This is to certify that the

dissertation entitled

The *in vitro* Effects of Soluble Yeast Mannan on
Mouse Peritoneal Macrophage Function.

presented by

Michael Peter Kolotila

has been accepted towards fulfillment
of the requirements for

Ph.D degree in Department of Botany and
Plant Pathology

Alvin Lee Rogers
Major professor
Alvin L. Rogers

Date 18 July 1985

THE *IN VITRO* EFFECTS OF SOLUBLE MANNAN ON MOUSE PERITONEAL MACROPHAGE FUNCTION.

By

Michael Peter Kolotila

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1985

AN ABSTRACT OF A DISSERTATION

THE *IN VITRO* EFFECTS OF SOLUBLE YEAST MANNAN ON MOUSE PERITONEAL MACROPHAGE FUNCTION.

by

Michael Peter Kolotila

A new method for assessing the candidacidal activity of macrophages using the supravital stain neutral red is described. This method is comparable to well established vital staining techniques, methylene blue and acridine orange, and to the lack of germ tube formation of *Candida albicans* as a method of assessing the candidacidal activity of phagocytic cells.

This study characterizes the inhibition by *Saccharomyces cerevisiae* mannan on the phagocytic activity and phagocytic capacity of Concanavalin A elicited peritoneal macrophages towards unopsonized *C. albicans* and *C. albicans* opsonized with C3. The interference by mannan on phagocytosis of C3 opsonized *C. albicans* has not been previously reported. The phagocytic activity and phagocytic capacity of Con A elicited peritoneal macrophages towards *C. albicans* opsonized with either normal human serum or immunoglobulins are unaffected by the presence of mannan. The inhibitory effect of mannan on phagocytic cells' candidacidal activity and superoxide generation are confirmed for macrophages and expanded to demonstrate that the inhibition of these functions is independent of the type of opsonization employed. Lastly, this study

demonstrates that phagolysosome fusion of macrophages is unimpaired by the presence of mannan which supports the previous observations that mannan does not interfere with neutrophil degranulation.

DEDICATION

To my parents, Mike and Vera Kolotila, who encouraged me to learn and who instilled in me a love of books and learning.

ACKNOWLEDGEMENTS

I would like to first acknowledge my parents, Mike and Vera Kolotila, without whose unfailing support I might not have not finished this dissertation. Next I would like to express my appreciated to the memebers, both past and present, of the Alex B. Filnow Memorial Symposium. You have made the good times better and the bad times more bearable. I would also like to thank Amy Douglass, Jim Hollers, Barbara Heerdt, Julie Stickle and other friends that I made here at MSU for their friendship, support and providing me with coffee that helped keep me going.

I would like to thank Dr. A.L. Rogers for serving as my major professor and for his critical editing of this manuscript. Also, I would like to thank Drs. E.S. Beneke, K. Baker, C.W. Smith and R.J. Patterson for serving on my committee and for their editing of this manuscript. I would like to express a special thanks to Dr. C.W. Smith for his generous use of his knowledge and laboratory during the initial stages of this dissertation's research. Thank you all for your help.

TABLE OF CONTENTS

TABLE OF FIGURES

TABLE OF TABLES

INTRODUCTION

Host defense against *Candida albicans* involves both cell mediated and humoral immunity as well as nonspecific immune mechanisms (Rogers and Balish, 1980). Both neutrophils (Diamond, 1981) and macrophages (Lehrer and Fleischmann, 1982) are capable of destroying *C. albicans* as a part of their nonspecific immune function against microbial invasion of the host. Macrophages may be stimulated to greater microbicidal activity against a wide range of microorganisms (Remington, 1982) which includes *C. albicans*.

Factors that aid *C. albicans* in establishing an infection have been the subject of renewed interest because of the increasing incidence of this organism as an opportunistic pathogen. Mannan (one of the main components of the cell wall of *C. albicans* (Sullivan *et al.*, 1983)) is one factor that affects not only cell mediated and humoral immunity but also nonspecific phagocytic cells, i.e. neutrophils and macrophages. Mannan was isolated from serum of infected individuals by Fischer *et al.* (1978). They were the first to show that this mannan would inhibit lymphocyte proliferation. This observation was expanded by other investigators with regard to the interaction of mannan and lymphocytes. Wright *et al.* (1983) demonstrated that mannan inhibits in a dose dependent fashion the myeloperoxidase mediated killing of *C. albicans* by neutrophils as well as killing in a cell free system.

Macrophages play an important role in the defense against infection

(Horwitz, 1982; Lehrer and Fleischmann, 1982; Murray, 1982; Remington, 1982) but little is known about the effects of mannan on these cells. Since mannan antigenemia is a finding in invasive candidiasis (Weiner and Yount, 1976) and macrophages are long lived phagocytic cells which are found throughout the body, it is important to determine the effects of mannan on these cells. One of the objectives of this study was to determine the effects of mannan on Con A elicited mouse peritoneal macrophages with respect to phagocytosis of *C. albicans* using three receptors, mannosyl/fucosyl glycoprotein, Fc and C3, alone and in combinations. As part of this objective, the intracellular destruction of *C. albicans* and phagocytosis were measured by a new candidacidal assay, neutral red. Another objective was to determine the affects of mannan on superoxide production and phagolysosome fusion by these macrophages.

LITERATURE REVIEW

Candidiasis is one the oldest diseases known to mankind. Thrush, an oral *Candida* infection, was described in debilitated patients by Hippocrates in his Epidemics (Odds, 1979; Rippon, 1982). The report by Hippocrates as well as by other early investigators emphasizes a major point about candidiasis which is its association with debilitated patients (Odds, 1979; Rippon, 1982). Since Hippocrates' times, *Candida* diseases have been the subject of intense scientific interest. These investigations have reinforced the observations of the intimate association between humans and members of the genus *Candida*, especially *Candida albicans* (Bodey and Fainstein, 1985; Odds, 1979; Rippon, 1982). Candidiasis may be defined as any infection, either primary or secondary, caused by a member of the genus *Candida*. The term candidosis is used by Odds (1979) to refer to infections caused by members of the genera *Candida*, *Torulopsis* and *Rhodotorula*. The term candidiasis will be used in this work and will refer to the infections caused by members of the genus *Candida*.

The genus *Candida* may be briefly defined as yeast that reproduce by blastoconidia and may form true hyphae or pseudohyphae, but do not produce carotenoid pigments, usually do not reduce urea and do not have a capsule (Rippon, 1982). The concept of the genus *Candida* has been enlarged to encompass the genus *Torulopsis* (Yarrow and Meyer, 1978). Also, van Uden and Buckley (1970) felt that the separation of these two genera on the basis that *Candida* produces true hyphae or pseudohyphae and that *Torulopsis* does not, was artificial. The combination of these two genera has been rejected by other mycologists (McGinnis *et al*., 1984).

Within the genus *Candida*, *C. albicans* is the most pathogenic member though other species in this genus are also pathogenic (Hurley, 1980; Odds, 1979; Rippon, 1982). Hurley (1980) presents a table consisting of seventeen species of *Candida* that are commonly associated with humans and another table consisting of eight more species that are occasionally isolated from humans. She also presents a list of nine species of *Candida* that show virulence for humans. The seven most virulent species of *Candida* in descending order are *C. albicans*, *C. tropicalis*, *C. stellatoidea* (which may be a variant of *C. albicans*), *C.* (*Torulopsis*) *glabrata*, *C. krusei* and *C. parapsilosis* (Hurley, 1980). Of these seven species *C. albicans* and *C. tropicalis* account for at least 80% of the yeast isolated from humans (Hopfer, 1985). *Candida albicans* comprises 90% of the yeast isolated from vaginal specimens (Hurley, 1980). *Candida parapsilosis* and *C. glabrata* account for between 10 and 15% of the yeast isolated from humans (Hopfer, 1985).

Candida albicans is part of the normal flora of humans, usually being associated with the alimentary tract and mucocutaneous surfaces (Odds, 1979; Rippon, 1982). The recovery of *C. albicans* from the human body increases at least two fold in a patient population as compared to healthy individuals with the notable exception of the skin which remains relatively the same regardless of the health of the individual (Odds, 1979). Passage through an infected birth canal may lead to oral candidiasis of the neonate. Infection rates have been recorded to be as high as 18%, but the average appears to be about 4% (Rippon, 1982).

It is because of this intimate relationship that C. albicans has with humans that Candida antigens are used to assess cellular immunity, either by lymphocyte transformation (Oppenheim and Schecter, 1976) or by delayed type hypersensitivity skin tests (Spitler, 1976). Humoral immunity is also involved in this relationship. Antibodies to C. albicans are found in most individuals without a known prior history of Candida infection (Gough et al., 1984; Lehnmann and Reiss, 1980; Weiner and Yount, 1976). Unfortunately, they have not been useful in the diagnosis of Candida infection because the titers from patients with Candida infections are often within the same range as those individuals without a Candida infection (Gough et al., 1984; Lehnmann and Reiss, 1980). Even in vaginal secretions antibodies of the IgE and IgG classes to C. albicans may be found (Gough et al., 1984). It is believed that the persistence of C. albicans in the gastrointestinal tract generates the antibody response found in most individuals (Lehnmann and Reiss, 1980). This is interesting because other microorganisms that also reside in the gastrointestinal tract in higher numbers than C. albicans do not invoke similar immune responses (Berg and Savage, 1972; Foo and Lee, 1972).

Since C. albicans is usually part of the normal flora of humans it has the opportunity to exploit any crack in the homeostatic mechanisms of its host. Rippon (1982) divided the causes that may predispose an individual to candidiasis into five major catagories: i) extremes in

age, ii) physiological change, iii) prolonged administration of antibiotics to which immunosuppressive agents may be added, iv) general debilitation and v) iatrogenic, mechanical and traumatic barrier breaks. Smith (1985) and Mitchell (1980) have each compiled a list of specific alterations of the host response that predisposes the host to candidiasis. The spectrum of disease caused by members of the genus Candida is quite varied, ranging from superficial colonization, mucocutaneous involvement, systemic infection, which may be life threatening, and allergies. Candidiasis leaves no organ system untouched, being reported to attack almost every part of the body (Bodey and Fainstein, 1985; Edwards et al., 1978; Hurley, 1980; Odds, 1979; Rippon, 1982).

One of the most interesting aspects of any microbial infection is the mechanism(s) that the microorganism employs to establish an infection. Since C. albicans is usually part of the normal flora of its host, an infection by this yeast is termed opportunistic. The mechanisms that the host uses to keep this microorganism under control and the mechanisms that the fungus uses to circumvent that control are an interesting area of study.

Candida albicans appears to employ several mechanisms to establish an infection. The most obvious one and one of the most controversial is the ability of the fungus to alter its growth form from an yeast like organism to a filamentous organism. There is still debate as to which form is the most pathogenic. The majority of investigators have found that the yeast form is most pathogenic (Evans, 1980; Evans and Mardon, 1977; Mardon et al., 1975; Rogers and Balish, 1978; Simonetti and

Strippoli, 1973; Vaughn and Weinberg, 1978) though some other investigators have found the mycelial form to be more pathogenic (Gresham and Whittle, 1961; Iannini _et al._, 1975; Salterelli _et al._, 1975; Young, 1958). Louria _et al._ (1963) in their study on the pathogenesis of _C. albicans_ could not make a correlation that either the yeast or mycelial form of the organism was more invasive. In tissue both forms of the organism are found (Odds, 1979; Rippon, 1982). In studies concerning oral candidiasis the necessity of filamentation of _C. albicans_ to penetrate the epithelial cells and establish an infection has been demonstrated (Howlett and Squier, 1980; Marrie and Costerton, 1981; Martin _et al._, 1984; Montes and Wilborn, 1968). Whittle and Gresham (1960) using a rabbit model of renal candidiasis found that the production of pseudohyphae was necessary for a fatal infection with _C. albicans_. If _C. tropicalis_, _C. parapilosis_ or _C. guilliermondi_ were used, they did not form pseudohyphae in the rabbit kidney and thus did not die. However, if they injected _C. tropicalis_ into either rats or mice they did die and pseudohyphae were present. Also, in _in vitro_ studies Hurley and Stanley (1969) showed that filamentation of different _Candida_ species caused a cytopathic effect in cultured cells. It is also this ability to become filamentous that some investigators believe allows _C. albicans_ to escape destruction in "professional" phagocytes (Arai _et al._, 1977; Louria and Brayton, 1964; Louria _et al._, 1963; Ozato and Uesaka, 1974; Stanley and Hurley, 1969).

Once a "professional" phagocyte comes into contact with _C. albicans_ as either a yeast form or mycelial form organism, a complex set of events is set into motion with the result being the destruction of the fungus. The main mechanism of destruction of *C. albicans* by "professional" phagocytes is through oxidative metabolism. In patients with a myeloperoxidase deficiency oxidative metabolism is lacking and their neutrophils and monocytes do not kill *C. albicans* while neutrophils and monocytes from normal donors do kill *C. albicans* (Lehrer and Cline, 1969). Sasada and Johnson (1980) demonstrated that macrophages killed *C. parapsilosis* more effectively than they did *C. albicans*. They also found that *C. parapsilosis* elicited a greater oxidative metabolic response from macrophages than did *C. albicans* and that compounds which interfere with oxidative metabolism decreased the killing of both organisms. This indicated that the generation of toxic oxygen metabolites is a major mechanism of intracellular killing of *C. albicans* (Sasada and Johnson, 1980). These studies were done with yeast form organisms. Diamond and Krzesicki (1978) examined the mechanism by which neutrophils killed the pseudohyphae of *C. albicans* and found that compounds which interfere with oxidative metabolism decreased the killing of the organism. They found that oxygen metabolites, notably superoxide, singlet oxygen and hydrogen peroxide acting with myeloperoxidase and halide, were involved in the destruction of pseudohyphae, but not the hydroxyl radical. In a latter study, Diamond *et al*. (1980a) further defined the role of these oxidative metabolites in the destruction of hyphae and pseudohyphae of *C. albicans* by neutrophils. They found that neutrophils from patients with chronic granulamatous disease and myeloperoxidase deficiency, both diseases show impaired oxidative metabolism, and neutrophils treated with inhibitors of oxidative metabolism exhibited a lowered candidacidal activity as compared to the controls.

The important oxidative metabolites, with respect to destruction of hyphae and pseudohyphae of C. albicans, were hydrogen peroxide, singlet oxygen and the hydroxyl radical. Superoxide did not play a direct role in killing but was important in generating hydrogen peroxide (Diamond et al., 1980a). These findings are in conflict with the earlier study with regard to the hydroxyl radical. This difference was attributed to the different hydroxyl radical scavengers used in the two studies. In the second study a much more effective hydroxyl radical scavenger, DMSO, was used instead of the mannitol and sodium benozate used in the first study (Diamond et al., 1980a).

