Discussion
DISCUSSION

6.1 INCIDENCE

Over past three decades the incidence of candidiasis has risen dramatically. There are two major forms of candidal infections, chronic mucocutaneous candidiasis and disseminated candidiasis (Edwards et al. 1978; Komshian et al. 1989). Chronic mucocutaneous candidiasis is not a common disease and its prevalence probably has not changed appreciably (Marsh et al. 1983). In contrast the prevalence of disseminated candidiasis has changed from rare (before 1960) to that of an important hospital acquired infection (Bodey 1984). Candida is now the fifth most common isolate from all blood cultures (Dyess et al. 1985; Komshian et al. 1989). Numerous excellent reviews have pointed out that this increasing prevalence coincides with the use of antibiotics (Smits et al. 1966), immunosuppressives (Weinstein et al. 1983), hyperalimentation (Moro et al. 1990), use of polythene catheters (Muenier-Carpentier et al. 1989), pressure monitoring devices (Plouffe et al. 1977), heroin abuse (Podzamezer et al. 1986), organ transplantation, abdominal surgery (Dennis et al. 1968; Marsh et al. 1983), prosthetic cardiac valves (Rose 1978) and AIDS (DeWit et al. 1989).

Presently Candida is recognised as one of the most frequently encountered fungal opportunistic and is now regarded as the commonest cause of serious fungal disease.
Age and sex distribution as well as clinical manifestations of candidiasis are markedly affected by varying predisposing factors. Because of these factors the normal equilibrium between *Candida* and host may be sufficiently upset to lead to a pathologic state.

**Occurrence of Candida in chronic lung disease patients**

Bronchial candidiasis is a chronic bronchitis with cough, production of sputum and medium coarse rales with linear fibrosis seen on radiologic examination. The etiologic significance of *Candida* in this disease is difficult to determine (Rippon. 1982). Many surveys have shown that the organism occurs, sometimes in considerable numbers in essentially chronic lung disease conditions (Maile 1943; Odds 1987). These may be present in considerable numbers in the direct preparations or in culture of sputum. According to Myerowitz *et al.* (1977), *Candida* may not be related at all to the disease process, or it may be a minor colonizer or an allergen. Recently more attention has been paid to the role of *Candida* in inciting allergic type diseases. Pulmonary candidiasis as a primary disease is extremely rare. The diagnosis of this disease must rest on unassailable evidence that rules out other etiologies of the pathologic process (Cohen 1953). *Candida* readily colonizes pre-existing pathologic conditions attributable to other infectious agents, neoplasms or chronic functional disorders. New born infants and children with cystic fibrosis have been reported to be more subject to primary pulmonary candidiasis than others (Dixon *et al.* 1978, Jenner *et al.* 1979). In our study the incidence of *Candida* in the adult chronic lung disease patients was higher
(75.0%) in the cases of cystic fibrosis, whereas it was lower (14%) in the cases of bronchiectasis.

Occurrence of *Candida* in our chronic lung disease patients shows a strong positive correlation with the duration of underlying illness (Rippon 1982). The list of disease syndromes associated with candidiasis is long and varied. According to Komshian *et al.* (1989) the most important underlying diseases are CNS disorders (30%), cardiovascular diseases (23%) followed by diabetes mellitus (17%). In our patients with chronic lung diseases 36.6% of cases were positive for *Candida*.

*C. albicans* is perhaps the most protean infectious agent that afflicts man. In addition to active infection *C. albicans* is also involved in several allergic conditions. In our study *C. albicans* accounted for 65.3% of isolates and *C. tropicalis*, in 18.0% of the cases.

Rippon (1982) indicated that extremes of age makes a person susceptible to *Candida* infection. In our population of patients with chronic lung disease, the highest incidence was noticed in the age group 31-40 years (42%). We observed that the occurrence of *Candida* did not correlate with advancing age.

Analysis of sex distribution showed that females were more susceptible to infection. Many of workers feel that there is no male/female predilection in candidiasis (Komshian *et al.* 1989).
Candida infection in vaginitis cases

The incidence of *C. albicans* in the normal vagina of healthy non-pregnant women is about 5% (Linares de and Marin 1978). Other studies showed no increase in the presence of *Candida* associated with oral contraceptives (Goldacre *et al.* 1979). They reported that there was a distinct increase in clinical vaginitis in gravid females. Most studies indicate a rate of candidiasis of about 18% for non-pregnant women with vaginal discharge but an average rate of 30% for gravid women and women on contraceptives (Goldacre *et al.* 1979). In our study, we noticed that 23.9% of cases had *Candida* infection. These types of studies have been carried out in many geographic locations under varied climatic conditions and such factors do not appear to contribute to the incidence of, or severity of disease (Odds *et al.* 1983). The analysis of factors influencing the *Candida* infection in leucorrhoea cases shows that incidence is higher in multiparous women.

