndrome, red and white oral lesion groups) (Table VI). The significances were observed in most of the techniques used, but not in the gram-staining (Fisher's exact test) and the swab technique (Kruskall-Wallis test).

Not all of the differences between Candida isolates of the controls were significant when the subjects were subdivided according to their underlying conditions, and the organisms were detected using the different sampling techniques (Table VI, VII). However, the mouthwash technique showed significant differences in detecting C. albicans from every group of patients as compared to the control group. The significances ranged from $P < 0.05$ to $P < 0.001$ (Table VI).

When the controls and the patients were compared for frequency of recovery of C. albicans from different sites (using the imprint method), the dorsal tongue was the most frequent site for C. albicans in the controls (1.3cfu/cm^3) while the lesion was the most frequent site of the organisms in the patient group (27 cfu/cm^3). But, the frequency of Candida counts at intra-oral sites varied when we subdivided the patients into subgroups (Table VII).

Considering that the density of Candida in the patient group was higher than that in the healthy controls and should be quantitated by the culture techniques, we had confirmed the hypothesis by determining whether the colony counts of Candida in the patients were reduced after antifungal treatment. The salivary, mouthwash, and the imprint techniques were used in 3 patients with signs and symptoms of oral candidiasis to determine the Candida counts.

The pilot study revealed improvement in signs and symptoms of Candida infection together with the great reduction of Candida colony counts taken 2 weeks after antifungal medicine had been applied. Likewise, the cultures at the latter 2 weeks, as the patients reported their experience discomforts, yielded higher colonies of the organisms compared with those taken during the treatment.

The distribution of Candida isolates in each patient are shown below.

Table a

C. albicans concentration changes with antifungal treatment

Samples	Before Treatment				During Treatment				After Treatment			
	I	II	III	mean	I	II	III	mean	I	II	III	mean
Undiluted* saliva	9070	1420	230	4433.3	270	170	100	180	1450	580	570	866.7
Diluted* saliva	825	70	510	468.3	10	40	20	23.3	230	52	0	94
Swab*	20	30	40	30	0	0	0	0	5	0	185	63.3
Mouth-wash*	710	340	80	376.7	10	170	0	60	110	0	0	36.7
Imprint**												
Buccal mucosa	5	13	0	6	0	0	0	0	0	3	0	1
Palate	15	9	5	9.7	0	0	0	0	6	7	1	4.7
Dorsal tongue	16	3	4	7.7	0	0	0	0	0	4	0	1.3
Lesion	6	30	3	13	1	2	0	1	0	0	0	0

* colony-forming units/ml

** colony-forming units/cm^3

I, II, III = Patient number

DISCUSSION

Many Candida species including C. albicans are commensals in the oral cavities of normal individuals (Berdicevsky et al. 1980, Arendorf and Walker 1980, Tapper-Jones et al. 1980) and of patients (Tapper-Jones et al. 1980, 1981, Walker et al. 1981, Epstein et al. 1981, Bastiaan and Reade, 1982, Dahlén et al. 1982, DeGregorio et al. 1982 a, b, Silverman et al. 1984 a, b). Some researchers (Campbell and Heseltine 1960, Epstein et al. 1980) have considered candidal pathogenecity in terms of its density. Therefore, we used 5 sampling techniques to quantitate and to qualitate Candida organisms in the oral cavity of our subjects.

The present investigation has confirmed and extended previous studies indicating that C. albicans is the main species (Barlow and Chattaway 1969, Walker et al. 1981, DeGregorio 1982a), and it is more prevalent in the mouths of patients than in those of healthy controls (Arendorf and Walker 1979, Tapper-Jones et al, 1981).

The statistical analysis also indicated significant differences between colony counts in samples taken from the healthy controls and from patients with oral diseases together with suspected oral candidiasis ($P < 0.0.5$). The results support a conclusion that carriers and patients with candidal infection may be distinguished on the basis of quantitative cultures for C. albicans. This was shown by the reduction of Candida counts in 3 patients who were treated for oral candidiasis. Because of the small sample size of the selected patient groups and the wide range of colony forming units, the cut-off point between

the controls and the patients was arbitrary and requires a well-designed prospective study with defined patient groups and regulated tests.

The gram-staining direct smear appeared to be inadequate. We were unable to identify blastospores or candidal hyphae in the oral mucosa of the healthy controls, and only 6/48 (12.5%) of our patients showed the blastospores and/or hyphae in their smear materials. The smear gave fewer positive results than cultures (Table IV) and the differences in the presence of candidal elements were not statistically significant. Arendorf and Walker (1980) have reported Candida hyphae in smears from 3 of their 54 healthy subjects. Therefore, the absence and/or presence of hyphae and blastospores in smears neither specifically means it is not an overgrowth of Candida or it is a pathologic state. Since we used the cotton swab to transfer the specimens to glass slides and the hyphae might have been lost.

In general, patients treated with radiotherapy for head and neck malignancies present with oral lesions associated with xerostomia and pain. These signs and symptoms minimize the efficiency of the direct smear technique, since a firm scraping and/or adequate saliva are necessary.

Another limitation of the smear technique relates to the fact that Candida in the mouth is not only one species. Four of 58 of our isolations were not C. albicans, and the direct smear could not identify those species unless used in combination with culture methods.

Relative to culture techniques, our results demonstrated that there is considerable variation in Candida counts. Therefore, the appropriate technique used must be selected on the basis of the measurement desired and patient source.

Using the Swab technique, Candida organisms were detected from 34 of 48 patients compared to 41 positive cases using undiluted saliva and mouthwash

tests. Not surprisingly, Candida counts isolated by swab technique were smaller since the swab samples were obtained from limited sites. The swab as well as the smear may also fail to satisfactorily transfer the organisms from oral mucosae to the culture media.

The Imprint technique yielded the highest number of positive cases in the patient groups (42/48). The only explanation would appear to be based on the efficacy of obtaining and transferring the fungus. There was no significant difference in the number of colony-forming units isolated from the red and white lesions, homosexuals and healthy controls. The results might indicate that Candida detected from patients were not involved in the pathogenecity of the observed and sampled lesions.

The mouthwash technique was the most effective method in detecting significant differences in the counts of Candida organisms from all patient groups. Furthermore, the colony forming counts were second only to those from the undiluted saliva, indicating usefulness also in differential quantitation. The mouthwash technique was easily standardized, further supporting its usefulness.

Undiluted samples of saliva yielded the highest incidence in both the frequency and the density of candidal organisms. Therefore, this technique is the most sensitive method for detecting Candida organisms in the oral cavity of both healthy and diseased individuals. This is contrary to the findings of Arendorf and Walker (1980) who stated the salivary samples yield less positive findings for Candida than the imprint technique. However, the majority of reports are consistent with our findings (Lehner 1966, 1967, Sharon et al. 1977).