If the formation of the hyphal form via germ tubes is a mechanism by which C. albicans may escape from a phagocytic cell then it would be expected that the hyphal form of this organism would be more resistant to killing by phagocytic cells. A study by Culter and Poor (1981) using in vivo chambers found that the hyphal forms of C. albicans were more resistant to destruction by neutrophils and macrophages than were the yeast form cells. The chambers, depending on the membrane pore size, would allow free diffusion of soluble host substances but would exclude host phagocytic cells or would allow both soluble host substances and host phagocytic cells to enter the chamber which housed the C. albicans. In an in vitro study, Schuit (1979) found that human blood monocytes were more effective than human blood neutrophils at killing the hyphal form of C. albicans. Schuit (1979) also found that the yeast form was killed to a greater degree than the mycelial form of the organism regardless of the phagocytic cell type. The differences seen between the monocytes' and neutrophils' ability to kill the hyphal form of C.

albicans was not due to diffences in phagocytosis between the two phagocytic cell types because both forms of the fungus were phagocytized to an equal degree by both types of phagocytic cells.

However, in *in vitro* studies with phagocytic cells from several body sites, Baccarini *et al*. (1985) found that the hyphal form of *C. albicans* was killed equally as well if not better than the yeast form. Cockayne and Odds (1984), using an *in vitro* technique, found that the hyphal form of *C. albicans* was more effectively killed than the yeast form by neutrophils. Both studies used a radiometric assay to detect the killing of the fungus so quantitation of phagocytosis by the phagocytic cells with respect to the two growth forms of *C. albicans* was not measured. This is perhaps a moot point because Diamond *et al*. (1978) demonstrated that neutrophils could damage pseudohyphae that were too large for them to completely engulf. The neutrophils spread over the surface of the pseudohyphae and lysosomes were degranulated with subsequent damage to the pseudohyphae.

Candida albicans possesses a repertoire of enzymes that may aid it in establishing an infection. The most widely studied of these enzymes are the phospholipases (Banno *et al*., 1985; Price and Cawson, 1975; Sammaranayake *et al*., 1984) which are believed to aid in the penetration of the hyphae of *C. albicans* into epithelial cells. Penetration of hyphae into epithelial cells have been described by Howlett and Squier (1980), Marrie and Costerton (1981), and Montes and Wilborn (1968). Both Marrie and Costerton (1981) and Montes and Wilborn (1968) have observed epithelial cell damage with the invasion of hyphae from *C. albicans*. Howlett and Squier (1980) found little damage to the epithelial cells and what damage was observed was limited to the site of invasion. The localization of the damage to epithelial cells corresponds to the observations of Pugh and Cawson (1977) who histochemically located phospholipase in the developing hyphae that had penetrated the epithelial cells. Samaranayake *et al*. (1984) found that only *C. albicans* produced phospholipase activity while *C. tropicalis*, *C. glabrata* and *C. parapsilosis* did not. This would be in accord with *C. albicans* being more pathogenic than the other three *Candida* species. Banno *et al*. (1985) demonstrated that the mycelial form of *C. albicans* produced roughly twice as much phospholipase activity as did the yeast form. This lends credence to the concept that the mycelial form of *C. albicans* is necessary to establish an infection. *Candid albicans* has at least one proteinase for which there is strong evidence that it is a virulence factor (Macdonald and Odds, 1983; 1980), out of the several proteinases that this yeast possesses (Ruchel, 1984). Macdonald and Odds (1980) detected antibodies to a proteinase in serum and were able to detect this same proteinase in infected tissue with indirect immunofluorescence. In a latter study, Macdonald and Odds (1983) developed a proteinase deficient strain of *C. albicans*. When this proteinase deficient strain was grown on a medium that induced proteinase activity it was phagocytized and killed by neutrophils more readily than its parent strain . However, when both strains were grown on a medium that did not induce proteinase activity both strains were phagocytized and killed to the same extent. This is supporting evidence to the observation made by Mardon *et al*. (1975) that the medium used to grow *C. albicans* on influenced its virulence. When Macdonald and Odds (1983) injected both strains into mice the proteinase deficient strain was less pathogenic than its parent strain. Ruchel (1984) cited unpublished results of an experiment where mice were immunized with a proteinase. The mice were protected from the proteinase secreting strain of _C. albicans_. Ruchel (1984), in reviewing the substrates of these proteinases, found that they attacked a large number of important proteins of the body. Perhaps one of the most important substrates of this proteinase in relation to pathogenicity is that it attacks immunoglobulins, particularly all classes of IgA (Ruchel _et al_., 1982). Ruchel (1984) tested seven _Candida_ species for protolytic activity and found that only _C. albicans_ and _C. tropicalis_ showed strong protolytic activity while only _C. parapsilosis_ exhibited weak activity and all the other _Candida_ species showed no activity.

Other enzymes have been implicated in the pathogenesis of _C. albicans_ but little work has been done with these enzymes. Elinov (1984) has suggested that carbohydrases may play a role in pathogenesis by removing sugars from large molecules. Hattori _et al_. (1984) reported on a keratolytic proteinase activity in _C. albicans_ and postulated that it may be important in the pathogenesis of the organism _in vivo_.

Pathogenic microorganisms are known for their ability to evade or suppress in some manner the immune response of the host (Densen and Mandell, 1980; Gotschlich, 1983; Horwitz, 1982; Mackowiak, 1984; Schwab, 1975; Sptiznagel, 1983). These authors cited research illustrating that the cell wall or cell wall components are involved in the immunosuppressive activity of the microorganisms. Since the cell wall is the first part of the invading organism to come into contact with the immune system of the host, the cell wall or components of the cell wall may decide the outcome of that incounter. The cell wall of *C. albicans* or cell wall components may influence the immune response of the host which may be an experimental animal.

San-Blas (1982) reviewed the literature concerning the cell walls of pathogenic fungi in humans. He came to two conclusion: 1) that much more work is necessary to establish a correlation between fungal cell wall structure or composition and pathogenicity and 2) that more work is necessary to determine why two fungi with similar cell wall structure and composition differ greatly in their respecitive pathogenicities. An example of the second conclusion is *C. albicans* and *Saccharomyces cerevisiae*. The latter rarely causes infections in humans (Eng *et al*., 1984; Rippon, 1982). San-Blas (1982) was able to classify fungi pathogenic in humans based on the cell wall classificaiton scheme of Bartnicki-Garcia (1968). These fungi fell into two catagories according to cell wall composition, those with cell wall composed of chitin-glucan and mannan-glucan (San-Blas, 1982). Respectively, these two groups are groups 5 and 6 according to Bartnicki-Garcia (1968). *Candida albicans* is included in group 6, mannan-glucan, along with the other members of the Cryptococcaceae and the Saccharomycetaceae (San-Blas, 1982).

Bishop *et al*. (1960) determined that the main components of the cell wall of *C. albicans* are mannan, glucan and chitin. Elorza *et al*. (1983) in investigating the regeneration of protoplasts of *C. albicans*. determined the amounts of mannan, glucan and chitin in both growth forms of the organism. Regardless of growth the amount of glucan remained relatively constant being 71% for the yeast form and 74% for the

mycelial form while mannan dropped from 19% in the yeast form to 4% in the mycelial form. This decrease in mannan was counteracted by an increase in the chitin content from 9% in the yeast form to 21% in the mycelial form. A slightly higher mannan content was found in the yeast form (about 22%) by Sullivan *et al.* (1983) and Cassone *et al.* (1979) as well as a slightly higher glucan content for the yeast form (about 79%) The increase in the amount of chitin from the yeast form to the mycelial form is in agreement with the observations of Chattaway *et al.* (1968) who postulated that this increase in chitin content may result in greater resistance of the mycelial form to the defense mechanisms of the host allowing for the persistence of the mycelial form *in vivo*.

The localization of mannan has been examined by investigators using a variety of techniques such as lectins (Con A), antibodies to mannan, biochemical techniques, histochemical techniques and combinations of any of the above. Montes and Wilborn (1968) in examining the invasion of oral epithelial cells by *C. albicans* noted a floccular material that was present on the extracellular yeast but not on the yeast cells invading the epithelial cells. They were not sure if this floccular material was of host or yeast origin but it was being detached during the invasion of the epithelial cells. They implied that the floccular material might be mannan based on the histochemical work of Mundkur (1960) on *S. cerevisiae*. Histochemically the localization of mannan in the outer cell wall of *C. albicans* has been confirmed by Djaczenko and Cassone (1971) using TAPO, and Poulain *et al.* (1978) using the polysaccharide staining technique of Thiery (1969). Evron and Drewe (1984) utilizing extraction and histochemical procedures also placed mannan in the outer cell wall. They analyzed the extraction fluid for cell wall components and found that the extraction fluid for the outer cell wall layer contained mannan and glucan. This outer layer of mannan is apparently easily shed based on the observations of Montes and Wilborn (1968) and Poulain _et al_. (1978). They observed that old cultures of _C_. _albicans_ had lost their outer cell wall layers which they said contained mannan. Due to this loss of outer cell wall layers Poulain _et al_. (1978) postulated that this could account for the mannan antigenemia described by Weiner and Yount (1976) in patients with invasive candidiasis. In culture filtrates, Diedrich _et al_. (1984) and Diamond _et al_. (1980b) isolated a glycoprotein that contained mannose which both groups thought to be mannan. Elorza _et al_. (1983) found that during the regeneration of protoplasts of _C_. _albicans_ radioactively labelled mannan was not associated with the protoplast indicating that mannan was not a structural polysaccharide of the cell wall as are glucan and chitin but that mannan is a matrix polysaccharide. This study is in agreement with Cassone _et al_. (1978) who concluded that mannan is located throughout the cell wall both over it and interwoven into the glucan-chitin framework.

Mannan is also located next to the plasma membrane as demonstrated by Poulain _et al_. (1978). A trilaminar staining pattern using antibodies to mannan was described by Meister _et al_. (1977) and Venezia and Lachapelle (1973). In both cases the staining was absent when the antibodies were treated with mannan. Tronchin _et al_. (1981) arrived at the same conclusion using Con A and a silver staining technique for polysaccharides.

All three of the above mentioned cell wall components have been shown to affect the immune system. Glucan is a stimulatory agent of the reticuloendothelial system (DiLuzio, 1975) and this stimulation of macrophages is seen under *in vitro* conditions (Burgaleta *et al.*, 1978). Glucan has also been reported to protect mice from experimental *Candida* infections (Williams *et al.*, 1978). Suzuki *et al.* (1984) demonstrated that immunizing mice with chitin increases their longevity when challenged with *C. albicans*. They also showed that chitin is a stimulator of oxidative metabolism in neutrophils and macrophages. The observations of Suzuki *et al.* (1984) would tend to support the proposal of Chattaway *et al.* (1968) that the chitin in the mycelial form plays a role in the infection process of *C. albicans*.

Mannan is the cell wall component most implicated in the suppression of the immune response. Mannan antigenemia has been reported in cases of invasive candidiasis by Weiner and Yount (1976). In a latter study, Weiner and Coats-Stephen (1979) confirmed, in patients and an animal model of candidiasis, the existence of mannan antigenemia in disseminated candidiasis. Segal *et al.* (1979) in an attempt to devise a reliable means of antemortum detection of invasive candidiasis found mannan concentrations ranging from 25 to 540 ng per ml of serum in autopsy proven cases of candidiasis. Wright *et al.* (1981) isolated from a patient with chronic mucocutaneous candidiasis a remarkable 2.7 mg of mannan per ml of serum.

Mannan appears to be the predominant polysaccharide cell wall antigen (Summers *et al.*, 1964). Antibodies to mannan can be found in serum of patients with candidiasis (Chew and Theus, 1967; Lehnmann and

Reiss, 1980; Weiner and Coats-Stephens, 1979, Weiner and Yount, 1976), in experimentally infected animals (Meister et al. 1977; Weiner and Coats-Stephens, 1979) and in normal uninfected individuals (Lehnmann and Reiss, 1980; Weiner and Yount, 1976). Circulating antigen-antibody complexes have been detected in the serum of patients with candidiasis (Burges et al., 1983) and immune complexes can be generated by adding mannan to human serum (Reiss et al., 1981). Immune complexes have been implicated in the lethality seen with Candida infections (Kasckin, 1974). He reported that immune mice when injected with Candida species developed vascular emboli due to immume complex deposition. Patients with high anticandidal antibody titers and normal complement levels are found to have dissemianted Candida infections (LaForce et al., 1975). They found that sera from these patients when used to opsonize C. albicans decreased intracellular killing by the neutrophils regardless of whether the neutrophils were from the patient or the uninfected individuals. Ultrastructurally, they found a heavy deposition around the opsonized yeast cell which seemed to protect it from being killed inside the neutrophil.

Fischer et al. (1978) found that mannan inhibited the in vitro Candida antigen induced T cell proliferation. Other investigators have found that Candida infection inhibits the immune response of T cell dependent antigens (Valdez et al., 1984; Vardinon and Segal, 1979). Later, Segal et al. (1980) demonstrated tht culture filtrates suppressed the immune response to T cell dependent antigens but not to the extent of whole yeast cells. The substance which was not defined by Segal et al. (1980) may very well be mannan based on the observations of Diedrich *et al.* (1984) and Diamond *et al.* (1980b) that mannan is released into the culture medium by the fungus. Piccolella *et al.* (1981a) found that suppressor cell were generated after exposure to a polysaccharide from *C. albicans*. They suggested that the suppression occured by two possible mechanisms: a direct suppression and an interaction with soluble helper factors. Human peripheral blood lymphocytes were found to become unresponsive to a second exposure of this polysaccharide but the unresponsiveness could be overcomed by the addition of fresh mitomycin C treated lymphocytes (Piccolella *et al.*, 1981b). Nelson *et al.* (1984) observed that mannan inhibits lymphocyte proliferation to a variety of antigens including the *Candida* antigen and that the inhibition was due to the copper associated with the mannan. If the copper was removed, then the inhibition of lymphocyte proliferation to all antigens except for the *Candida* antigen was abolished. The copper was used to isolate the mannan. Nelson *et al.* (1984) proposed a different mechanism for the inhibition of lymphocyte proliferation based on the observation that mannan stimulates hydrogen peroxide generation by neutrophils and monocytes. They based this mechanism on the observation that hydrogen peroxide has been demonstrated to suppress lymphocyte proliferation *in vitro* (Metzger *et al.*, 1980).

The invading microorganism must be able to mediate adherence to the host surfaces. However, the same mechanism that allows adherence to the host surface may be used by the host to phagocytize the invading organism (Ofek and Silverblatt, 1982). Mannose mediated adherence of *C. albicans* to human buccal cells has been suggested by Sandin *et al.* (1982). Both macrophages and neutrophils possess receptors that allow them to phagocytize unopsonized C. albicans which is inhibited by mannan or mannose (Sung et al., 1983; Warr, 1980; Diamond et al., 1980b).

Mannan has been shown to inhibit the phagocytosis of zymosan by macrophages (Sung et al., 1983). They also demonstrated that mannan isolated from different species of yeast differed in their ability to inhibit the phagocytosis of zymosan. Oda et al. (1983) found that a galactomannan and a rhamnomannan from Sporothrix schenckii inhibited the phagocytosis of the yeast form of this organism. Diamond et al. (1980b) isolated a substance from the culture medium of C. albicans which they identified as mannan which inhibited the attachment of neutrophils to the pseudohyphae and hyphae of C. albicans. Prior to this study, Diamond and Krzesicki (1978) demonstrated that mannan isolated from serotypes A and B of C. albicans were able to inhibit the attachment of neutrophils to the pseudohyphae of C. albicans. These authors thought that the dose of mannan necessary to inhibit attachment of C. albicans to phagocytic cells in vitro was too high (1 mg) for mannan to be an inhibitor of attachment in vivo. Wright et al. (1981) found no inhibition of phagocytosis of zymosan particles opsonized with normal human serum by neutrophils. Sung et al. (1983) examined the effect of mannan on the phagocytosis of IgG and C3b opsonized erythrocytes by macrophages. In both cases no inhibition of phagocytosis was observed (Sung et al., 1983).