We have observed that there was high incidence (50%) of cases in females of below 20 years of age. Similar opinion has been expressed by Goldacre *et al.* (1979). In our study *C. albicans* accounts for most of the vaginitis cases with some cases being caused by *C.krusei, C.tropicalis, C.stellatoidea* and other species and this is in accordance with that of most reports (Soll *et al.* 1987).
Candida infection in burn wounds

Candida spp. is a common opportunistic pathogen in burned patients (Menier et al. 1981). According to Edward et al. (1972), the early diagnosis was difficult and therapy of established infection was often unsuccessful. Suppression of Candida infection has been attempted with varying results (Desai et al. 1985). The number of sites colonized and heavier colonization have been positively correlated with the risk of invasive infection. In our study we have investigated 40 patients with varying degree of burn injury to find out possible mechanisms, which contribute to increased incidence of Candida in burn patients.

The review of this subject by Seelig (1966) indicated that the use of broad spectrum antibiotics and a longer duration of hospital stay were definite predisposing factors in the development of candidiasis. Further, as reported by William et al. (1971), this is also true of patients treated by hyperalimentation or by any long term intravenous therapy especially with concomitant antibiotic therapy. In our study the average hospital stay was high in the patient group having 21-60% TBSA burns. The same group had more Candida infection (62.5%). Patients with large burns who survive the immediate post burn period usually develop complications like septicemia, burn wound sepsis, pneumonia and urinary tract infection (Law et al. 1972). Among Candida infections in burn wounds C. parapsilosis was the most predominant pathogen followed by C. albicans and C. tropicalis. A similar finding has also been reported by Law et al. (1972).
ABO blood group and *Candida* carriage in normal healthy population

Adherence of *Candida* to mucosal epithelial cells is thought to be the primary event necessary in order to establish colonization. *Candida* species adhere more readily to buccal and vaginal epithelial cells from some donors than others suggesting phenotypic differences in the susceptibility to colonization. Correlation between ABO blood groups and infectious diseases including mycotic infection are well documented (Deresinki *et al.* 1979) and blood group determinants have been shown to function as microbial receptors (Burford-Mason *et al.* 1988). The ABO blood groups are not confined to erythrocytes but are present on many cells including mucosal epithelial cells. In this study we examined the relation between ABO blood groups and oral carriage of *Candida* in normal healthy subjects.

*Candida* spp. are not uniformly distributed in the mouth and oral swabs are unsuitable for studying colonization. Mouth wash techniques are used to give a quantitative estimation of *Candida* carriage and have been shown to be easy to perform and are as sensitive as more complicated techniques (Burford-Mason *et al.* 1988). In our study non-carrier status was assigned to subjects if they failed to grow *Candida* colonies from 0.1 ml of 10 ml mouth wash. The mouth wash samples remained negative when culture was repeated after centrifugation. Oral carriage of *Candida* species is stable in uninfected mouths (Oliver *et al.* 1984). Hence we did not use mouth wash samples taken on three successive days to detect the carriage rate.
The H antigen on RBC and mucosal epithelial cells is known to function as a receptor for *Candida* strains. Subjects of blood group O who possess large quantities of H antigen on their cell surfaces would therefore be most susceptible to colonization (Burford-Mason *et al.* 1988). We found that 55.9% of O group subjects had oral *Candida* carriage, in terms CFU/ml, the groups had significantly higher count than the other blood groups. Based on our observation we concluded that O blood group subjects have a greater risk in the development of *Candida* infection.

**Occurrence of *Candida* in antibiotic associated diarrhoea patients**

Enteric candidiasis is one of the most controversial clinical disease attributed to *Candida*. The diagnosis is very difficult to establish and the implication of *Candida* as the primary agent of disease is at best tenuous. *Candida* enteritis follows administration of antibiotics and occurs also when the yeast population is supposedly suppressed by concomitant use of antifungal agents (Danna *et al.* 1991). The mycelial elements are also seen in fecal smears and indicate invasion of intestinal wall by *Candida* (Wingard *et al.* 1979).