The Candida density when using diluted saliva was not statistically different between the controls and the patients with oral lesions (P=0.2138). Although this served the purpose of simplifying colony counts, it dampened the

quantitative differences being sought. Less severe dilutions would probably solve both problems.

A wide number of variables found in our patients affected the Candida counts. An interesting observation was a direct relationship between the density of colonies and elderly patients. The effect of age on Candida is not clear, but changes in immune parameters (Weksler and Hansman 1982) and the increased number of denture wearers in the elderly (Budtz-Jörgensen et al. 1975) are important factors.

Smoking may be another factor influencing increased Candida counts among our patients (Beasley 1969, Arendorf and Walker, 1980, Holmstrup and Bessermann 1983).

Marijuana, a favorite recreational drug among homosexual patients, may in part account for the high counts of Candida found in the homosexual group. This may be due to the fact that cannabinoids cause xerostomia (Domino 1981) and exhibit an antibiotic effect (Nahas 1973) that may provide a favorable environment for Candida growth. Additionally, a high percentage of homosexual men may be at risk for immunosuppression.

In patients with malignant disease, cytotoxic drugs and irradiation induce mucosal ulceration (mucositis) and xerostomia which support fungal overgrowth (Chen and Webster 1974, Silverman et al. 1984b).

The above factors explain and the support the differences found in the patient groups.

SUMMARY AND CONCLUSIONS

1. Five tests for detecting and/or quantitating Candida were studied on 48 patients with oral disease and 10 healthy controls.

2. All subjects have been found to have Candida organisms on at least one of the tests. None of the controls were symptomatic, while all of the patients had signs and/or symptoms of candidiasis.

3. Candida were identified as albicans species in 97 percent (56/58) of the subjects, and other species of the genus in 7 percent.

4. The undiluted saliva was evaluated as the most overall sensitive tests, being positive for Candida in 51 of 58 individuals. This technique also gave the highest colony levels.

5. Using diluted saliva made colony counts easier but reduced the accuracy of quantitation for differentation between certain patient groups and controls.

6. The mouthwash technique was fairly accurate (83%) with smaller counts than the undiluted salivary technique. This caused some lack of differentation between some groups. However, the mouthwash technique is the test of choice for the patients with xerostomia.

7. The imprint technique was excellent for quantitating Candida colony counts for specific intraoral areas.

8. While the swab technique was the easiest test for obtaining specimens and innoculating plates, it was less accurate than either the saliva or mouthwash techniques.

9. Direct smears and gram-staining was the least sensitive for detecting Candida (6/58). In addition, the test cannot be used for quantitation or identification of strains.

10. Asymptomatic controls had the smallest colony counts which indicated the possibility of differentiating carriers from pathogenic states of candidal infection.

11. While conclusions could not be drawn regarding Candida counts and levels of pathogenicity, with a properly designed study, this identification remains a possibility. Additionally, along with signs and symptoms, the quantitation of colonies appears to be a potentially useful adjunct in assessing management progress.

12. The heavy Candida counts isolated from homosexual men may indicate the role of immunosuppression.

13. Low salivary flow rates and xerostomia influence and support higher oral Candida counts.

14. Age, dentures and tobacco use also probably influence an increased occurrence and pathogenic levels of Candida.

15. Correlations between candidal counts and either blood counts or blood sugar levels were not apparent

16. Epidermal response to Candida skin test antigen challenges could not be related to the candidal counts. However, diminished or absent reactivity in the response should warn the clinician of a possible defect in immunity.

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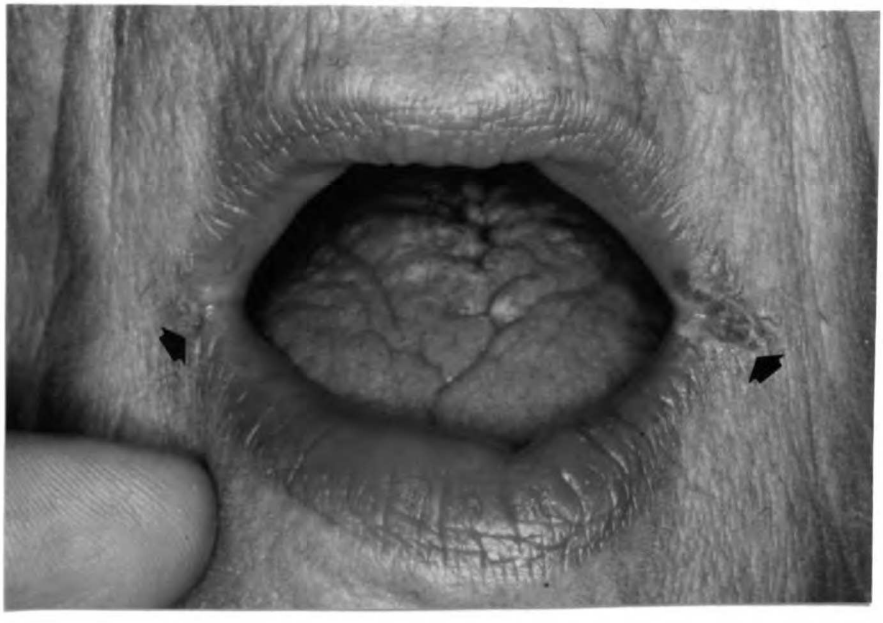
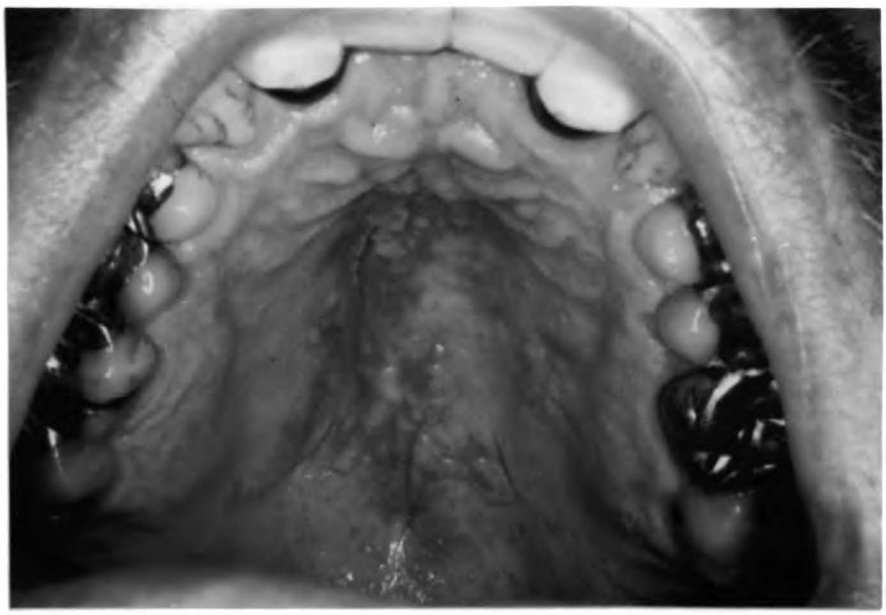
Figure 1.