Hilger and Danley (1980) demonstrated that whole yeast cells affected the ability of neutrophils to generate hydrogen peroxide. This effect was dependent on whether or not the yeast cells were viable. Increasing concentrations of opsonized or unopsonized killed C. albicans caused increasing amounts of hydrogen peroxide to be released. However, if opsonized or unopsonized viable _C_. _albicans_ were used increasing concentrations of yeast cells demonstrated decreasing amounts of released hydrogen peroxide. The authors suggested that viable blastospores somehow were able to neutralize the hydrogen peroxide. Viable blastospores elicited a greater metabolic response from the neutrophils than did the killed blastospores as measured by the reduction of nitroblue tetrazolium and chemiluminescence. In a latter study, Danley and Hilger (1981) demonstrated that mannan can stimulate hydrogen peroxide production and iodination by neutrophils, but that mannose the major sugar of mannan inhibits these reactions and reduces oxygen consumption, carbon dioxide generation and superoxide generation by neutrophils. They were uncertain as to why mannan and mannose should have opposing effects on the neutrophils. In their study, Danley and Hilger (1981) reported that _C_. _albicans_ is unable to neutralize even low amounts of hydrogen peroxide so that mannan in the cell wall is not a mechanism for the reduced hydrogen peroxide generation seen in neutrophils. Nelson _et al_. (1984) confirmed the observations of Danley and Hilger (1981) that mannan increases the amount of hydrogen peroxide generated when neutrophils are exposed to mannan. Superoxide generation by neutrophils exposed to mannan was measured by Nelson _et al_. (1984) and Wright _et al_. (1981) and in both studies, mannan reduced the amount of superoxide. Wright _et al_. (1981) proposed that mannan acts as a substrate to scavenge superoxide. This proposal was based on the observations that mannan reduced the amount of superoxide generated in hypoxanthine and xanthine oxidase system and when neutrophils were exposed to mannan their superoxide generation was reduced but mannan did not inhibit the oxygen uptake by the neutrophils. Nelson et al. (1984) demonstrated that mannan has a superoxide dismutase-like activity and that this activity is dependent of the association of copper with the mannan. When they removed the copper from the mannan it lost all superoxide dismutase-like activity. Danley et al. (1983) demonstrated that neutrophils modulate the respiration of C. albicans, as measured by carbon dioxide release, but this inhibition is only 30% of the respiration of the yeast cells alone. Candida albicans was shown in their study to be able to generate hydrogen peroxide and in the presence of iodine and lactoperoxidase the yeast was able to iodinate itself. This is a rather self-destructive facet of C. albicans since the myeloperoxidase-halide-hydrogen peroxide system has been shown to be a potent antimicrobial system by Klebanoff (1968) and Lehrer (1969). In addition to the generation of hydrogen peroxide, C. albicans is capable of generating superoxide which Danley et al. (1983) thought might be a mechanism by which the fungus may enhance invasiveness. While hydrogen peroxide and superoxide are toxic to the fungus they are also toxic to mammalian cell as well. Danley et al. (1983) postulated that since C. albicans can tolerate low levels of hydrogen peroxide and inhibit higher levels of hydrogen peroxide that would be detrimental to the yeast, then this could be a mechanism by which the yeast may establish an infection.

Lysosomes have been shown to participate in the killing of S. cerevisiae (D'Arcy Hart, 1981) and may play a role in the killing of C. albicans. Fontenla de Petrino and Sirena (1984) measured the release of three lysosomal eznymes from rat peritoneal macrophages and found that with time, after a single exposure to killed C. albicans blastospores, the amount of these enzymes released into the supernatant increased up to six hours and decreased thereafter. Since the yeast were killed prior to addition to the macrophages the decrease in the release of the lysosomal enzymes was presumably due to the decrease in the amount of antigen to which the macrophages were exposed (Fontenla de Petrino and Sirena, 1984). Wright et al. (1981) also measured the release of lysosomal enzymes, but their system employed mannan and neutrophils. In contrast to Fontenla de Petrino and Sirena (1984) they found no difference in the release of acid phosphatase and beta-glucuronidase. Wright et al. (1981) found a decrease in the amount of myeloperoxidase in the supernatant of neutrophils exposed to mannan as compared to the controls. They determined that the myeloperoxidase was binding to the mannan and that the mannan was binding to the neutrophils resulting in myeloperoxidase being unavailable for reaction in the assayed when the supernatant was used. They bound mannan to Sepharose beads and exposed these beads to isolated myeloperoxidase in their assay. The myeloperoxidase was associated with the Sepharose beads (Wright et al., 1981). Later, Wright et al. (1984) demonstrated that mannan will bind to neutrophils and that the binding sites for myeloperoxidase and neutrophils to mannan were different. Wright et al. (1983) found that the binding of myeloperoxidase to the yeast cell surface is necessary for the killing of the yeast and that mannan inhibited killing in a dose dependent manner. They postulated that the way the yeast may escape killing is that the soluble mannan interferes with the myeloperoxidase mediated killing by binding the myeloperoxidase away from the cell surface because the mannan-myeloperoxidase complex still retained the myeloperoxidase activity. Wright _et_ _al_. (1983) suggested that the superoxide scavenging role of mannan did not play a role in the protection of _C_. _albicans_ from killing. Also, they suggested that the serum levels of mannan were not sufficient to inhibit killing but that at the site of infection mannan levels might be high enough to inhibit the killing of _C_. _albicans_. The binding site of mannan to myeloperoxidase was also investigated by Wright _et_ _al_. (1984) who found that the phosphate attached to the outer chain of mannan was necessary for binding of myeloperoxidase to the mannan. They also reported that serine and threonine linked oligosaccharides of the mannan molecule are necessary for the binding of mannan to the neutrophils.

MATERIALS AND METHODS

Abbreviations

Buffer, Dulbecco's phosphate buffered saline, pH 7.2; TCM, M199 + 25mM HEPES; FITC, fluorescein isothiocyanate; Con A Concanavalin A; EDTA, disodium ethylenediaminetetraacetic acid; HEPES, N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid; PEC, peritoneal exudate cells; SDA, Sabouraud's dextrose agar; NBT, nitroblue tetrazolium.

Source of Materials

Sigma Chemical Co., mannan (M 3640), NBT, Con A, HEPES, EDTA, neutral red, Wright stain; Allied Chemical Co., acridine orange, methylene blue; GIBCO, M199; Difco, SDA, trypticase soy broth; U.S. Biochemical, FITC labelled antiserum to Fc portion of IgG and to C3; American Scientific Products, Pro-Texx.

Mice

CBA/J littermate breeding pairs were purchased from the Jackson Laboratory (Bar Habor, ME). The mice were housed at the Medical Mycology Research Laboratory at Michigan State University and were maintained by brother-sister matings. In the neutral red candidacidal assay studies both male and female mice from 8 to 16 weeks old were used. In the mannan studies only female mice from 8 to 16 weeks old were used. The mice were individually housed and fed standard laboratory chow and acidified water *ad libitum*.

Peritoneal Macrophage Monolayer Preparation

Resident peritoneal macrophages were obtained by washing out the peritoneal cavity of mice with 3 ml of cold TCM with gentle massage to dislodge any loosely adherent cells. The Con A elicited peritoneal macrophages were obtained by injecting 100 micrograms of Con A suspended in 0.1 ml of buffer intraperitoneally and harvesting the PEC four days later. The macrophages were removed by washing out the peritoneal cavity with 3 ml of cold TCM with gentle massage to dislodge any loosely adherent cells. Both types of macrophages were withdrawn from the peritoneal cavity with an 18 gauge needle and syringe. Then 0.3 ml of the peritoneal fluid was layered onto 9 x 22 mm glass coverslips. The number of macrophages from the peritoneal cavity of female mice injected with Con A layered onto the coverslips was about 1×10^6. The macrophages were allowed to adhere for 45 minutes at 37 C after which the monolayers were washed in warm buffer, placed into Leighton tubes and used immediately. Total and differential cell counts of the PEC were performed. Mice whose PEC contained more than 5% neutrophils were discarded.

Yeast Culture

Candida albicans (MSU-1), a fecal isolate, was maintained by monthly transfers on SDA slants. The inoculum was grown on SDA slants for 48 hrs at 37 C. A loopful of this culture was aseptically transferred to a 250 ml flask containing 100 ml of trypticase soy broth supplemented with 4% D-glucose and incubated for 15 hours at 37 C on a rotary shaker (180 rpm). This brought the culture to stationary phase. The yeast cells were harvested by centrifugation and washed twice with sterile saline. The viability of the yeast culture was determined by the methylene blue dye technique using 0.01% methylene blue. Cultures with less than 95% viability were discarded.

Opsonization Procedures

Four opsonization procedures were utilized to selectively opsonize the yeast cells. 1) Unopsonized yeast cells were used after being washed in sterile saline and resuspended in buffer. 2) Yeast cells were opsonized in normal human serum for 30 minutes at 37 C. The normal human serum used had an agglutination titer to *C. albicans* of 1:2. These yeast cells were opsonized with both immunoglobulins and complement (Morrison and Culter, 1981). This was demonstrated by fluorescence with FITC labelled antiserum to the Fc portion of IgG and to C3. 3) Yeast cells were opsonized with complement by a modification of the technique of Roos *et al*. (1981). The complement pathways of freshly collectly normal human serum were inactivated with EDTA (10mM final concentration). To 10 ml of this inactivated serum 5 grams of heat killed *C. albicans* were added to remove anticandidal antibodies. The *C. albicans* was killed by boiling the culture in saline for 30 minutes. The yeast cells were washed twice with saline and stored frozen until used. The mixture of the heat killed yeast cells and inactivated serum were incubated for 30 minutes on a rotating shaker at room temperature. The yeast cells were removed by filtration and the serum was stored at -20 C until used. This serum was used within 2 days of preparation. The serum was reactivated with $MgCl_2$ (10mM final concentration). Viable *C. albicans* were added to this reactivated serum and incubated for 30 minutes at 37 C. The yeast cells were washed twice in buffer and resuspended in buffer. Prior to use the selectivity of the opsonization procedure was screened with FITC labelled antiserum to Fc portion of IgG and to C3. In this procedure only the yeast cells exposed to the anti-C3 antiserum fluoresced. 4) The above procedure was used to opsonized the yeast cells with immunoglobulins. Normal human serum was inactivated with EDTA (10mM final concentration). The yeast cells were washed twice in 20mM EDTA and then the inactivated serum added. The mixture was then incubated for 30 minutes at room temperature on a rotating shaker. The yeast cells were washed twice and resuspended in buffer. Prior to use the selectivity of the opsonization procedure was screened with FITC labelled antiserum to Fc portion of IgG and to C3. In this procedure only the yeast cells exposed to the anti-Fc antiserum fluoresced. In all of the opsonization procedures the yeast cells were resuspended in buffer to a concentration of 5.5 x 10^7 (as a stock yeast suspension) and kept on ice until used.

Candidacidal Assay

To the monolayers of resident and Con A elicited peritoneal macrophages from male and female mice in Leighton tubes 0.9 ml of TCM and 0.1 ml of a stock yeast cell suspension (4.5 x 10^7/ml normal serum opsonized) were added, which completely covered the coverslip. The macrophages and the yeast cells were allowed to incubate together for 1 hour at 37 C.

A fresh solution of neutral red indicator dye in buffer (10mg/ml) was made up fresh every day (Hammond et al., 1980). Fifteen minutes before the end of the incubation period 10 microliters of the neutral red solution was added per Leighton tube. At the end of the incubation period the coverslip was removed, washed in warm buffer and examined immediately. One hundred phagocytizing macrophages were scored for the number of red (dead) and colorless (viable) yeasts.

A duplicate coverslip was removed at the same time as the neutral red coverslip. The duplicate coverslip was washed in warm buffer, air dried, fixed with absolute methanol, and stained with Wright's stain and mounted with Pro-Texx. One hundred phagocytizing macrophages were counted for the number of yeast cell with and without germ tubes.

The neutral red technique was compared to two standard candidacidal assays: acridine orange and methylene blue. The acridine orange method was that of Pantazis and Kniker (1979). A modification of the methylene blue technique of Schmid and Brune (1974) was employed where methylene blue (0.1 ml of a 0.1% solution in buffer) was added to the Leighton tube 15 minutes before the end of the incubation.

The phagocytic capacity and candidacidal activity of the macrophages were determined by the following formulae:

phagocytic capacity = no. of yeast cells/100 phagocytizing macrophages

candidacidal activity = no. dead yeast cells/100 phagocytizing macrophages

Mannan Treatment Experiments

Mannan was dissolved in 0.9 ml of TCM in the following amounts 0.0, 0.5, 1.0 and 2.0 mg and added to monolayers of Con A elicited peritoneal macrophages. The macrophages were incubated with the different mannan concentrations for 15 minutes at 37 C after which time 4.5 x 10^4 unopsonized *C. albicans* were added. The macrophages and the yeast cells were incubated together for 30 minutes at 37 C. The coverslips were washed in warm buffer and 50 macrophages were scored for the number of yeast cells per macrophage and the percent macrophages containing yeast cells. The lenght of time for treatment with 1 mg of mannan prior to the additon of the yeast cells was determined by incubating the macrophages with mannan for 5, 10, 15, 20, and 30 minutes at 37 C. The same number of yeast cells were added to the Leighton tubes. The macrophages (50) were scored for the number of yeast cells per macrophage. The viability of the macrophages was determined by eosin Y dye exclusion (Mishell et al., 1980). Detachment of the macrophages from the coverslips was determined by examining the culture medium on a hemacytometer.

Phagocytic and Candidacidal Studies

Monolayers of Con A elicited peritoneal macrophages were incubated for 15 minutes at 37 C with either TCM or TCM plus 1 mg of mannan. After this incubation 5.5×10^6 yeast cells (regardless of the opsonization procedure) were added to each Leighton tube. The macrophages and the yeast cells were incubated for 30 minutes at 37 C. Fifteen minutes prior to the end of this incubation 10 microliters of neutral red in buffer were added to each Leighton tube. The monolayers were washed in warm buffer and mounted in a drop of buffer, sealed with fingernail polish and examined immediately. The macrophages were scored on three parameters modified form Simpson et al. (1979). They are:

phagocytic activity = (no. macrophages with yeast cells/100 macrophage) x 100

phagocytic capacity = no. of yeast cells/100 macrophages

candidacidal activity = no. of dead yeast cells/phagocytic capacity

Mannan and Fc and Complement Receptors

The effects of mannan on these receptors were determined by using decreasing concentrations of *C. albicans* opsonized with either immunoglobulins or complement. The concentrations of yeast cells were 5 x 10^6, 2.5 x 10^6, and 1.25 x 10^6 per ml. The monolayers of Con A elicited peritoneal macrophages were incubated for 15 minutes at 37 C either with TCM or TCM plus 1 mg of mannan prior to the addition of the yeast cells. The macrophages and the yeast cells were incubated together for 30 minutes at 37 C. The coverslips were washed in warm buffer, air dried, fixed in absolute methanol, and stained with Wright's stain. The coverslips were mounted with Pro-Texx and 100 macrophages were scored for their phagocytic activity and phagocytic capacity.