A quantitative increase in the population of *C.albicans* following antibiotic therapy or change in diet may also cause chronic diarrhoea (Holti 1966). In our study we analysed degree of colonization of *Candida* in antibiotic associated diarrhoea cases by the method of Danna *et al.* (1991). 70% of the isolates had the score of $> 3+$ in a simple gram stained smear.
From the above observation we concluded that AAD patients had heavier colonization in their GI tract. Moreover, the presence of large number of yeast cells in direct smear in patients with diarrhoea furnishes clinical clue to candidal etiology of the enteritis.

Some researchers suggest that stool smear and culture are obviously inconclusive and additional diagnostic criteria are required to establish the diagnosis of *Candida* enteritis (Talwar *et al.* 1990).

β-lactamases are enzymes which inactivate β-lactam antibiotics. These enzymes are nearly ubiquitous among bacterial species. Although β-lactamases are widely distributed throughout the microbial kingdom there are few reports of β-lactamase production in yeasts (Mehta and Nash 1978). Little is known about the β-lactamase production of *Candida* species and quantitative determination of β-lactamase production in AAD has not been reported.

*Candida* species are known to colonize the gut in increased numbers in patients who are on antibiotic therapy. In the present study we isolated *Candida* in 78.3% of children with AAD. 65.7% of *Candida* isolates were found to produce β-lactamase, only when incubated with penicillin for 24 hrs, which clearly indicated that β-lactamase production is enhanced in the presence of an inducer. The genetic information for β-lactamase production can be transferred on plasmids or transposons and is a possible mechanism for spread of resistance to sensitive microorganisms (Kitzis *et al.* 1988). Another important clinical problem is that the production of β-lactamases by these strains would inactivate penicillin group of drugs and cause failure in the
therapy directed towards pathogenic organisms (O'Brien et al. 1987). Hence β-lactamase producing Candida species would contribute greatly to the disease process by interfering with antibiotic therapy directed against other organisms.

6.2 DIFFERENTIATION OF CANDIDA SPECIES

Conventional and commercial systems for the identification and differentiation of Candida species are based on different properties that include colony morphology, microscopical appearance, fermentation and assimilation reactions (Emmons et al. 1977). The fermentation of sugars with the production of acid or gas is so dependent upon elimination of endogenous carbohydrate, amount of inoculum, temperature of incubation and interval before reading, that carbon utilization tests as standardized by Lodder and Kreger Van Rij (1967) are probably more definitive and essential adjuncts to the other methods of species differentiation. These methods are labour intensive, time consuming and expensive, alternate rapid and reliable methods are needed.

Disks containing chemicals and antibiotics have long been used for the routine detection of biochemical reactions and antibiotic sensitivity of bacteria. For example identification of gram negative anaerobic bacilli by antibiotic sensitivities alone has been described (Finegold et al. 1967; Essers 1982). Yeasts are known to be sensitive to dyes, an observation affording a possible basis for differentiating species. Accordingly, the potential of a disk diffusion test for the identification of yeasts had already been investigated by Sobczak (1985). The results presented here reveal that the disk diffusion test identified
all but few (0.7%) of 448 clinical isolates of *Candida*. There was good agreement between the results obtained by disk diffusion method and conventional methods (98.8%).

The method described here is technically straightforward and gives reliable results within 18 hrs for most species. Because the inhibition profiles to the chosen chemicals and dyes (group A) may be tested with seven impregnated disks on only one plate, the method is inexpensive. Furthermore, the disks and stock solutions remain stable for up to one year and results are not subjected to minor technical variations in the application of the system. Sobczak (1985) suggested that the inoculum used to seed the Sabouraud’s agar is not critical, reproducible results were obtained even over a wide inoculum range (5 x 10³-10⁶ cells). Similarly, the method of seeding the agar did not influence the results. We also observed that the zones of inhibition obtained for each species showed no more than slight deviation, indicating that the interaction between *Candida* and dyes was essentially constant, a characteristic of the system that simplified evaluation. Discrepancies in differentiation of *Candida* species were however observed with overlapping of codes.

In the minority of cases in which one code (1200500) identified two species, *C.albicans* and *C.krusei*, these species were readily identified by microscopy, colonial morphology and growth characteristics in Sabouraud’s glucose broth.
In group B chemicals the available codes for each species could not be used for species differentiation, since profile overlapping was very common in all *Candida* species studied.