Xerostomia and angular cheilitis (arrows) in an irradiated head and neck cancer patient. The lesions disappeared after appropriate antifungal therapy.

Figure 2



Inflammatory palatal hyperplasia in clinical appearance (upper). Cultures for Candida were positive and the lesions disappeared after anti-fungal therapy (lower).

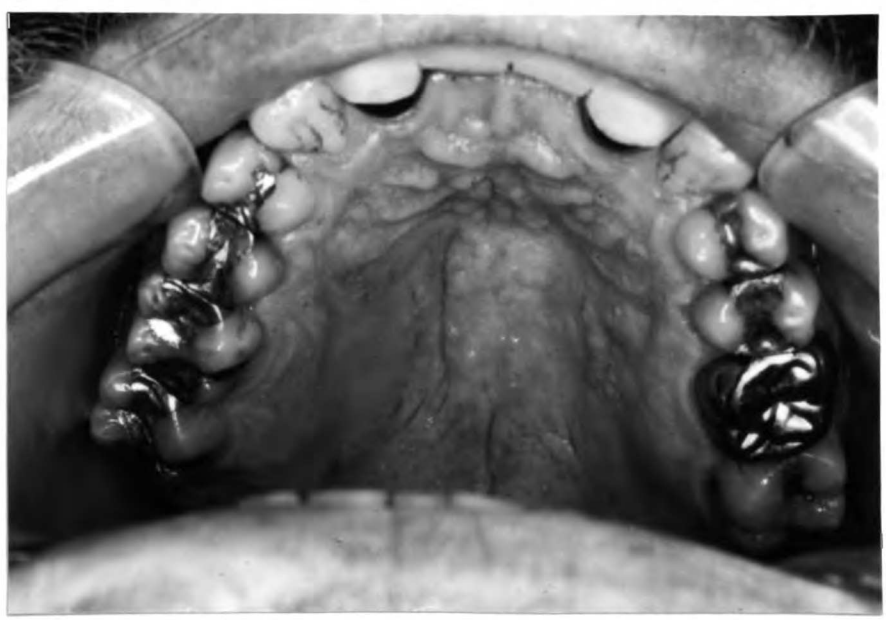
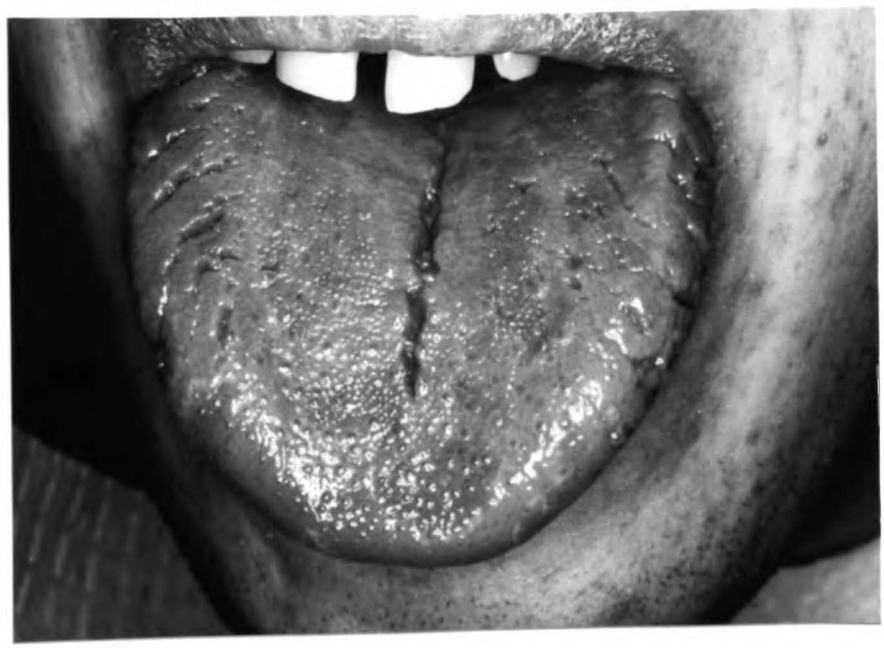


Figure 3

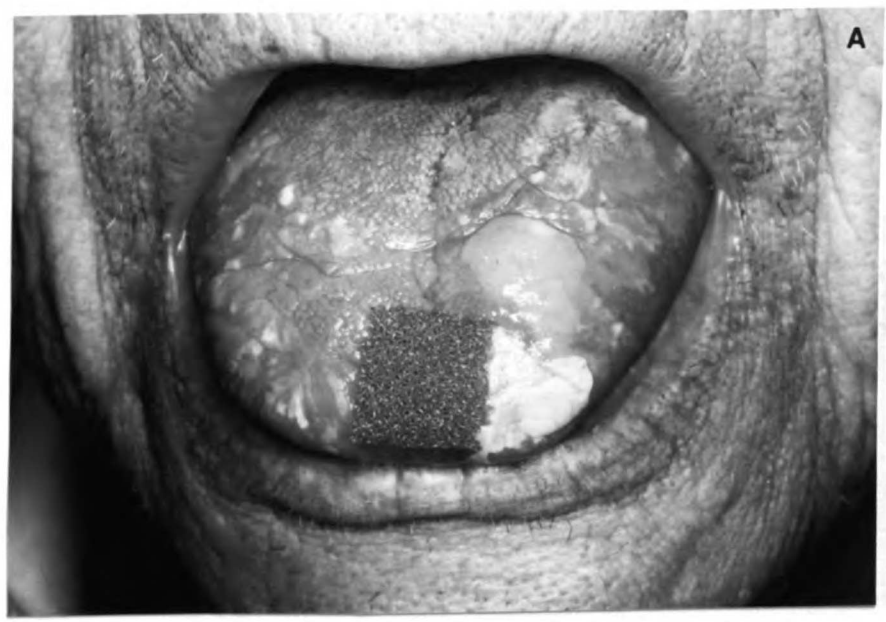


An erythematous and xerostomic mouth of a Sjögren's syndrome patient who yielded very high colony counts of C. albicans.