NBT Reduction

A stock solution of nitroblue tetrazolium was prepared by a modification of the technique of Cline (1981). A 0.1% stock solution of NBT in TCM was prepared, filter sterilized and stored at 4 C until used. The monolayers of Con A elicited peritoneal macrophages were incubated for 15 minutes at 37 C either with TCM or TCM with 1 mg of mannan. This medium was removed from the Leighton tubes and replaced with the appropiate TCM (with or without mannan), normal human serum opsonized *C. albicans* (5.5 x 10^{6}) and NBT stock solution to yield a final concentration of NBT of 0.05%. The macrophages and the yeast cells were incubated for 30 minutes at 37 C. The coverslips were then removed from the Leighton tubes, washed in warm buffer, air dried, fixed with absolute methanol and counterstained with 0.2% safranin O. The coverslips were mounted with Pro-Texx and 100 phagocytizing macrophages were scored for the presence or absence of a dark blue precipitate which indicates the reduction of the NBT (Cline, 1981).

Phagolysosome Fusion

The Con A elicited peritoneal macrophages were allowed to adhere for 1 hour to glass coverslips at 37 C. The monolayers were washed with warm buffer and then labelled with acridine orange according to the method of Kielian and Cohn (1980). The monolayers were washed in warm buffer and simultaneously incubated with either TCM or TCM with 1 mg of mannan and 5 micrograms of acridine orange for 15 minutes at 37 C. The macrophages were washed with warm buffer and placed into Leighton tubes to which TCM or TCM with 1 mg of mannan and 4 x 10^6 of normal human serum opsonized *C. albicans* were added. This concentration of yeast cells yielded 4-5 yeast cells per macrophage. The macrophages and the yeast cells were incubated together for 30 minutes at 37 minutes. Then the coverslips were washed in warm buffer, mounted in a drop of buffer, sealed with fingernail polish and examined with a Zeiss epifluorescent microscope. One hundred phagocytizing macrophages were scored for fluorescent and nonfluorescent yeast cells. Fluorescent yeast cells indicated phagolysosome fusion.

Statistics

The statistical analyses were done using the paired Student's "t" test and an one way ANOVA (comparison of the different candidacidal assays). The data were transformed accordingly: percentages by the arcsin square root of the percentage and distribution by the square root for the statistical analyses but the data are presented in the table and figures as nontransformed data.

RESULTS

Figure 1 illustrates that neutral red concentrates in the lysosomes of macrophages. In the macrophage with an arrow in Figure 1 three neutral red stained C. albicans yeast cells are inside a phagolysosome. Figure 2 illustrates a macrophage with numerous ingested yeast cell some of which are uniformly stained with neutral red indicating that they are nonviable while viable yeast are not stained or only have their vacuole stained with neutral red. Next to this macrophage is another macrophage that has not ingested any yeast but has its lysosomes stained with neutral red. The macrophage with small arrow in Figure 3 illustrates a variation on the staining pattern of nonviable yeast cells. The yeast is stained uniformly but a central circular structure is not as intensely stained. The macrophage with large arrow in Figure 3 presents neutral red stained yeast cells and neutral red stained lysosomes. The lymphocytes with arrows in Figure 4 have not taken up neutral red to an appreciable degree and usually do not stain with neutral red. Viable C. albicans within macrophages may produce germ tubes as seen in Figure 4 (large arrows).

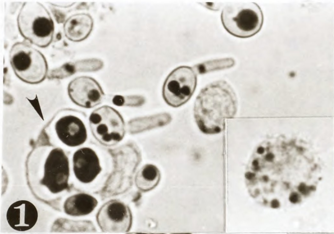
There was an uniform degree of phagocytosis of C. albicans opsonized with normal human serum (Table 1.) which permitted the post phagocytic events to be clearly studied. The phagocytic capacity (number of yeast cells per macrophage) was not statistically different between all four groups of macrophages. The phagocytic capacity of

Figure 1. Macrophage with neutral red stained yeast cells within a phagolysosome. Insert: a macrophage with neutral red concentrated in the lysosomes.

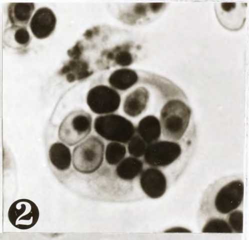
Figure 2. Macrophage with numerous yeast cells within a large phagolysosome showing both neutral red stained, nonviable, and unstained, viable, yeast cells.

Figure 3. Macrophage that illustrates a different staining pattern for neutral red stained yeast cells (small arrow). A macrophage with both neutral red stained yeast cells and lysosomes (large arrow).

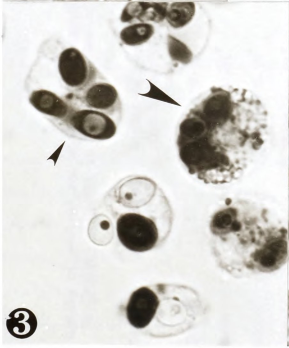
Figure 4. Lymphocytes (small arrows) that have not taken up neutral red. Macrophages (large arrows) that have ingested yeast cells which have formed germ tubes.



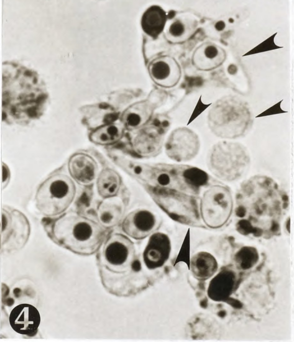
1



2



3



4

Table 1.

Uptake of neutral red stain[a] and intracellular germ tube formation by *Candida albicans* in phagolysosomes of peritoneal macrophages.

Sex	Stimulant	Number of mice	Number of yeast[b] per macrophage	% red staining[c] yeast	% yeast without[d] germ tubes
female	Con A[e]	5	3.64 ± 0.57*	82.0 ± 5.0**	80.1 ± 5.2**
female	none	5	3.44 ± 0.81*	34.3 ± 4.4**	32.0 ± 3.7**
male	Con A	5	3.88 ± 0.59*	80.5 ± 3.6**	78.6 ± 3.9**
male	none	5	3.46 ± 0.28*	32.8 ± 6.0**	31.9 ± 5.2**

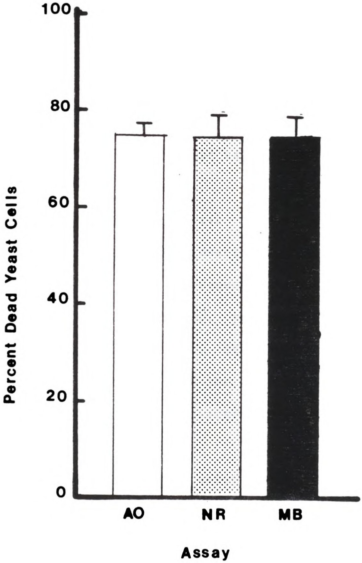
a. Neutral red stain (10 mg/ml of buffer).
b. Number of yeast cells per 100 phagocytizing macrophages.
c. Number of red staining yeast cells in 100 phagocytizing macrophages x 100.
d. Number of yeast cells without germ tubes in 100 phagocytizing macrophages x 100.
e. 100 micrograms (0.1 ml of 1 mg/ml of buffer) injected intraperitoneally.
* No significant statistical differences between the number of yeast cells per macrophage regardless of sex or stimulant as determined by the Student "t" test.
** No significant statistical differences between the % red staining yeast cells and the % yeast cells withouth germ tubes as determined by the Student "t" test.

resident macrophages from female mice was 3.44, 3.46 for resident macrophages from male mice, 3.64 for Con A elicited macrophages from female mice and 3.88 for Con A elicited macrophages from male mice. The candidacidal activity of the resident macrophages from male and female mice was the same regardless if measured by the neutral red technique (32.8% and 34.3%, respectively) or by lack of germ tube formation (34.3% and 32.0%, respectively). The candidiacidal activity of Con A elicited macrophages from male and female mice was the same whether measured by neutral red (80.5% and 82%, respectively) or by lack of germ tube formation (78.6% and 80.1%, respectively). When the candidacidal activity of resident macrophages from female mice (34.3% as measured by neutral red) was compared to the candidacidal activity of Con A elicited macrophages from female mice (82% as measured by neutral red) a statistically significant difference (P>0.01) is seen. The same observation was made when the candidacidal activity of resident macrophages from male mice (32.8% as measured by neutral red) was compared to the candidiacidal activity of Con A macrophages from male mice (80.5% as measured by neutral red), that a statistically significant difference (P>0.01) is seen.

Neutral red was a comparable candidacidal assay method to other methods, methylene blue and acridine orange, which require the differential staining of yeast cells to determine viability. Figure 5 illustrates this point. The candidacidal activity of macrophages was 74.2 % when measured by neutral red and methylene blue and 74.9% when measured by acridine orange. These results are not statistically different.

Mannan did not affect the viability of Con A elicited macrophages

Figure 5. Candidacidal activity of Con A elicited macrophages from female mice as measured by different candidacidal assays. NR represents neutral red (10 mg/ml of buffer), AO represents acridine orange (1:10,000 in buffer), MB represents methylene blue (0.1% in buffer).


100
80
60
40
20
0
Percent Dead Yeast Cells
AO
NR
MB
Assay

at any concentration or incubation times over 15 minutes. The viability of the control macrophages and the macrophages exposed to 0.5, 1.0 and 2.0 mg of mannan remained at 98% as determined by eosin Y exclusion. Incubation of the macrophages with 1 mg of mannan for 20 and 30 minutes did not alter their viability (100% and 98% viability, respectively). At no time during the incubation period with different concentrations of mannan or incubation for different lenghts of time with 1 mg of mannan, nor with the control macrophages was there any detachment of the macrophages from the glass cover slips.

The inhibition of the phagocytic capacity (number of yeast cells/macrophage) of the macrophages towards unopsonized C. albicans by mannan was seen to progress in a dose dependent manner (Figure 6). The lowest concentration of mannan ,0.5 mg, exhibited the least amount of inhibition of the phagocytic capacity (68% of the control) while the highest concentration ,2.0 mg, showed the most inhibition at 24% of the control and 1 mg of mannan showed an intermediate amount of inhibition at 30% of the control.

The inhibition of the phagocytic activity (percent macrophages with yeast cells) of the macrophages towards unopsonized C. albicans by mannan was also seen to progress in a dose dependent manner (Figure 7). The least amount of inhibition by mannan on the phagocytic acitivity was seen with 0.5 mg of mannan which was 71% of the control, and the most inhibition was exhibited by the highest concentration of mannan at 36% of the control while 1 mg of mannan showed and intermediate amount of inhibition at 46% of the control.

Since the time period of treatment with mannan was arbitrarily set

Figure 6. The influence of different concentrations of mannan on the phagocytic capacity (number of yeast cells per macrophage) of Con A elicited macrophages from female mice using 4.5 x 10^6 unopsonized C. *albicans*. The line is the control value and the open bars are the different mannan treatment values. Each data point represents at least two experiments.

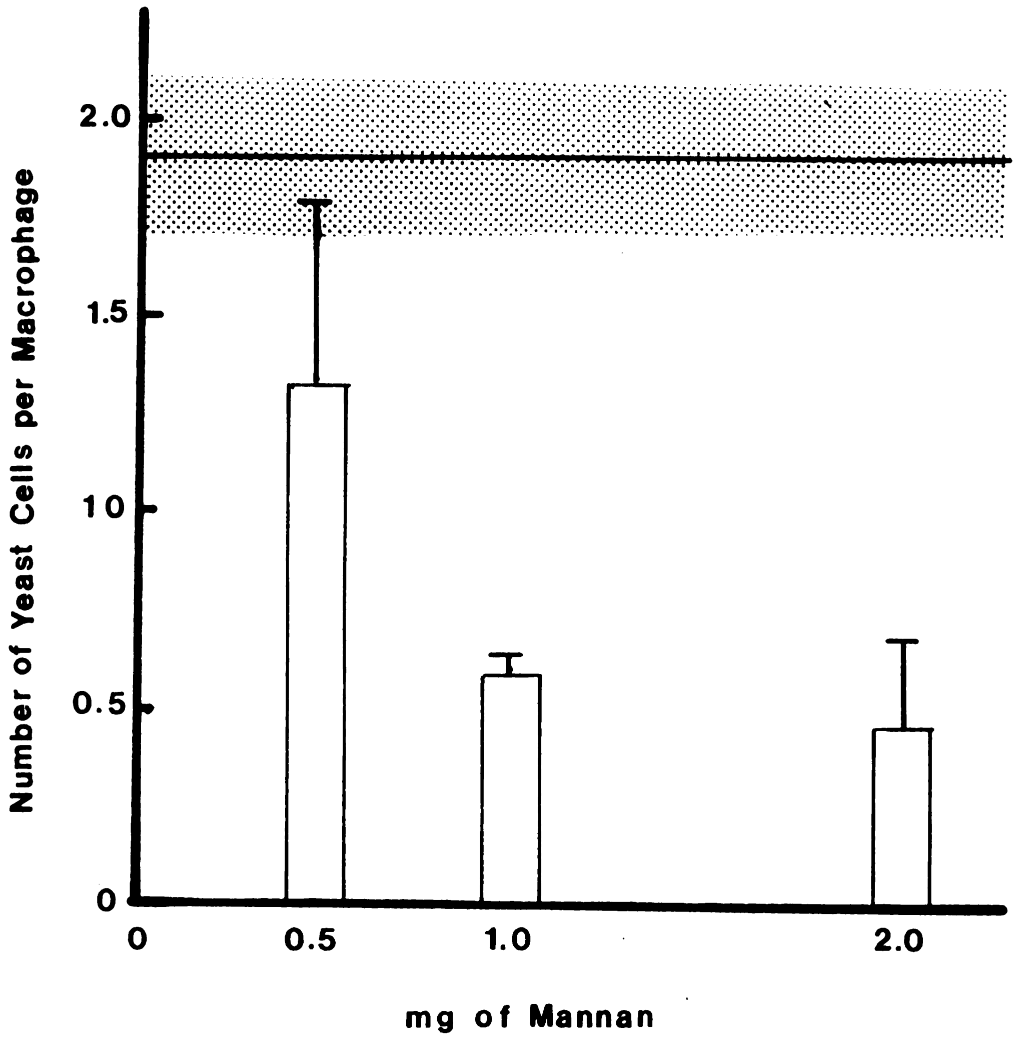
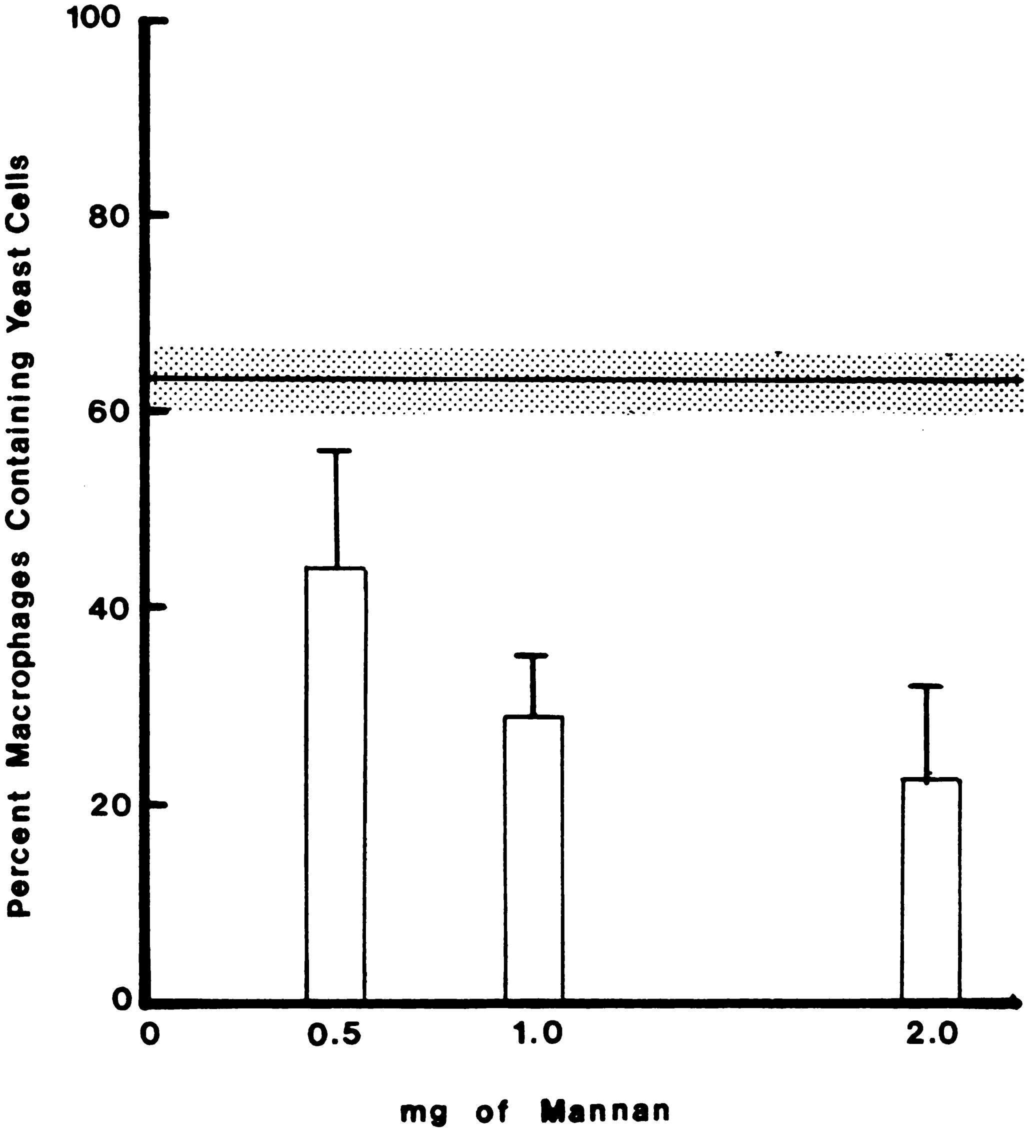