The disk diffusion method described offers, therefore, a reliable means for the identification of *Candida* species from clinical specimens. 98.8% of isolates were identified at the first attempt by the combination of disk diffusion tests and additional criteria. The potential of this simple test is high especially because it can be applied in small laboratories without need for extensive and expensive facilities. It is also clear however that further detailed examination, including test with larger number of named reference strains, is required for some species, such as *C.krusei*.

6.3 SDS-PAGE PROTEIN PROFILES

The identification of fungal form genera and species is based on a body of morphologic and physiologic characters. Species identification is based on a number of phenotypic characteristics. Variability in these characters is expected and accepted by most mycological taxonomists (Barnett et al. 1983). For example, although *C.albicans* is defined, in part, by its ability to form germ tubes under specific environmental conditions, a small percentage of this yeast like fungus may fail to form these distinctive structures and other *Candida* species also may produce germ tubes. In addition all *Candida* spp. vary in their ability to assimilate one or more carbohydrate sources. Consequently, such variability in the characters used to differentiate these species does not in itself negate the entire body of information accumulated for
individual taxa, nor does it invalidate the taxonomic status of a given species (Lehmann et al. 1989). They also suggested that, where there is relatively low number of such phenotypic characters, species may be separated in some instances, on the basis of single character.

Several workers have demonstrated the usefulness of electrophoretic separation of proteins in defining variations within or between fungal species. Many groups of fungi have been studied taxonomically by electrophoresis including dermatophytes (Schecter et al. 1966), Candida (Schecter, 1972), Aspergillus (Sorenson et al. 1971) and Fusarium (Glynn 1969). Comparative electrophoresis, isoelectric focussing and numerical taxonomy of some isolates of Fonsasae pedrosoi and allied fungi were carried out by Oumaima Ibrahim Granel in 1985.

We have studied the cellular protein profiles of seven Candida species namely C.albicans, C.krusei, C.tropicalis, C.stellatoidea, C.guillermondii, C.parapsilosis and C.pseudotropicalis. In this study, we observed seven species specific proteins which were common to all isolates of Candida species studied. Differences, if any, were very minor and included the species specific proteins. Similar investigations have been used to a limited extent to distinguish Candida species (Axelson et al. 1976; Ahearn et al. 1977; Schecter 1972; and Schecter et al. 1973). Cellular protein profiles were found to be similar in C.albicans and C.stellatoidea, however differences were noted in the species specific proteins unique to these species. In addition, minor differences were found in a few proteins between these two species. However, although the differences may be viewed as being significant taxonomically and could suggest
that the *C. stellatoidea* isolates belong to a distinct species or variety rather than being variants of *Candida albicans* in agreement of the finding of Kobayashi *et al.* (1984).

Indeed, DNA homology would seem to argue for a close similarity between *C. stellatoidea* and *C. albicans*. Meyer (1984) reported that an isolate derived from the same material used to prepare the nomenclatural type of *C. stellatoidea* showed a very close DNA homology with *C. albicans*. Furthermore, studies carried out by Lehmann *et al.* 1989 show that the protein profiles of *C. stellatoidea* isolates are observably different from those of other *C. albicans* isolates.

The cellular protein profiles of *C. krusei, C. tropicalis, C. guillermondii, C. parapsilosis* and *C. pseudotropicalis* were found to be virtually indistinguishable, except for the species specific protein. Such similarities are to be expected with PAGE when closely related organisms are compared (Glynn, 1969), Sorrenson *et al.* 1971; Tucker *et al.* 1990). However, there remain several characters by which these organisms may be distinguished from each other.

Protein patterns analysed by SDS-PAGE successfully applied to bacterial classification (Millership *et al.* 1989) and epidemiology have been used in retrospective analysis of 7 systemic *C. albicans* infections in a neonatal intensive care unit. 2 strains were found to be involved, the result was confirmed by restriction endonuclease digestion of whole cell DNA by EcoRI (Naudry 1988). Cross infection was suspected as the strains were isolated from
patients linked spatially and temporally in the unit. Others have found PAGE patterns to be too variable for use in either taxonomic or epidemiological studies (Cunningham 1989).

We have investigated the use of SDS-PAGE protein patterns in typing of *Candida* strains isolated from the burns unit. In our study we found that, there was similarity in all protein patterns obtained from both clinical and environmental isolates. Moreover, no consistent differences were seen, which would enable the strains from various sources to be distinguished from each other. We also concluded that SDS-PAGE protein profiles could not be used for typing of *Candida* strains.