Figure 4

Presence of C. albicans blastospores on epithelial cells detected from a hairy leukoplakia lesion using gram- staining technique.

Figure 5



Imprint cultures from a patient with erythroleukoplakia and glossopyrosis (A). The culture foam pads were innoculated onto a mycobiotic agar plate at room temperature (B).

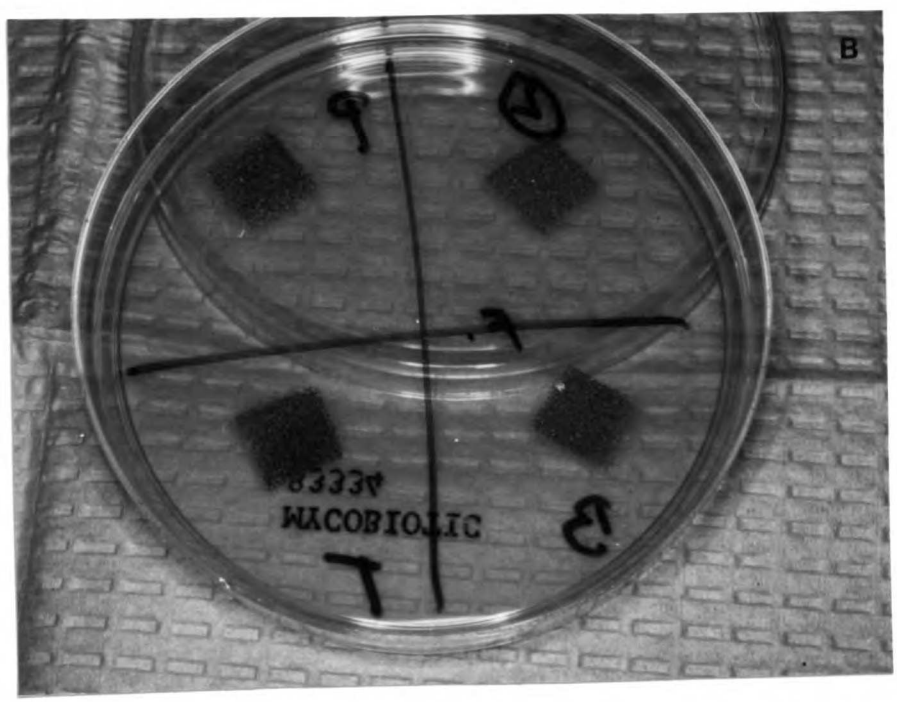


Figure 5

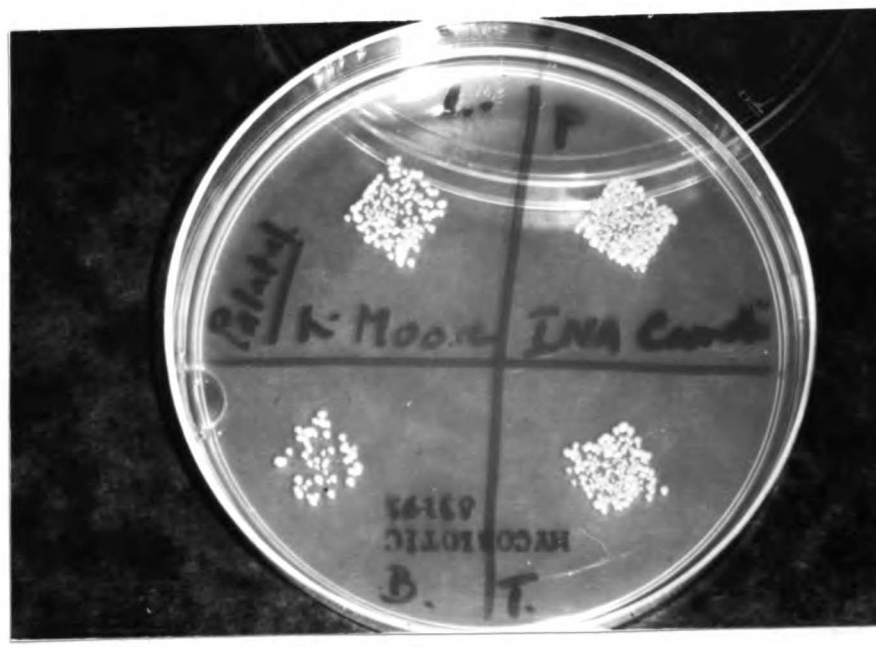


Fig 5C The mycobiotic agar plate shows very large numbers of yeast colonies taken from the specific intra-oral sites after imprint materials were incubated at 37°C. for 48 hours.