Number of Yeast Cells per Macrophage
2.0
1.5
10
0.5
0
0
0.5
1.0
2.0
mg of Mannan

Figure 7. The influence of different concentrations of mannan on the phagocytic activity (percent macrophages containing yeast cells) of Con A elicited macrophages from female mice using 4.5 x 10^6 unopsonized C. albicans. The line is the control value and the open bars are the different mannan treatment values. Each data point represents at least two experiments.


Percent Macrophages Containing Yeast Cells
100
80
60
40
20
0
0
0.5
1.0
2.0
mg of Mannan

at 15 minutes it was necessary to determine the effect of length of time of treatment with 1 mg/ml of mannan on the phagocytic capacity of macrophages towards unopsonized C. albicans (Figure 8). The effect of the length of time of mannan treatment did not vary greatly from 5 to 20 minutes. The inhibition of the phagocytic capacity of the macrophages at times of 5 to 20 minutes was about 49% of the control. Only at 30 minutes was there a decrease in the phagocytic capacity larger (30% of the control) than previously seen. On the basis of these experiments, an incubation time of 15 minutes at 37 C with 1 mg of mannan prior to the addition of the yeast cells, regardless of the opsonization procedure, was adopted.

The effects of mannan on the phagocytic activity of macrophages towards C. albicans opsonized by different means is shown in Figure 9. The phagocytic activity of macrophages towards C. albicans opsonized with normal human serum was not affected by the presence of mannan, 96.9% for the control macrophages and 96.1% for the mannan treated macrophages. The phagocytic activity of the control macrophages being 96.9% and the mannan treated macrophages phagocytic activity being 96.1% towards C. albicans opsonized with immunoglobulins. Mannan affected the phagocytic activity of these macrophages to a statistically significant degree ($P>0.01$). The mannan treated macrophages had a phagocytic activity of 91.7% as compared to the controls which had a phagocytic activity of 92.9% towards C. albicans opsonized with complement. The phagocytic activity of these macrophages was also decreased by a statistically significant amount ($P>0.05$). The greatest difference was seen when unopsonized C. albicans were employed to measure the effect of

Figure 8. The phagocytic capacity (number of yeast cells/macrophage) of Con A elicited macrophages from female mice treated with 1 mg/ml of mannan for 5, 10, 15, 20, and 30 minutes prior to the addition of 4.5 x 10^6 unopsonized *C. albicans*. The line is the control value and the open bars are the mannan treatment values. Each data point represents at least two experiments.

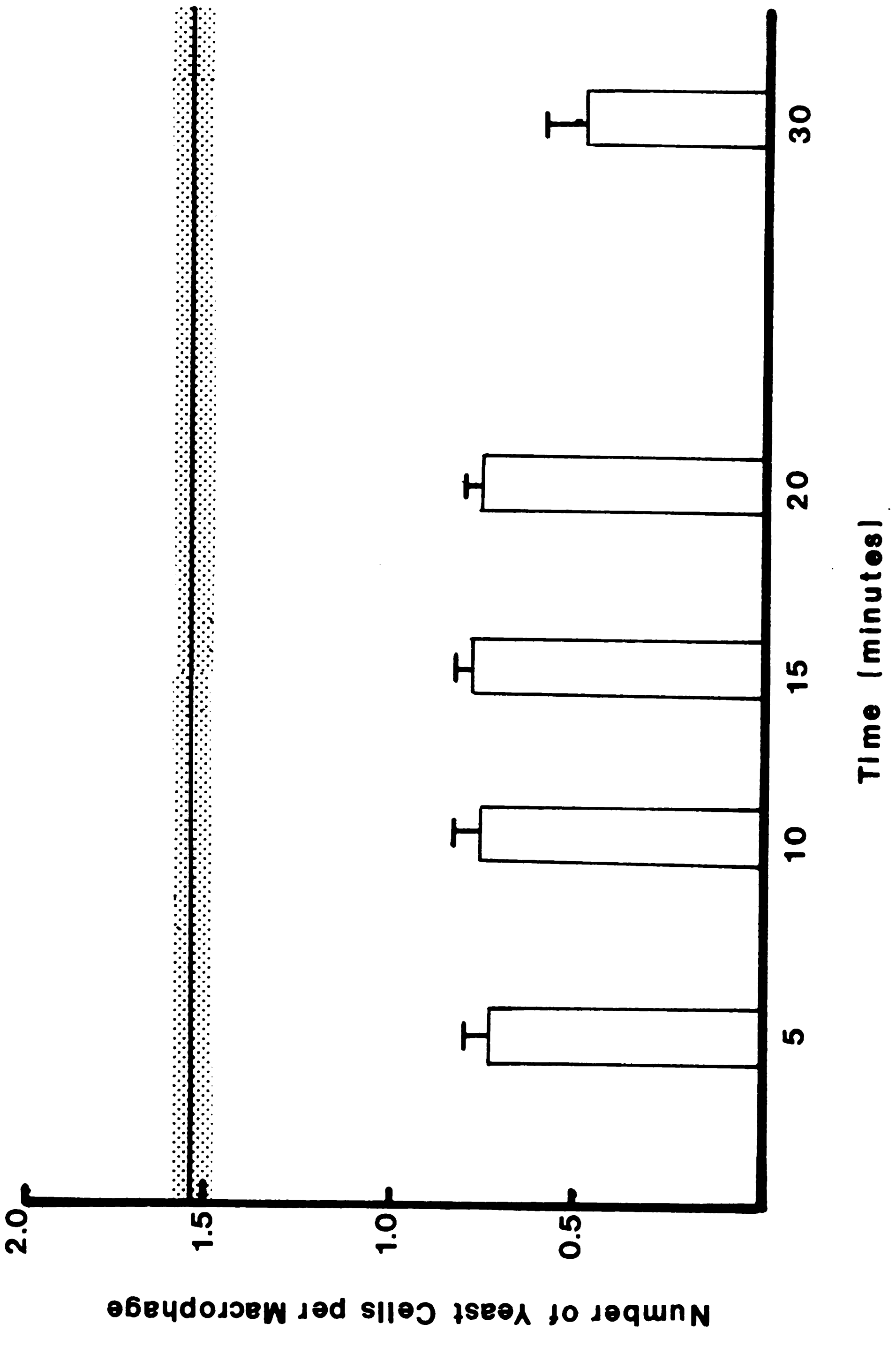
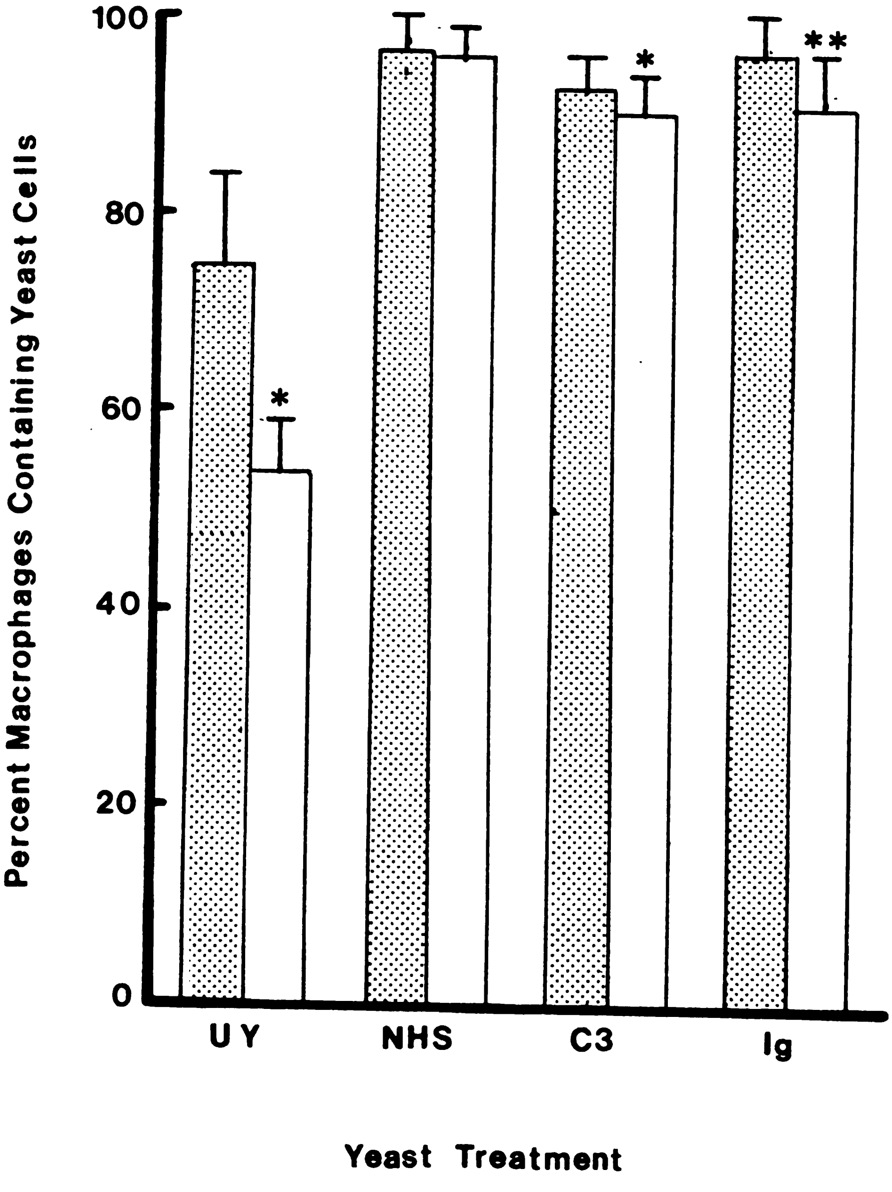

Number of Yeast Cells per Macrophage
2.0
1.5
1.0
0.5
5
10
15
20
30
Time (minutes)

Figure 9. Phagocytic activity (percent macrophages containing yeast cells) of Con A elicited macrophages from female mice treated with 1 mg/ml of mannan prior to the addition of 5.5 x 10^6 C. albicans opsonized by various means. The shaded bars represent control values and the open bars are the mannan treatment values. Each bar represents at least seven experiments. One * represents P>0.05 and two * represents P>0.01. UY = unopsonized yeast cells, NHS = normal human serum opsonized yeast cells, C3 = yeast cells opsonized with complement, Ig = yeast cells opsonized with immunoglobulins.