### 6.4 CANDIDA DNA TYPING

Increasing frequency of systemic candidiasis outbreaks in burn patients deserves more attention. Prevention and greater control of further epidemics depends on the increasing awareness of health care personnel. When a case of systemic candidiasis occurs and endogenous source have been excluded, exogenous source should be clinically suspected and epidemiologically investigated with particular respect to the hospital environment (Moro *et al.* 1990). Adherence to standard protocol for drug treatment and clean environment should get priority in each hospital, given the high case fatality rate associated with fungemia.

Moro *et al.* (1990) also described an outbreak of *C.albicans* systemic infection involving eight patients in an Italian geriatric hospital. They
investigated the outbreak by DNA restriction pattern method, a single strain was responsible for all, and a nurse was found to harbor the same strain. DNA finger printing provided a reliable system for typing the strain of *C. albicans*. This was the first outbreak of *C. albicans* systemic infection associated with parenteral nutrition.

In our study, we have noticed one strain of *C. tropicalis* was isolated from the patient attendant in 1st week of January and the same strain was isolated from patient A subsequently. A strain of *C. albicans* was isolated from the dressing room tap during II week of January and similarly from the patient B a few days later. Patient C and D admitted in February were also infected with *C. albicans* and all the three isolates of *C. albicans* were found to be similar to the isolates from the dressing room tap. Patients E,F,G and H who were admitted to the hospital during the months of March and April were found to be infected with a strain of *C. parapsilosis* in the month of May. A strain of *C. parapsilosis* was also isolated from the dressing room tap in the month of May. The strains analysed by DNA restriction pattern were found to be similar with the isolate from the dressing room tap.

According to the DNA pattern groups obtained, a single strain of *C. albicans* was responsible for two cases and a single strain of *C. parapsilosis* was responsible for 4 cases. Moreover, the same strain was isolated from the dressing room tap. The DNA finger printing techniques have been used to type the strains from *Candida* outbreak (Scherer and Stevens et al. 1987). Our results also show that this technique is useful to type strains in an outbreak. A report has been published on the application of DNA finger printing in the
study of an outbreak of candidiasis (Vaundry et al. 1988). They investigated a cluster of systemic *C. albicans* infections in a neonatal intensive care unit, typing the five available clinical strains with the electrophoresis patterns and DNA profiles.

Burnie et al. (1988) assessed the validity of DNA finger printing with 45 previously characterized *C. albicans* isolates from five different outbreaks and with 96 unrelated isolates from a mixed control population. DNA analysis discriminated better than any of the other typing methods used including serotyping (Hasenclever et al. 1981) and biotyping (Odds and Abbott, 1980). Reproducibility was high and all isolates was typable within 48 hours (with only one restriction enzyme was used EcoRI). Based on our experience and the aforementioned studies, DNA analysis provides a reliable means of delineating strains of *Candida* isolates. Moreover, the method is reproducible and relatively easy to perform. According to Moro et al. (1990) combining genotype and phenotype methods could enable a more precise discrimination. The protein profiles obtained with SDS-PAGE could not be used for strain discrimination. Hence we have utilized only DNA typing methods.

Although the ultimate source of infection cannot be entirely proved, the finding that (according to DNA analysis) the patient attendant and dressing room tap harbored the same strain as the infected patients, supports that contamination occurred during dressing the patients. Our findings clearly emphasize the need for more accurate identification of microbial species and the use of epidemiological markers for the prevention, control and monitoring of nosocomial infections.
6.5 SUSCEPTIBILITY TO ANTIFUNGAL AGENTS

*Candida* infection is an increasingly common and serious problem among immunocompromised patients. The current therapy of candidiasis depends mainly on antifungal agents belonging to three different clinical groups, the imidazoles, thiocarbamates and antibiotics such as amphotericin B. *Aazole* antifungal agents have generated substantial interest as new therapeutic agents for many of the mycoses (Borgers 1980; Larsen 1990). The compounds offer the advantages of relatively broad spectrum activities without excessively severe side effects or toxicity to the host (Georgopoulos *et al.* 1981). Dupont and Drouhet (1988) reported that the number of antifungal agents for candidiasis are small and most are toxic. Fluconazole is the new triazole antifungal with a novel pharmacokinetic profile such as water solubility, high bioavailability, low protein binding (10%), a long half life (25 hr), diffusion into CSF and rapid elimination by the kidney as an active drug. In our study we have examined the efficacy of fluconazole in *in vitro* and *in vivo* methods and compared with 5 flurocytosine, amphotericin B and miconazole.