Figure 6

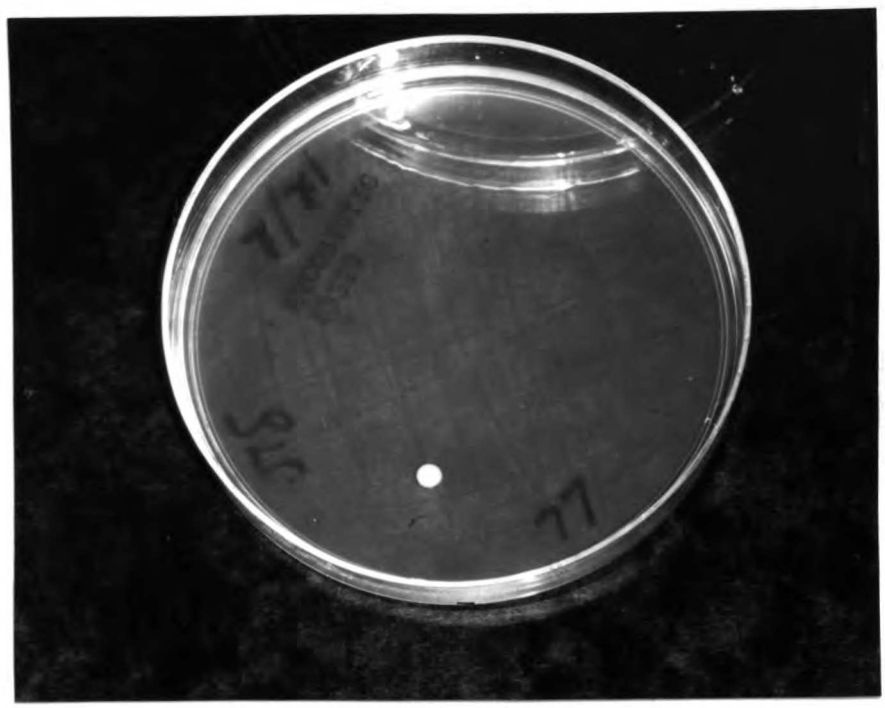
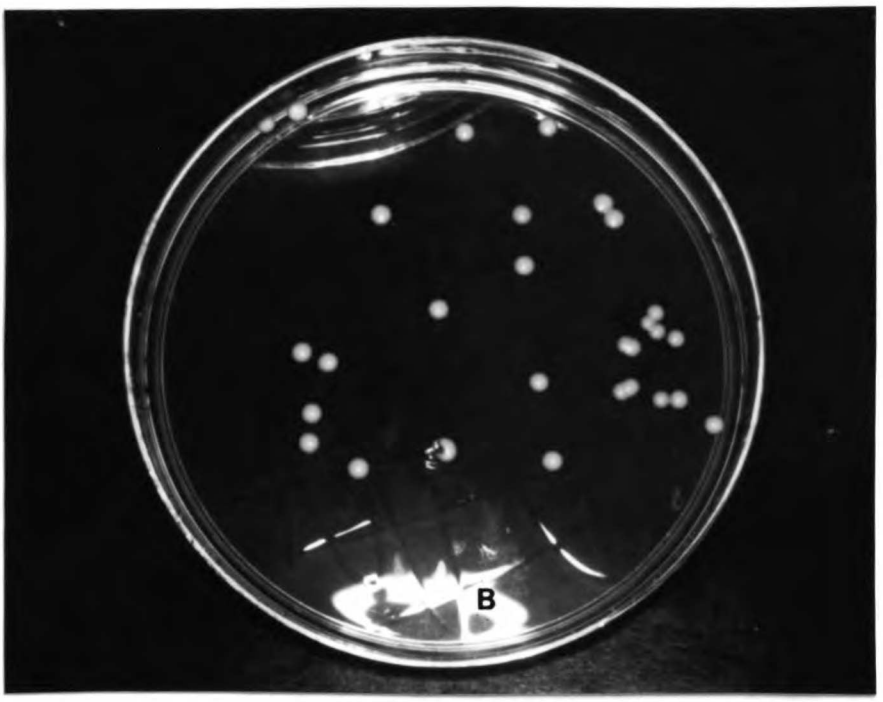


Fig 6A. Only a very few colonies of C. albicans were isolated from a swab obtained from a healthy individual.

Figure 6



The agar plates show moderate (Dilute saliva source) (B) and heavy (undiluted saliva source) (C) growth of C. albicans isolated from a patient with oral lesions, using quantitative culture techniques.

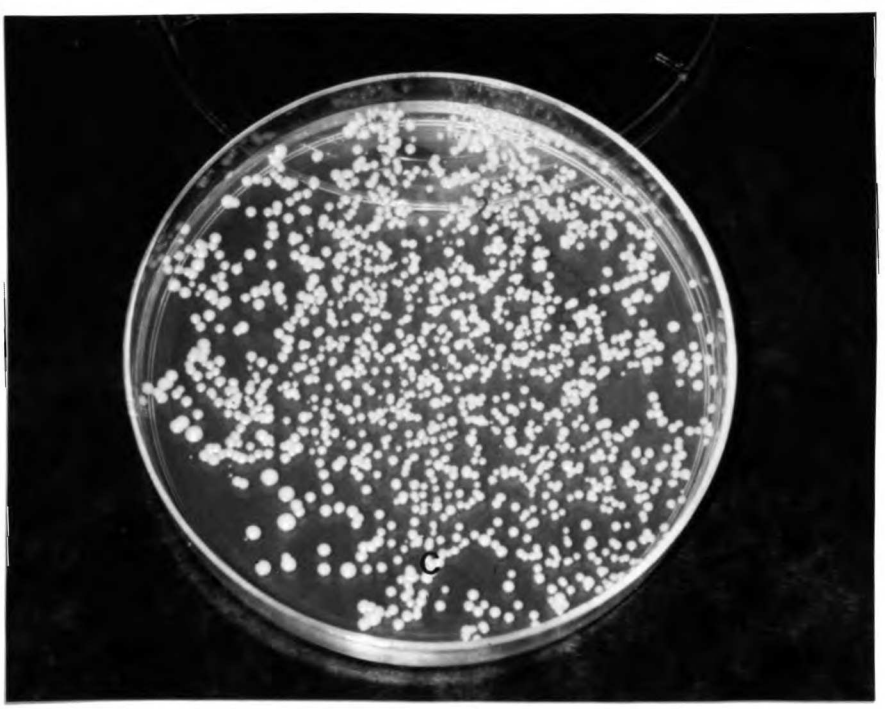
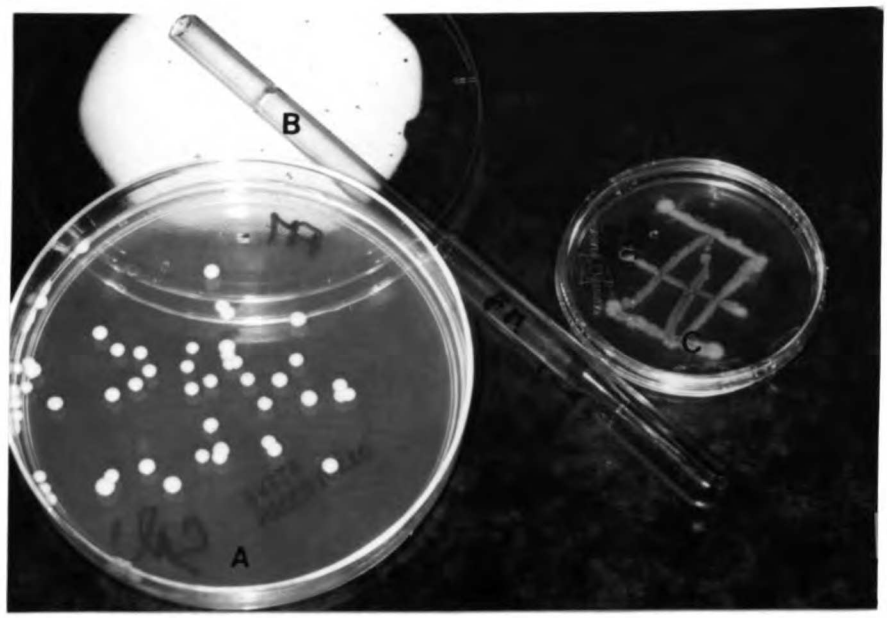


Figure 7



(Upper) Presumptive tests for Candida albicans using gross-surface morphology (A), germ tube formation (B) and chlamydospore formation (C). (Below) Yeast assimilation for Candida species via Uni-Yeast-Tek. (A) Before and (B) after incubation.