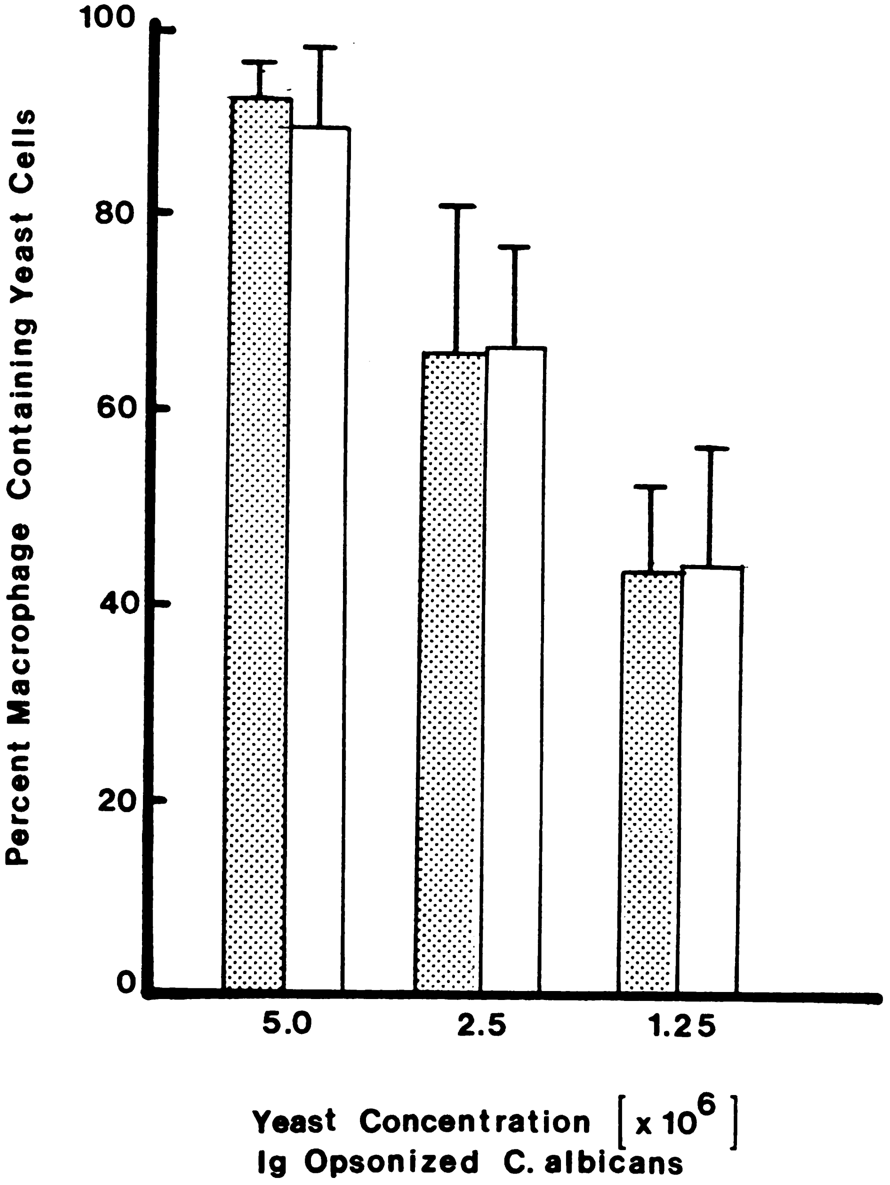

Percent Macrophages Containing Yeast Cells
100
80
60
40
20
0
*
*
**
U Y
NHS
C3
Ig
Yeast Treatment

mannan on the phagocytic activity of macrophages. The phagocytic activity decreased from the control value of 75.6% to 53.5% for the mannan treated macrophages. This is a statistically significant difference $(P>0.05)$.

Since the differences between the means of the phagocytic activity of the macrophages, whether or not treated with mannan, towards C. albicans opsonized with either complement or immunoglobulins was small, this was examined in closer detail using dilutions of the yeast cells. Under these conditions the inhibitory effect of mannan on the phagocytic activity of macrophages towards C. albicans opsonized with immunoglobulins was abolished (Figure 10). The phagocytic activity of the control macrophages and the mannan treated macrophages at 5×10^6 was 91% and 88%, respectively, at 2.5×10^6 was 65% and 65%, respectively, and at 1.25×10^6 was 43% and 44%, respectively. However, with C. albicans opsonized with complement the decrease in the phagocytic capacity of the macrophages remained (Figure 11). The phagocytic activity of the macrophages treated with mannan was 82% of the control at 5×10^6, 65% of the control at 2.5×10^6, and 57% of the control at 1.25×10^6. The inhibition of the phagocytic activity by mannan was statistically significant at each concentration $(P>0.01)$.

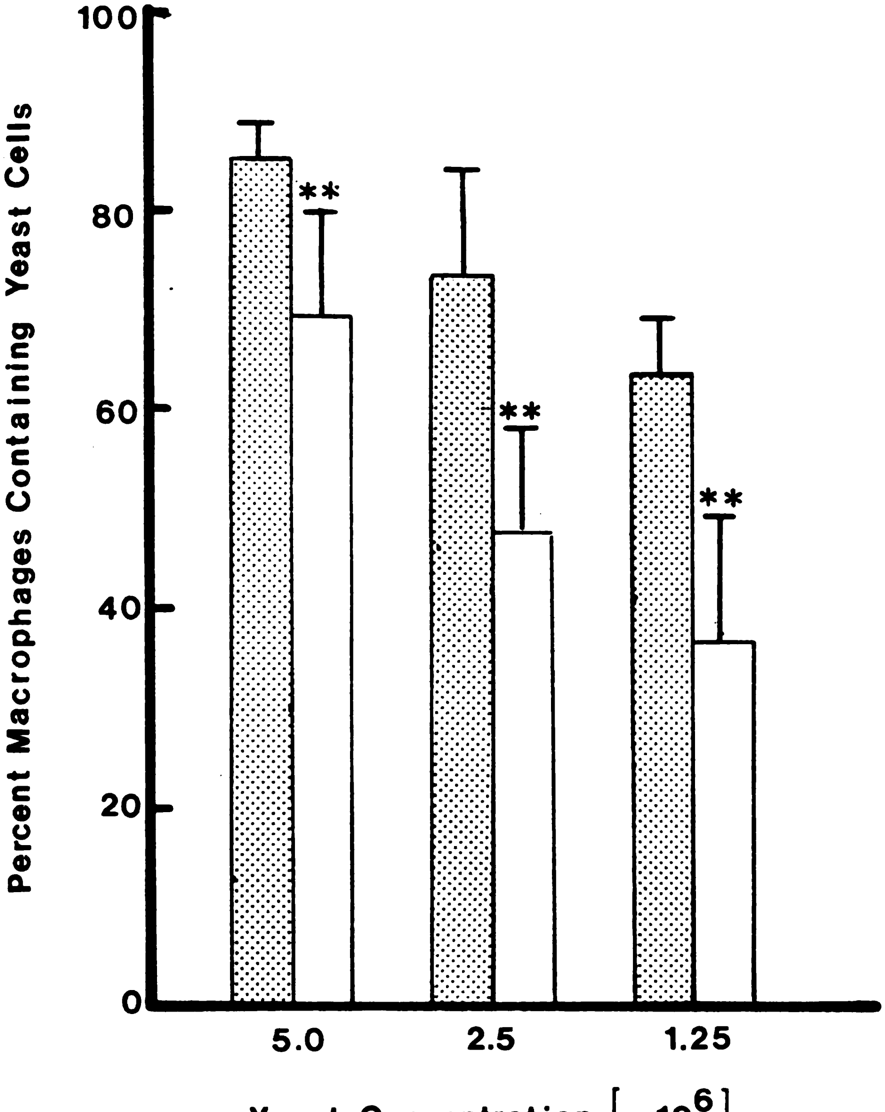
Mannan treatment had the greatest effect on the phagocytic capacity of macrophages towards unopsonized C. albicans (Figure 12). The phagocytic capacity was reduced from 3.19 yeast cells per macrophage for the control macrophages to 1.48 yeast cells per macrophage for the mannan treated macrophages $(P>0.01)$. Mannan treatment had no statistically significant effect on the phagocytic capacity of macrophages towards C.

Figure 10. The phagocytic activity (percent macrophages containing yeast cells) of Con A elicited macrophages from female mice treated with 1 mg/ml of mannan prior to the addition of different concentrations of *C. albicans* opsonized with immunoglobulins. The shaded bars represent the control values and the open bars represent the mannan treatment values. Each bar represents seven experiments.



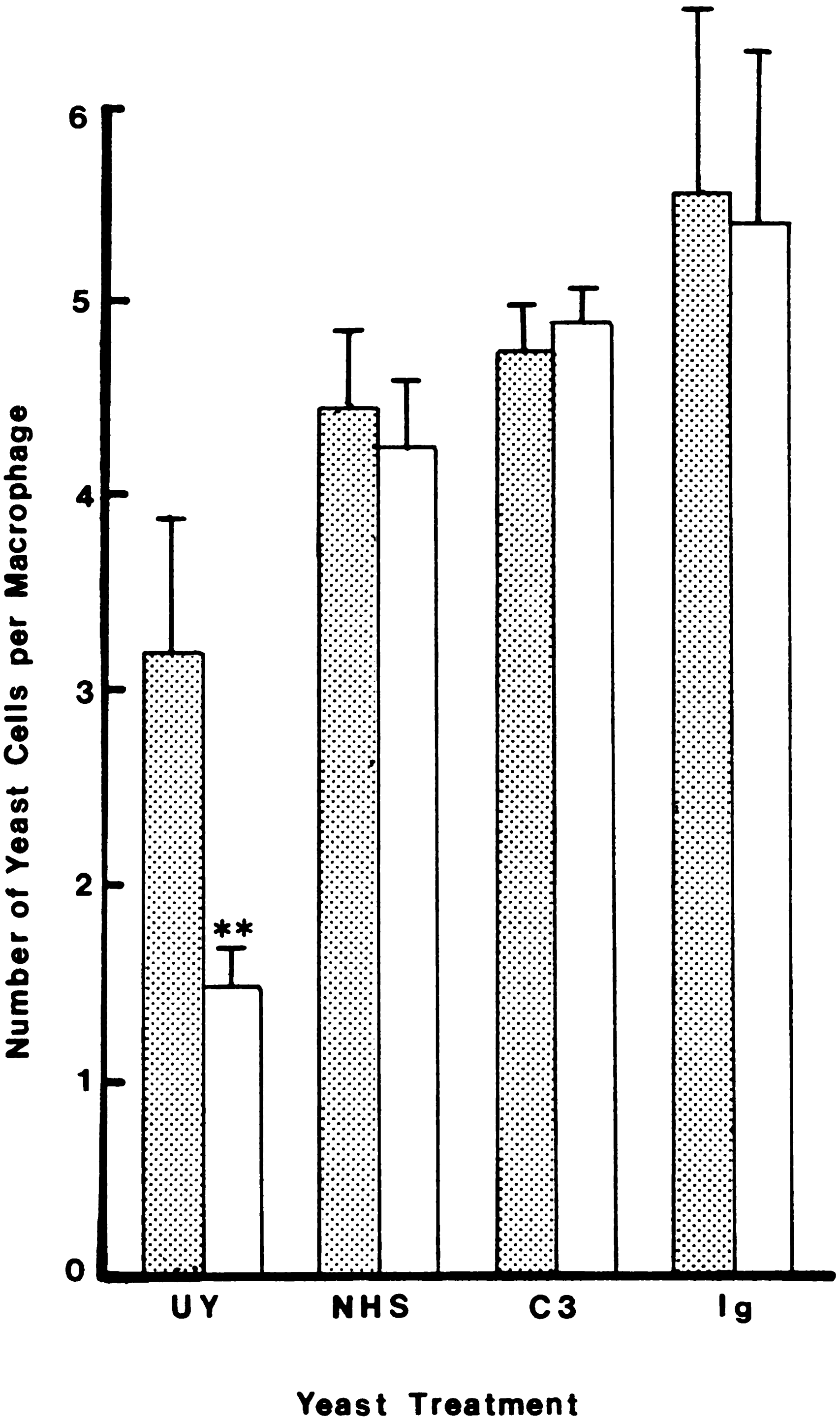
100
80
60
40
20
0
Percent Macrophage Containing Yeast Cells
5.0
2.5
1.25
Yeast Concentration [x 10^6]
Ig Opsonized C. albicans

Figure 11. The phagocytic activity (percent macrophages containing yeast cells) of Con A elicited macrophages from female mice treated with 1 mg/ml of mannan prior to the addition of different concentrations of *C. albicans* opsonized with complement. The shaded bars represent the control values and the open bars represent the mannan treatment values. Two * indicates a P>0.01. Each bar represents eight experiments.



C3 Opsonized C.albicans

Figure 12. The phagocytic capacity (number of yeast cells/macrophage) of Con A elicited macrophages from female mice treated with 1 mg/ml of mannan prior to the addition of 5.5 x 10^6 *C. albicans* opsonized by different means. The shaded bars are the control values and the open bars are the mannan treatment values. Two * indicate a $P>0.01$. Each bar represents at least seven experiments. UY = unopsonized yeast cells, NHS = normal human serum opsonized yeast cells, C3 = yeast cells opsonized with complement, Ig = yeast cells opsonized with immunoglobulins.


6
5
4
3
2
1
0
Number of Yeast Cells per Macrophage
**
UY
NHS
C3
Ig
Yeast Treatment

albicans opsonized with normal human serum, complement and immunoglobulins (Figure 12). The phagocytic capacity of macrophages, control and mannan treated, towards *C. albicans* opsonized with normal human serum are 4.45 and 4.23 respectively, for complement opsonized *C. albicans* are 4.76 and 4.85 respectively, and for immunoglobulin opsonized *C. albicans* are 5.56 and 5.39.

The phagocytic capacity of macrophages towards *C. albicans* opsonized with complement and immunoglobulins at different yeast cell concentrations was also measured. The presence of mannan had no effect on the phagocytic capacity of macrophages towards immunoglobulin opsonized *C. albicans* (Figure 13). The phagocytic capacity of these macrophages, control macrophages and mannan treated macrophages, was for 3.50 and 3.34, respectively with 5 x 10^6, 1.57 and 1.57, respectively with 2.5 x 10^6 and 0.80 and 0.79, respectively for 1.25 x 10^6. In contrast to the previous finding that mannan had no effect on the phagocytic capacity of macrophages towards complement opsonized *C. albicans* (Figure 12) when dilutions of the complement opsonized yeast cells were employed a statistically significant difference ($P>0.01$ at each concentration) was detected (Figure 14). At 5 x 10^6 yeast cells the inhibition of the phagocytic capacity of macrophages by mannan was 56% of the control while 2.5 x 10^6 and 1.25 x 10^6 the inhibition was 38% and 43% of the control, respectively.

The candidacidal activity of macrophages exposed to mannan was decreased, regardless of the opsonization procedure utilized, as compared to the controls (Figure 15). The greatest inhibition of the candidacidal activity of the macrophages by mannan was seen when *C.*

Figure 13. The phagocytic capacity (number of yeast cells/macrophage) of Con A elicited macrophages from female mice treated with 1 mg/ml of mannan prior to the addition of different concentrations of *C. albicans* opsonized with immunoglobulins. The open bars represent the control values and the shaded bars represent the mannan treatment values. Each bar represents seven experiments.