6.5.1 *In vitro* studies

Selection of medium for *in vitro* antifungal susceptibility is very important, since the composition of medium appears to be an important factor in susceptibility testing with imidazole compounds (Shadomy 1982). The influence of medium on the antifungal effects on azole compounds was well studied by Shadomy *et al.* (1991). They described the effect for miconazole on *C.albicans* stating "the richer the medium, the greater the effect". Shadomy
et al. (1982) compared five imidazoles under variety of test conditions and found that MICs obtained with peptone glucose agar were approximately $\log_{10}$ higher than those obtained on yeast nitrogen base glucose agar. Testing of 5FC requires a medium free of antagonists. This excludes use of peptone and meat extract. Some organisms are reluctant to grow on a medium whose only source of nitrogen is inorganic (Collins et al. 1987). Growth is improved by using antifungal assay medium. Hence we have used antifungal assay medium which was prescribed by Collins et al. (1987).

We also found that the antifungal assay medium inhibited fluconazole activity since the pH of the medium was 6.8. Reports by Troke et al. (1985) indicated that high resolution medium (HR Medium pH 7.2) is useful medium to get realistic in vitro results. Therefore, we have used HR medium for determination of MIC of Candida to fluconazole.

Previous studies also indicated that MICs of antifungal agents were markedly influenced by the starting inoculum size (McIntyre et al. 1989). In our study we have used $10^3$ organisms in each spot inoculation, which has been recommended by Troke et al. (1985). McIntyre et al (1989) studies showed that inadequate inoculum leads to measurable delays in the onset of their inhibitory effects. This delay is also consistent with mode of drug action which requires metabolic processings to exert an antifungal effect as seen with azole compounds (Cope 1980; Saag et al. 1988). We have got wide range of MICs for miconazole.
The susceptibility of a limited number of yeast species to antifungal agents has been reported (Shadomy et al. 1991). We have expanded upon these observations by performing susceptibility tests on Candida species. In our study, a total of 204 clinical isolates of Candida were tested, of which majority of the isolates were C. albicans. C. albicans was more susceptible to fluconazole and 5FC, whereas it was resistant to amphotericin B and miconazole. We also found that fluconazole was most active with C. albicans. A standard strain of C. albicans (Y01-09), supplied by Pfizer Central Research had an MIC 0.77 µg/ml in our study, which was not significantly different from that (1.56 µg/ml) indicated by the reference laboratory. There by findings shows our test system was working properly. We also observed that high MICs of amphotericin B (3µg/ml to 24 µg/ml) and miconazole (12.5 µg/ml - 100 µg/ml). This was higher than the recommended dose for clinical treatment (Oblack et al. 1981). The high MICs of the above drugs was probably due to factors associated with the medium. It is difficult to predict the susceptibility of an isolate to an antifungal drug based on its in vitro results (Speller et al. 1986).

6.5.2 In vivo studies

Efforts to produce persistent systemic infection with C. albicans in animals have been based on the use of compromised animals (Turner et al. 1976; Bannatyne et al. 1992). Oral intragastric route of infant mice leads to systemic spread, lethality or long term colonization of the gastrointestinal tract (Field et al. 1981). These characteristics make mice a useful model for the study of gastrointestinal and systemic candidiasis (Guentzel et al. 1982; Berestein et al. 1984). Infant mice have been used successfully in several
investigations as a model for studying the efficacy of antifungal agents. In this study we have given fluconazole treatment to mice infected with *C. albicans* and results were compared with amphotericin B and 5-fluorocytosine treated mice.

The model of candidiasis in mice used in our experiments is one of a subacute systemic infection that is usually well tolerated by animals for several weeks but is not cleared spontaneously (up to 30th day). This model, with treatment delayed for several days, mimics human infection. In these animals which had well established visceral infections fluconazole, 5-fluorocytosine and amphotericin B treatments produced microbiological cures in 3 out of 6 mice, which was not significant with this sample size. These data suggest that in this model fluconazole may be as effective as amphotericin B and other drugs.