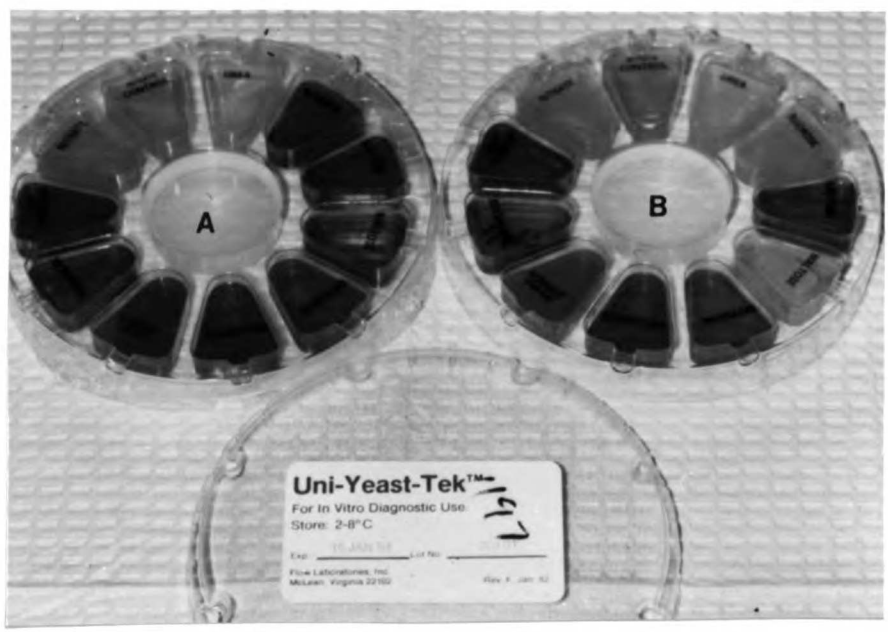
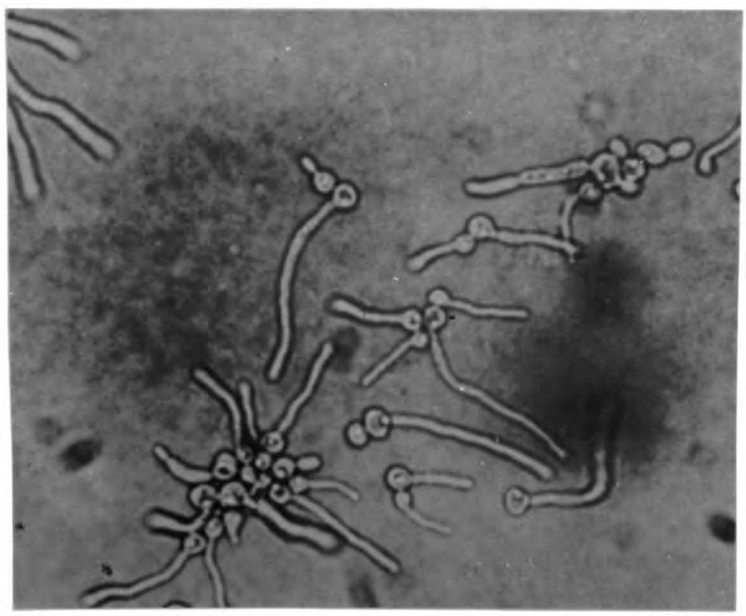
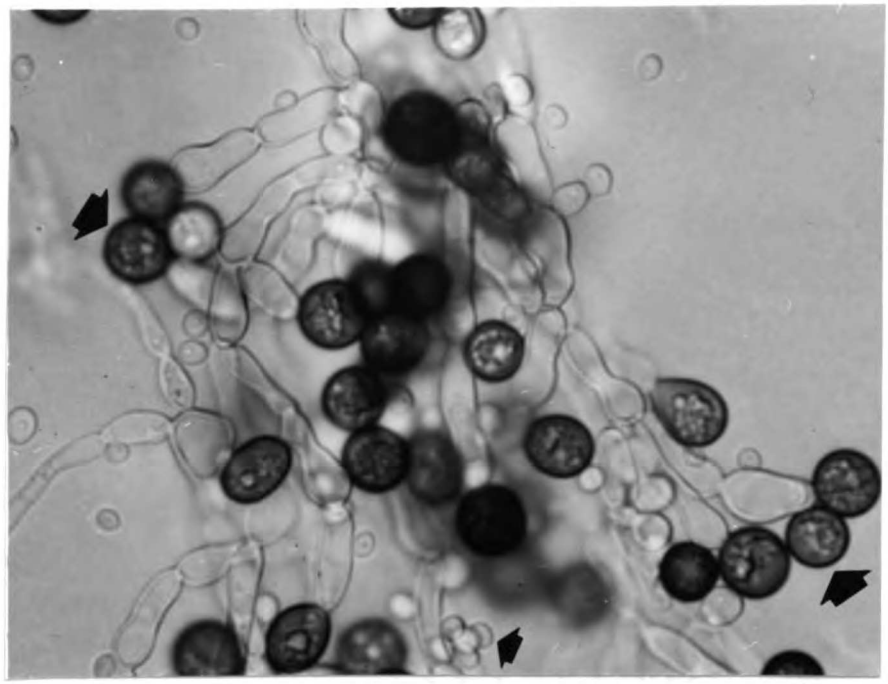


Figure 8



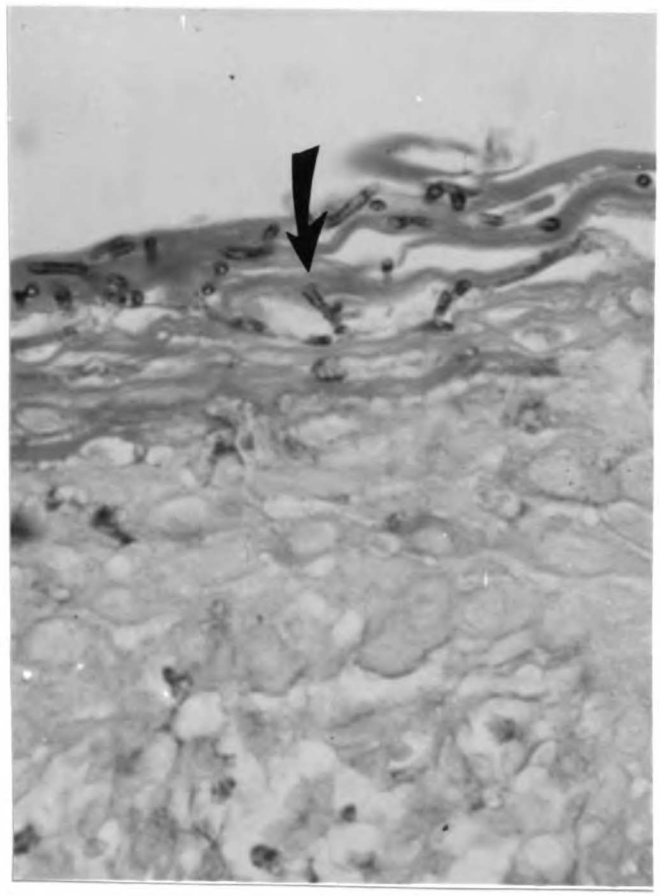
Germ tubes of C. albicans after 3 hour incubation at 37°C. Note formation of hyphae.