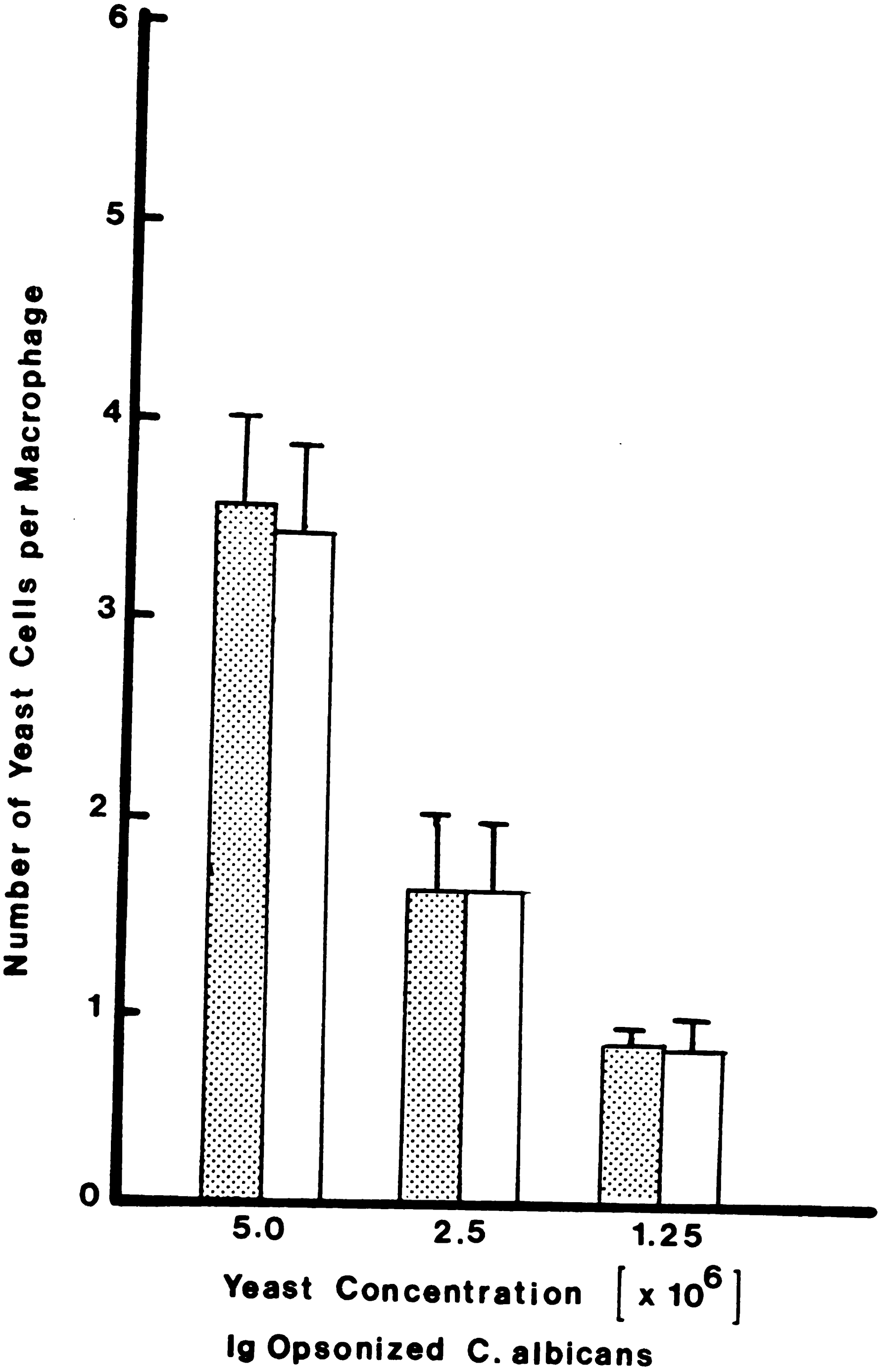
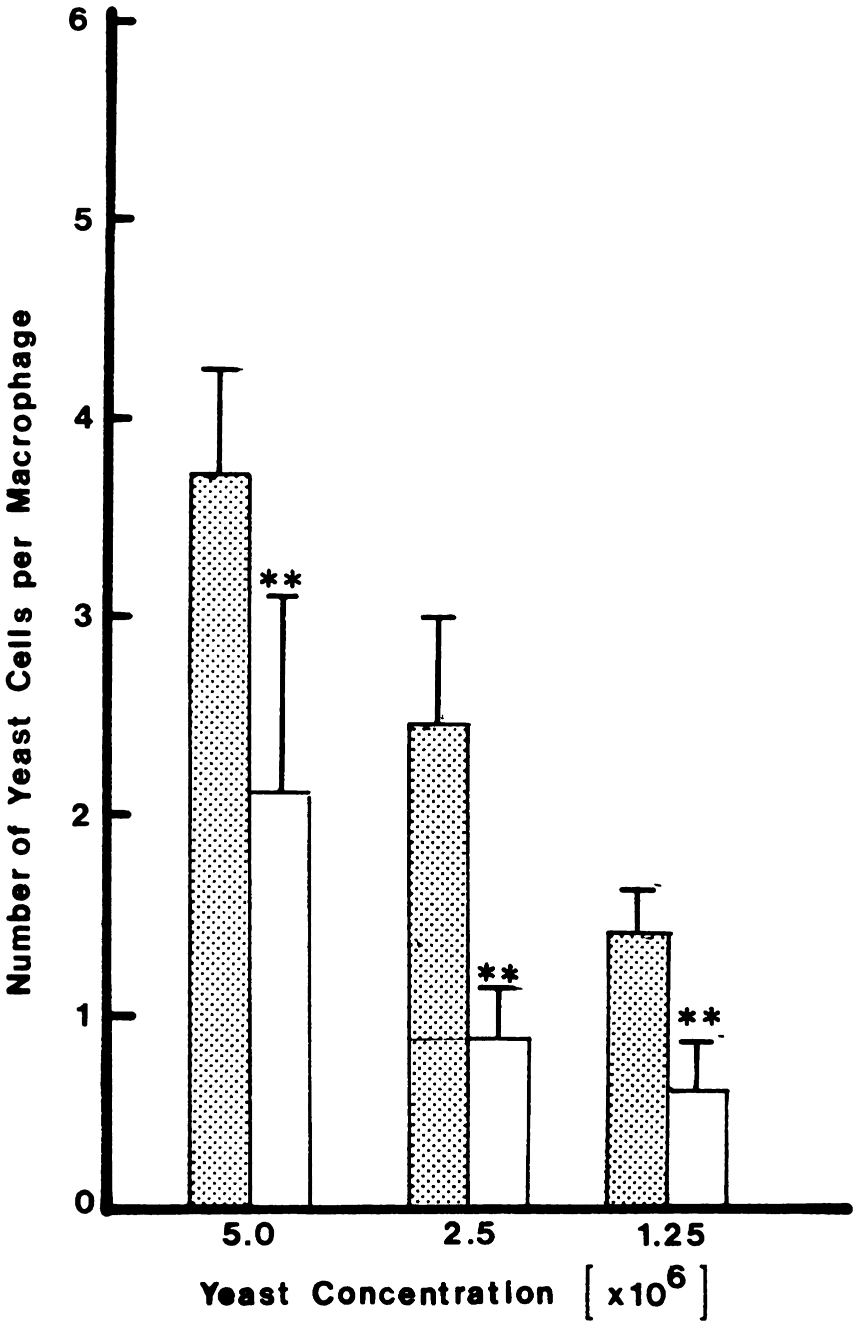
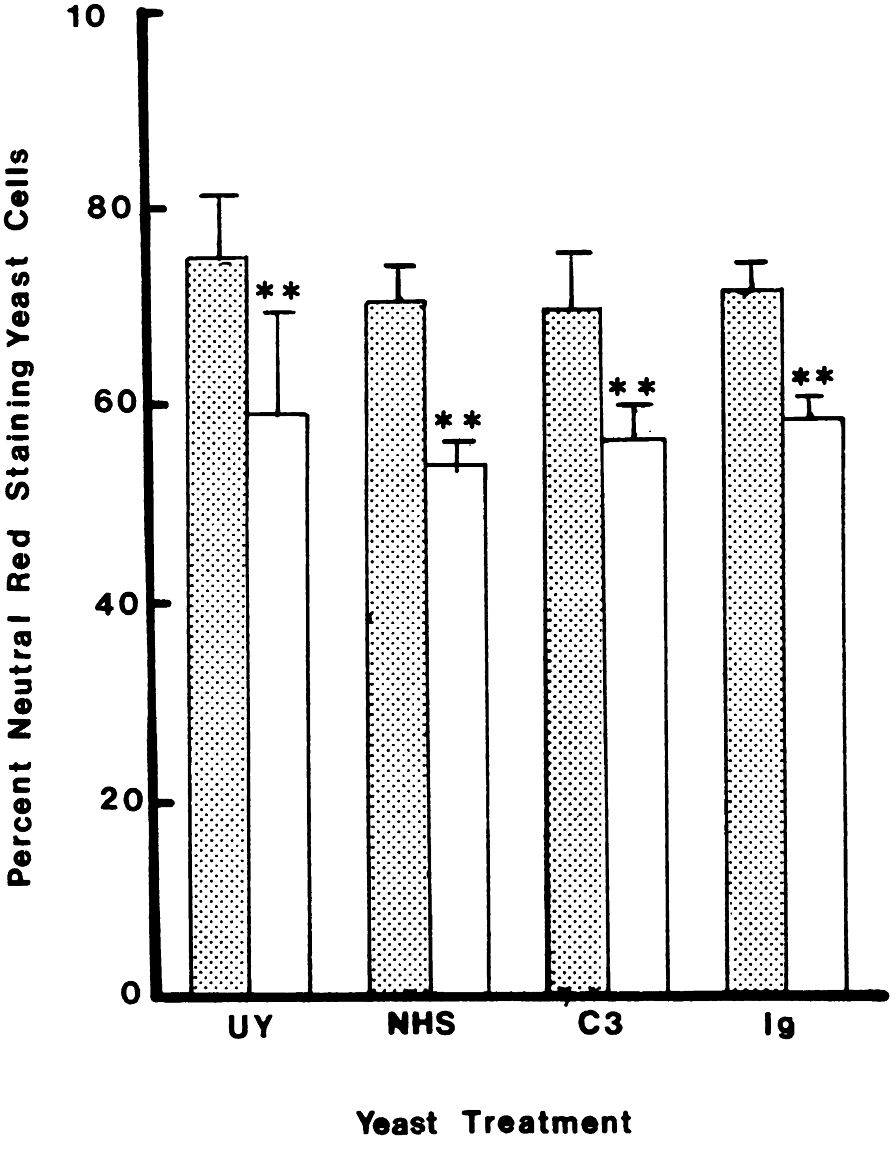

Number of Yeast Cells per Macrophage
6
5
4
3
2
1
0
5.0
2.5
1.25
Yeast Concentration [x 10^6]
Ig Opsonized C. albicans

Figure 14. The phagocytic capacity (number of yeast cells/macrophage) of Con A elicited macrophages from female mice treated with 1 mg/ml of mannan prior to the addition of different concentrations of *C. albicans* opsonized with complement. The shaded bars represent the control values and the open bars represent the mannan treatment values. Two * indicate a P>0.01. Each bar represents eight experiments.



C3 Opsonized C. albicans

Figure 15. The candidacidal activity of Con A elicited macrophages from female mice towards 5.5×10^6 C. albicans opsonized by different means. The shaded bars represent the control values and open bars represent the mannan treatment values. Two * indicates a $P>0.01$. Each bar represents eight experiments. UY = unopsonized yeast cells, NHS = normal human serum opsonized yeast cells, C3 = yeast cells opsonized with complement, Ig = yeast cells opsonized with immunoglobulins.


10
80
60
40
20
0
Percent Neutral Red Staining Yeast Cells
**
**
**
**
UY
NHS
C3
Ig
Yeast Treatment

albicans was opsonized with normal human serum at 75% of the control (P>0.01). The inhibitory effect of mannan of the candidacidal activity of macrophages towards C. albicans opsonized with complement or immunoglobulins or unopsonized C. albicans was 80%, 81% and 79% of the control value, respectively (P>0.01 for each opsonization).

Mannan had an inhibitory effect on the superoxide present in macrophages which had ingested C. albicans that were opsonized by different means (Figure 16). The pattern of inhibition of the reduction of superoxide did not follow the same pattern as seen in the reduction of the candidacidal activity of the macrophages (Figure 15). The least amount of reduction of superoxide was seen in macrophages which had ingest C. albicans opsonized with normal human serum at 73% of the control (P>0.01) and the most reduction of superoxide was seen with macrophages which had ingested C. albicans opsonized with immunoglobulins at 43% of the control (P>0.05). Unopsonized and complement opsonized C. albicans showed an intermediate reduction of superoxide at 55% of the control (P>0.05) and 53% of the control (P>0.01), respectively.

The effects of mannan on the phagolysosome fusion of macrophages was examined using acridine orange and C. albicans opsonized with normal human serum (Figure 17). The macrophages were incubated with C. albicans so that differences in phagocytosis would not influence the assay because mannan did not have an effect on the phagocytic capacity of macrophages. The phagocytic capacity of the control macrophages and mannan treated macrophages was 3.81 and 3.72 yeast cells per macrophage, respectively. There was no statistical difference between these two values. Mannan was determined to have no effect on the phagolysosome

Figure 16. The percent Con A elicited macrophages from female mice reducing NBT after addition of 5.5 x 10^6 C. albicans opsonized by different means. The shaded bars represent the control values and the open bars represent the mannan treatment values. One * indicates a P>0.05 and two * indicates a P>0.01. Each bar represents at least five experiments. UY = unopsonized yeast cells, NHS = normal human serum opsonized yeast cells, C3 = yeast cells opsonized with complement, Ig = yeast cells opsonized with immunoglobulins.

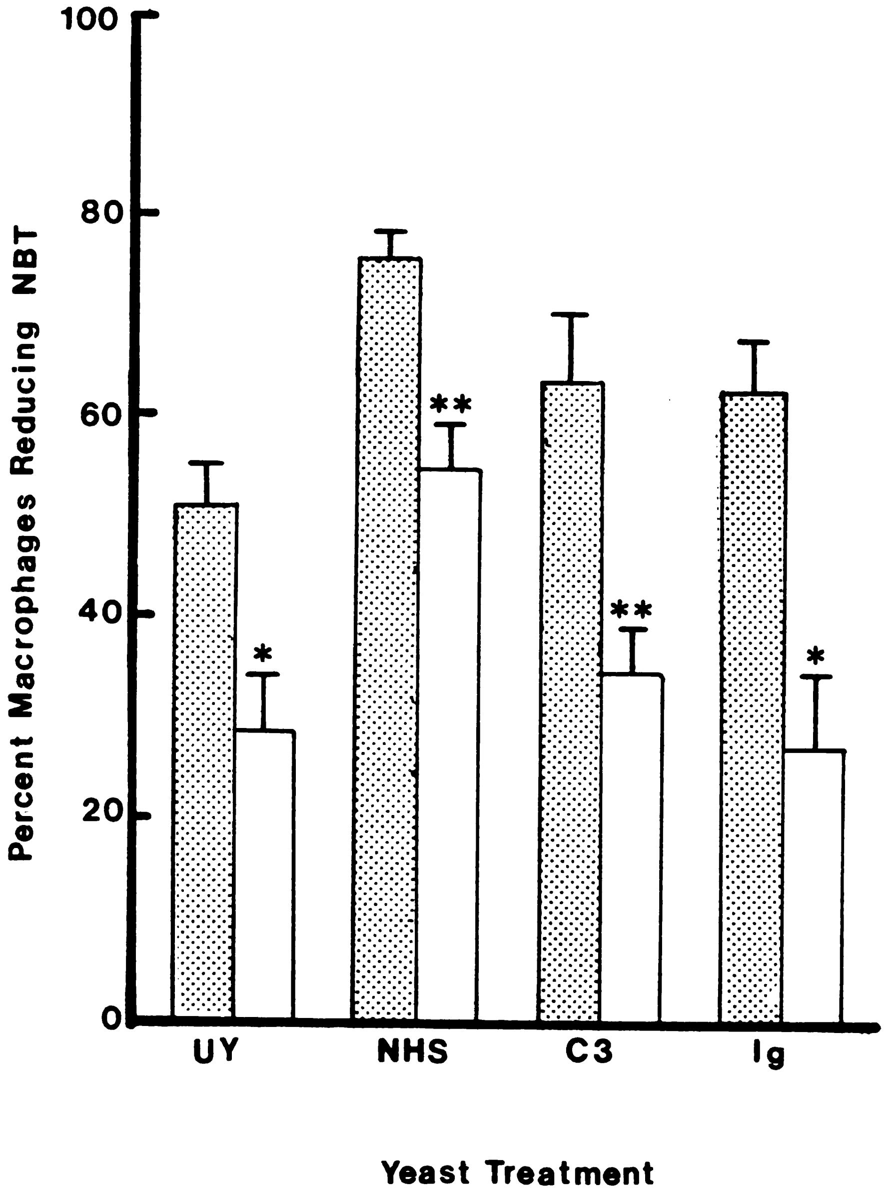
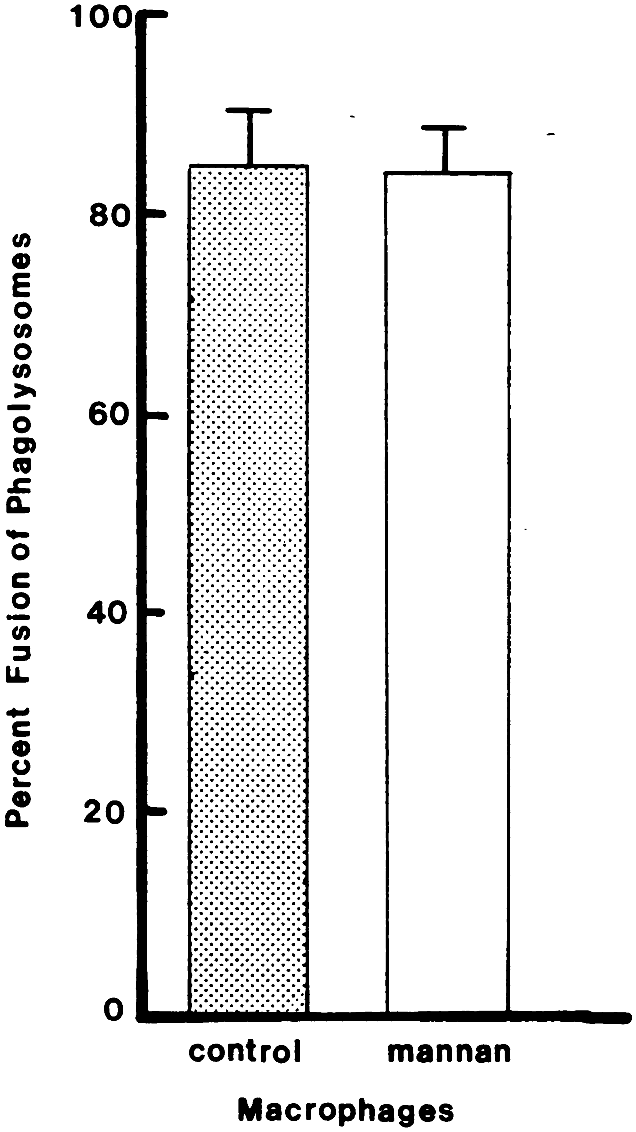