The results suggest that in infant mice, *C. albicans* after intragastric challenge establishes long lasting intestinal colonization. The organism is also shown to spread from the digestive tract in a large percentage of animals and may cause systemic infections. *C. albicans* was essentially cleared from extra-intestinal intra-abdominal organs (liver, kidney and spleen) in survivors of oral intragastric challenge examined at 20 days age or later. Immunosuppressive treatment of the animals before or after inoculation is not required to establish the persistent infection. Short term therapy with the three antifungal drugs (fluconazole, 5-fluorocytosine and amphotericin B) significantly reduced colony counts in the spleen. Treatment with amphotericin B gave a reduction in CFU in all three organs (liver, spleen and kidney).
The AMB has renal toxicity and other harmful side effects. The 5 FC is known to cause resistance in the management of disease (Walsh et al. 1990). Based on these results we found that there was no difference between fluconazole and other drugs used for treatment (AMB and 5 FC). The fluconazole treated mice had significantly lower CFU in the stomach homogenates than mice treated with 5 FC and AMB.

The organism was totally cleared from spleen in all drug treated animals. However treatment of persistently infected animals initiated in infancy, leads to little tissue invasion and dissemination of the organism from the gastrointestinal tract. Although amphotericin B is a proven drug for systemic candidiasis, it has found limited usage due to its renal toxicity and side effects. Our observation suggests that fluconazole is a useful drug in the treatment of gastrointestinal candidiasis.

**Comparison in vivo and in vitro tests**

We have observed that by in vitro tests fluconazole and 5 FC are equally good, where as in in vivo system the amphotericin B and fluconazole are better drugs. Kobayashi et al. (1986) noticed that the high MIC of the antifungal agents when tested in synthetic amino acid medium fungal did not correlate with the efficacy of the drug in their model. It has been shown that when complex media used for in vitro testing of fluconazole, the MIC results may be falsely high and may not correlate with clinical efficacy (Perfect et al. 1986 and Saag et al. 1988). Complex media appears to contain substances which may inhibit antifungal agents in vitro (Fisher et al. 1989). Furthermore,
in vitro susceptibility testing of fluconazole is trouble some and difficult to reproduce in different laboratories even when same growth medium is used (Troke et al. 1985). In our study testing fluconazole with defined medium, the MIC was still high, which emphasizes the continued problem of discrepancies between in vitro and in vivo susceptibilities.

Because of discrepancy between the efficacy of the triazoles and their limited activity in vitro found in our studies, the possibility of antagonizing effect of the culture medium must be considered. However, this seems unlikely since the same medium proved to be particularly useful for comparison of the relative activities of various azoles in vitro. Moreover when we performed MIC determinations in the same medium we found values were not comparable to those found by others in same media (Troke et al. 1985). Lastly, if there were antagonizing substances in the medium, one should expect that the effects of these substances could be overcome by raising the concentration of the drug (Simonetti et al. 1989).

Finally we suggest that the in vivo animal models of fungal infections appear to be the best method of assessing the relative antifungal effectiveness of the drug before use in humans. We also observed that fluconazole has good activity against C. albicans in this animal model but is slightly inferior to that of amphotericin B. Major advantages of the use of fluconazole in humans include oral administration and low toxicity profile found in the studies that have been done to date (Simonetti et al. 1991). It is also said that a longer duration of therapy with other antimicrobial agents may be required to attack mycological cure of established renal and hepatic candidiasis.
6.5.3 Effect of fluconazole on germ tube formation

Many morphological forms of *C. albicans* have been identified, besides the yeast form. These include chlamydospores which are formed under nutrient deficient conditions and pseudohyphae formed in serum containing medium (Johnson and Nickerson 1970). Another morphological from which consists of short stunted mycelia which are bulbous at the tip is produced, when cell are grown under conditions that induce mycelial formation in the presence of azole antifungals, at levels below the minimum inhibitory concentration (Borgers 1980). In our study, we have investigated the effect of fluconazole on germ tube formation and cell structure. When grown in the presence of fluconazole, *C. albicans* cells displayed shortened germ tube, which were rounded at the tip, whereas in the absence of the drug the organism showed normal mycelial development. The number of germ tube forming cells were significantly decreased in drug treated cells. The use of electron microscope allowed a temporal study of growth in fluconazole containing serum and it was found that once elongation of hyphae had ceased, swelling of hyphal tips and budding occurred on the mother cell. The presence of fluconazole in the serum appears to inhibit germ tube development and cells continued to produce stunted mycelium. These observations demonstrates its potent antifungal activity and correlate with other reports (Cannon *et al.* 1988).