Figure 9



Pseudohyphae of C. albicans on chlamydospore agar showing clusters of blastospores (small arrow) and terminal chlamydospores (large arrows).

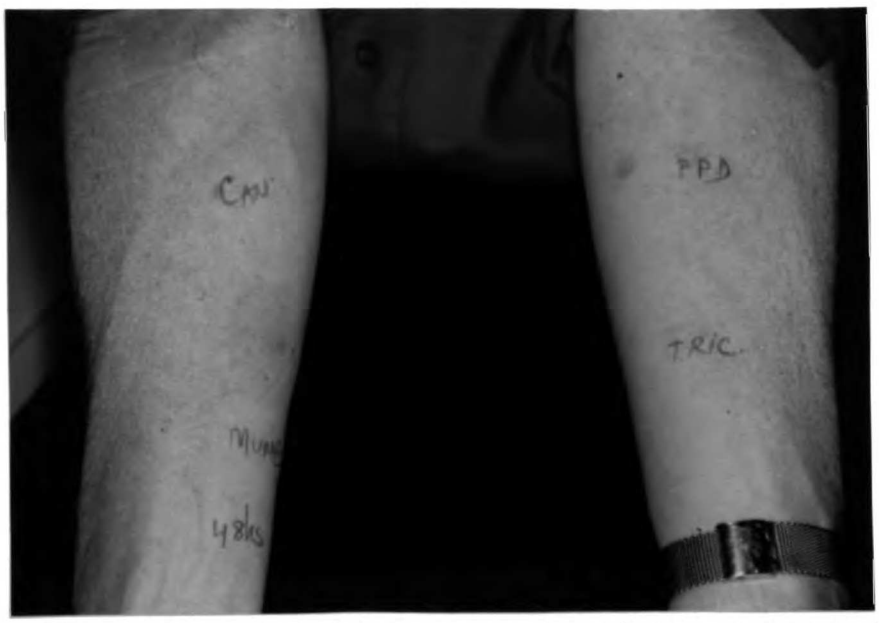
Figure 10



Microscopic appearance of Candida hyphae (pseudomycelia) in oral mucosal surface epithelium of an infected patient.

Figure 11

a) Skin test demonstrating intraepidermal challenge technique.



At 48 hrs. only mumps antigen was positive (> 5 mm induration). Seven of 10 patients tested for delayed hypersensitivity were Candida antigen negative.

Table I

PARTICIPANTS IN THE STUDY (n = 58) GROUPS

ACCORDING TO THEIR IDENTIFIED UNDERLYING CONDITIONS

Group	No. Subject	Sex F:M	Age (yrs) mean	Age (yrs) range
Healthy heterosexual controls	10	7:3	35.7	23-54
Homosexual men				
without lymphadenopathy	10	0:10	33.4	25-52
with lymphadenopathy	5	0:5	33.6	22-65
with Kaposi's sarcoma	3	0:3	41.3	31-54
Patients with red & white oral lesions	14	10:4	54.6	28-48
Head & neck cancer patients				
Irradiated	10	3:7	56.7	34-79
Non/pre-irradiation	3	3:0	73.7	67-78
Patients with Sjogren's syndrome	2	1:1	34.0	20-48
Patients with glossopyrosis	1	1:0	73.0	73-

Table II

SMOKING HABIT AND DENTURE WEARING IN GROUP PARTICIPANTS

Group	Tobacco smoking number of cases	Denture wearing number of cases
Heterosexual controls	3/10	1/10
Homosexual men		
without lymphadenopathy	9/10	2/10
with lymphadenopathy	5/5	0/5
with Kaposi's sarcoma	1/3	0/3
Patients with red & white oral lesions	7/14	5/14
Head and neck cancer patients		
Irradiated	3/10	4/10
Non/pre-irradiation	1/3	1/3
Patients with Sjögren's syndrome	1/2	0/2
Patient with glossopyrosis	0/1	1/1

Table III

FREQUENCY OF POSITIVE RESPONSE TO SKIN TEST ANTIGENS IN 9 HEALTHY HETEROSEXUAL CONTROLS AND 10 HOMOSEXUALS WITHOUT LYMPHADENOPATHY SYNDROME

Skin-test antigen	Induration to skin-test antigens (at least 5 mm induration)		
	Controls		Homosexuals
Candida (1:100)	7/9	P = 0.07	3/10
PPD (5UT)	3/9	P = 0.08	0/10
Mumps	8/9	P = 0.001	1/10
Trichophyton (1:30)	0/9	P = 1.000	1/10

: Statistics using Fisher's exact test. P = exact probability

Table IIIa.

MEAN OF REACTIVITIES IN MILLIMETERS OF INDURATION TO SKIN-TEST ANTIGENS IN 9 HEALTHY HETEROSEXUAL CONTROLS AND 10 HOMOSEXUALS WITHOUT LYMPHADENOPATHY SYNDROME

Group	Intermediate strength				
	Candida 1:100 mean ± SD	PPD mean ± SD	Mumps mean ± SD	Trichophyton mean ± SD	Total mean ± SD
Controls	8.4±6.26	3.7±5.7	11.7±6.22	0.6±1.36	24.3±11.88
Homosexuals	3.6±5.61	0	1.2±2.03	1.3±3.95	6.1±8.40

SD = standard deviation

Table IV

POSITIVE ORAL *CANDIDA* FINDINGS IN 58 INDIVIDUALS

WHO WERE POSITIVE FOR ORAL CANDIDAL CULTURE ON AT LEAST 1 TEST

POSITIVE FINDINGS OF *CANDIDA* IN THE ORAL CAVITY

	number of subjects	Salivary technique: Gram-staining cases	(%)	Undiluted cases	(%)	Diluted cases	(%)	Swab cases	(%)	Mouth-wash cases	(%)	Imprint cases	(%)
Healthy controls	10	0	(0)	10	(100)	6	(60)	2	(20)	7	(70)	6	(60)
Patients	48	6	(12.5)	41	(85.4)	39	(81.3)	34	(70.8)	41	(85.4)	42	(87.5)
TOTAL	58	6	(10.3)	51	(87.9)	45	(77.6)	36	(62.1)	48	(82.8)	48	(82.8)

Table V

MEANS IN COLONY-FORMING UNITS OF C. ALBICANS ISLOATED FROM THE ORAL CAVITY OF 10 HEALTHY CONTROLS AND 45 PATIENTS* USING DIFFERENT SAMPLING TECHNIQUES

	Salivary technique cfu/ml		Swab cfu/ml	Mouth-wash cfu/ml	Imprint cfu/cm^3			
	Undiluted mean (range)	Diluted mean (range)	mean (range)	mean (range)	B mean (range)	P mean (range)	T mean (range)	L mean (range)
Healthy controls	147 (10-820)	24 (0-155)	7 (0-45)	13.5 (0-50)	0.4 (0-1)	0.1 (0-1)	1.3 (0-6)	0.7 (0-3)
Patients	2167.1 (0-6940)	698.2 (0-HG1)	341.3 (0-6160)	865.6 (0-8235)	13.6 (0-120)	20.7 (0-171)	25.8 (0-127)	27.1 (0-160)

* excluded 3 cases of other Candida species

HG1 = heavy growth = 6000 cfu/ml

cfu = colony-forming units

B = right buccal mucosa

P = palate

T = dorsal tongue

L = lesion in patients, left buccal mucosa in controls

Table VI

MEANS IN COLONY-FORMING UNITS OF C. ALBICANS ISOLATED FROM 4 VITAL STATISTIC GROUPS ACCORDING TO SAMPLING TECHNIQUES

	Colony-forming units/ml of C. albicans							
	Undiluted saliva		Diluted saliva		Swab		Mouth-wash	
Group	mean	(range)	mean	(range)	mean	(range)	mean	(range)
Controls	147.0	(10-820)	24.0	(0-155)	7.0	(0-45)	13.5	(0-50)
Irradiated	3717.5	(790-HG1)	2038.0	(60-HG1)	1654.3	(0-6160)	2259.3	(150-HG1)
Homosexuals**	1732.0	(130-HG1)	396.2	0-2292)	175.0	(0-1075)	1182.0	(0-8235)
Red & white oral lesions	1816.2	(0-HG1)	442.5	(0-2720)	86.8	(0-535)	196.8	(0-1340)
ANALYSIS OF VARIANCE AMONG 4 GROUPS								
F-ratio	4.053		4.228				3.492	
significance	0.015		0.012		NS*		0.025	
COMPARISON BETWEEN EACH GROUP OF THE PATIENTS AND THE CONTROL GROUP USING MEDIAN TEST								
Controls vs Irradiated								
Median			25.000		0.000		35.000	
significance	NS		0.007		0.015		0.0004	
Controls vs Homosexuals **								
Median	245.000		52.500				42.500	
significance	0.023		0.001		NS		0.001	
Controls vs Red & White Oral Lesions								
Median	120.000						10.000	
significance	0.036		NS		NS		0.047	

NS* = no significance using Kruskal-Wallis test

**Homosexuals without lymphadenopathy syndrome

HG1 = heavy growth as 6000 cfu/ml

cfu = colony-forming units

Table VII

THE CANDIDA COUNT /CM^3 ISLOATED AT INTRA-ORAL SITES FROM GROUPS OF CONTROLS OF CONTROLS AND SELCTED PATIENTS USING THE IMPRINT TECHNIQUE

	Colony-forming units/cm^3							
	Right Buccal Mucosa		Palate		Dorsal tongue		Lesions/Left Buccal mucosa	
	mean	(range)	mean	(range)	mean	(range)	mean	(range)
Healthy controls	0.4	(0-1)	0.1	(0-1)	1.3	(0-6)	0.7	(0-3)
Irradiated	34.7	(0-HG2)	39.1	(2-HG2)	64.1	(14-HG2)	39.9	(0-HG2)
Homosexuals*	15.4	(0-88)	30.3	(0-109)	33.1	(0-125)	23.9	(0-118)
Red & white lesions	7.1	(0-65)	7.8	(0-53)	10.3	(0-67)	23.9	(0-160)
DIFFERENCES AMONG 4 GROUPS USING KRUSKAL-WALLIS TEST								
Chi-square	8.6021		16.8943		17.5526		9.5624	
significance	0.0351		0.0007		0.0005		0.0227	
COMPARISON BETWEEN EACH GROUP OF THE PATIENTS AND THE CONTROL GROUP USING MEDIAN TEST								
Control vs Irradiated								
Median	1.0000		0.0000		4.0000		1.0000	
significance	0.0034		0.0004		0.0004		0.0152	
Control vs Homosexuals*								
Median	1.0000		0.0000		2.0000			
significance	0.0108		0.0055		0.0230		NS	
Controls vs Red & white oral lesions								
significance	NS		NS		NS		NS	

NS = no significance

* Homosexuals without lymphadenopathy

HG2 = heavy growth as 120 cfu/cm^3

Table VIII

MEAN COLONG-FORMING UNITS OF CANDIDA CONTRIBUTED TO CLINICAL MANIFESTATION IN THE PATIENTS

Oral manifestation	number of cases	colony-forming units/ml				colony-forming units/cm^3			
		undiluted saliva	diluted saliva	swab	mouth-wash	B	P	T	L
THRUSH / MUCOSITIS									
Homosexuals*	1	2810.0	355.0	1075.0	415.0	2.0	1.0	47.0	12.0
Red & white lesion gr.	1	5530.0	270.0	210.0	540.0	1.0	13.0	10.0	11.0
Irradiated	5	4263.3	3176.7	2305.0	3091.0	48.0	50.4	78.8	54.8
RED MUCOSAE									
Homosexuals*	3	1220.0	198.3	16.7	838.3	3.3	39.7	22.0	39.3
Red & white lesion gr.	4	237.5	5.0	0.0	0.0	0.0	0.0	0.3	0.3
Sjogren's	2	6470.0	3120.0	35.0	1370.0	5.5	25.5	12.0	30.0
RED & WHITE LESIONS									
Homosexuals*	6	1808.3	502.0	104.2	1481.7	23.7	30.5	36.3	18.2
Red & white lesion gr.	10	1914.4	591.5	100.5	221.5	9.9	9.3	13.4	32.2

*Homosexuals = Homosexuals without lymphadenopathy syndrome