100
80
60
40
20
0
Percent Macrophages Reducing NBT
*
**
**
*
UY
NHS
C3
Ig
Yeast Treatment

Figure 17. Percent fusion of phagolysosomes of Con A elicited macrophages from female mice after the addition of 4.0 x 10^6 normal human serum opsonized C. albicans. The shaded bar is the control value and the open bar is the mannan treatment. Each bar represents five experiments.


100
80
60
40
20
0
Percent Fusion of Phagolysosomes
control
mannan
Macrophages

fusion using the acridine orange technique. The percent fusion of phagosomes and lysosomes was 85.5% for the control macrophages and 84.1% for the mannan treated macrophages.

DISSCUSION

The use of neutral red as a candidacidal assay is described in this study. Neutral red was chosen for the candidacidal assay for three reasons. First, neutral red accumulates in the lysosomes of macrophages, as well as in other cell types, such as fibroblasts, neutrophils, mast cells, and eosinophils (Bulychev _et_ _al_., 1978; Cohn and Wiener, 1963; Hammond _et_ _al_., 1980; Ogawa, 1961). Secondly, neutral red exhibits low toxicity towards macrophages (Hammond _et_ _al_., 1980). Lastly, neutral red has been used to determine the viability of yeast cells (Mills, 1941). The staining pattern is the same as seen by Mills (1941) where the nonviable yeast cells are uniformly stained red and only the vacuole of viable yeast cells stained red (Figures 1-4). A second staining pattern for nonviable yeast cells is described where the yeast cells are uniformly stained red except for a circular central structure which is not intensely red (Figure 3).

The use of neutral red to determine the candidacidal activity of macrophages is comparable to other methods that depend on the differential staining of yeast cells (Figure 5). The other methods are acridine orange (Pantazis and Kniker, 1979) and methylene blue (Lehrer and Cline, 1969; Schmid and Brune, 1974). Methylene blue is dependent on the metabolic state of the yeast cell since metabolically inactive yeast cells stain a light blue similar to dead yeast cells with no RNA (Lehrer, 1981). Acridine orange is a rapid and simple method to deter-

mine the microbicidal activity of macrophages (Pantazis and Kniker, 1979) but it requires an UV microscope while both methylene blue and neutral red require only a light microscope. Also, acridine orange is a mutagen as shown by the Ames assay (McCann _et_ _al_., 1975). The lack of germ tube production has been used to measure the candidacidal activity of phagocytic cells and in this study, neutral red was demonstrated to be comparable to this technique (Table 1).

These studies were designed to examine the influence of soluble _S. cerevisiae_ mannan on the macrophage's ability to ingest and kill selectively opsonized _C. albicans_ yeast cells and on the intermediate events of phagolysosome fusion and superoxide generation. One of the first method(s) by which mannan may affect the macrophage is toxicity. It was found in this study that mannan was not toxic to macrophages in concentrations up to 2 mg/ml nor was it toxic at 1 mg/ml when incubated with macrophages for up to 1 hour. This is in agreement with the observations by Stahl and Gordon (1982) who reported that 2 mg/ml of mannan were not toxic to macrophages or their hybrids even after 48 hours of culture.

There are three receptors on the surface of macrophages that have been implicated in host defense against _C. albicans_. They are the mannosyl/fucosyl glycoprotein receptor (Warr, 1980), the Fc receptor and the C3 receptors (Ferrante and Thong, 1979; Morrison and Culter, 1981; Pereira and Hosking, 1984; Schmid and Brune, 1974). The present study demonstrates that Con A elicited mouse peritoneal macrophages, like mouse alveolar macrophages (Warr, 1980), are capable of phagocytizing unopsonized Candida yeast cells. Inhibition of phagocytosis of unopsonized C. albicans, whether measured by phagocytic activity (Figures 7 and 9) or by phagocytic capacity (Figures 6, 8, and 10) by macrophages is depressed in the presence of mannan. Mannan may accomplish this depression of phagocytosis in two ways. The first method being the physical blocking of the receptor so that the unopsonized yeast cell cannot bind to the receptor. The second method is that mannan causes the internalization of the mannosyl/fucosyl glycoprotein receptor which perphaps is the most likely method. Sung et al. (1985) cite unpublished observations that S. cerevisiae mannan increases the fluid phase pinocytosis of thioglycollate elicited macrophages which result may internalation of this receptor.

Mannan was found not to interfere with the phagocytosis of normal human serum opsonized C. albicans by macrophages (Figures 9 and 12). This is consistant with the findings of Wright et al. (1981) that mannan not does interfere with the phagocytosis of serum opsonized zymosan. It is unclear whether or not the opsonzied zymosan used by Wright et al. (1981) was opsonized with both immunoglobulins and complement. It is likely that the zymosan was opsonized with immunoglobulins and complement based on the observation of Roos et al. (1981) who found immunoglobulins to zymosan in pooled normal human serum.

Collectively, there appeared to be no decrease in the phagocytosis of normal human serum opsonized C. albicans by macrophages in the presence of mannan. This does not mean that either the Fc or C3 receptors were not affected or that the one receptor is compensating for the other. When immunoglobulin or complement opsonized C. albicans cells were incubated with macrophages that had been treated with mannan a decrease in the phagocytic activity was seen (Figure 9). The difference between the control and mannan treated macrophages was small but statistically significant. Further studies using decreasing concentrations of selectively opsonized C. albicans were undertaken to determine if this was a real event (Figures 10 and 11). The results of these experiments with the Fc receptor were in contrast to results obtained by Sung et al. (1983) who found no difference in phagocytosis of IgG coated erythrocytes by macrophages plated onto S. cerevisiae mannan coated coverslips as compared to control macrophages. Further studies did not confirm the earlier results for the differences in the phagocytic activity of the control versus the mannan treated macrophages the decreasing concentrations of C. albicans. In neither case (Figures 12 and 13) was a decrease of the phagocytic capcaity of the macrophages seen in the presence of mannan. In a later study, Sung et al. (1985) found that Kloeckera brevis mannan was capable of causing a decrease in the phagocytosis of IgG coated erythrocytes by macrophages. They suggested that K. brevis mannan has a much higher affinity for the mannosyl/fucosyl glycoprotein receptor than does S. cerevisiae mannan and that the K. brevis mannan caused the internalization of the mannosyl/fucosyl glycoprotein receptor and the co-internalization of the Fc receptor. The results of Sung et al. (1985) suggest that these experiments be repeated using C. albicans mannan as it may have a higher affinity for the mannosyl/fucosyl glycoprotein receptor than does S. cerevisiae mannan.

The decrease in the phagocytic activity (Figure 9) of macrophages phagocytizing C3 opsonized *C. albicans* in the presence of mannan was very small as compared to the control macrophages. Additional studies using decreasing concentrations of C3 opsonized *C. albicans* demonstrated that mannan can interfere with the phagocytic activity of macrophages to a very large degree (Figure 11) and that this interference can be extended to the phagocytic capacity (Figure 14) of macrophages where an interference was not seen before (Figure 12). These results are in contradiction to those obtained by Sung *et al*. (1983) who demonstrated no difference in the phagocytosis of C3 coated erythrocytes by thioglycollate elicited peritoneal macrophages plated on mannan coated coverslips as compared to the control macrophages. The most likely explanation for this difference in results is that two different C3 receptors were being examined. Macrophages have several different C3 receptors on their surface (Fearon and Wong, 1983). In neither study, this one or Sung *et al*. (1983), was the type of C3 split product deposited on the particle surface identified. It may be that Sung *et al*. (1983) were examining the CR1 receptor which has its highest affinity for C3b and the present study examined the CR3 receptor which has its highest affinity for iC3b. The reason that CR3 receptor is suspected of being inhibited in this study is based on the results of Ross *et al*. (1985) who demonstrated that the CR3 receptor is composed of two functional sites. One site binds iC3b and the other is a lectin-like site that binds unopsonized zymosan and *S. cerevisiae*. The implications of this complement receptor having two binding sites is intriguing. Zymosan and yeast cells are capable of activating complement (Smith *et al*., 1982). It is possible that both sites on the CR3 receptor function in the binding and ingestion of iC3b coated yeast cells so that blockage of the lectin-like site would inhibit ingestion. This is suggested by the results of Ross et al. (1985) where they demonstrated that mannan (20 mg/ml) did not interfere with the binding of iC3b coated erythrocytes to neutrophils. However, they did test the mannan against the lectin-like site which is the site that is involve in the ingestion of particles while the iC3b is important in binding. Mannan may function to promote the infection by inhibiting the ingestion of the yeast cells by phagocytic cells even though the yeast cells are bound to the phagocytic cells by the iC3b site of the CR3 receptor. The questions of whether or not this lectin-like component of the CR3 receptor is different from the mannosyl/fucosyl glycoprotein receptor or if it is able to bind and promote the uptake of mannan have yet to be answered. There remains a great deal of work necessary to clarify the interaction of mannan, especially from C. albicans, and the Fc and complement receptors.

Con A elicited peritoneal macrophages are capable of a high degree of intracellular killing of C. albicans yeast cells regardless of the type of opsonization of the yeast cells (Figure 15). This indicates that these macrophages have only one level of candidacidal activity which is not influenced by the opsonization of the yeast cells. These results are in conflict with the results presented by other investigators (Pereira and Hosking, 1984; Schmid and Brune, 1974; Yamamura and Valdimarsson, 1977) who reported that complement was necessary for maximum killing of C. albicans. The difference between their results and these results may be the different phagocytic cell types used, neutrophils versus macrophages, respectively. Mannan reduced the intracellular killing ability of macrophages to a similar extent regardless of the opsonization of the yeast cells. Thus mannan is an inhibitor of macrophage as well as neutrophil candidacidal activity.

The mechanisms by which macrophages and monocytes kill *C. albicans* are not well understood. Lehrer (1975) described both a myeloperoxidase dependent and independent mechanisms of candidacidal activity for monocytes. Patterson-Delafield *et al.* (1980) described cationic proteins from alveolar macrophages as having candidacidal activity. Sasada and Johnson (1980) has suggested that superoxide is a major mechansim by which macrophages kill *C. albicans*. Mannan has been shown to interfere with myeloperoxidase both in neutrophils and in a cell free system by Wright *et al.*, (1983). However, mature macrophages lack myeloperoxidase (Murray, 1984) so another mechanism(s) must come into play. The presence of superoxide in macrophages exposed to mannan was measured by nitroblue tetrazolium blue reduction (Figure 16). Mannan was shown to decrease the amount of superoxide in macrophages regardless of the opsonization of the yeast cells.The decrease in superoxide of the macrophages could be accomplished by two means: a decrease in the oxygen uptake of the macrophages or by the dismutation of superoxide into hydrogen peroxide. Nelson *et al.* (1984) demonstrated that mannan has a superoxide dismutase like activity.

Another level that mannan may interfere with the candidacidal activity of macrophages is by inhibiting phagolysosome fusion. There were several reasons for pursuing this mechanism. First, D'Arcy Hart (1981) demonstrated that phagolysosome fusion is necessary for the killing of *S. cerevisiae* by macrophages. Second, the presence of cationic proteins as a potent microbicidal substance and in the absence of oxidative metabolism may provide a secondary line of defense for the host. Finally, *in vitro* fusion of neutrophil phagocytic vesicles and lysosomes could be inhibited by the presence of mannose (Amano and Mizuno, 1981). Since it is possible that mannan may be internalized by the mannosyl/fucosyl glycoprotein receptor and that the intracellular fate of mannose terminal glycoproteins is not known it is possible that these glycoproteins may be degraded and released into the cell or in some way interfere with phagolysosome fusion. The results obtained in this study (Figure 17) indicate that mannan does not interfere with the phagolysosome fusion. This indicates that in Con A peritoneal macrophages that mannan interferes with superoxide and that lysosomal contents plays little or no role in the intracellular killing of *C. albicans*.

The results of this study indiate that mannan is capbale of affecting the ability of macrophages to phagocytize *C. albicans* via the mannosyl/fucosyl glycoprotein receptor and the complement receptor, probably the CR3, which may be an important pathogenic mechanism in patients with low levels of immunoglobulins. Also, mannan might influence pathogenicity because it diminishes the amount of superoxide present in macrophages thus reducing the ability of macrophages to kill the ingested yeast cells. It is unlikely that phagocytic cells are exposed to such high concentrations of mannan in the host with the exception of sites of infection or in patients with chronic mucocutaneous candidiasis where serum mannan level of 2.7 mg/ml have been reported (Wright _et_ _al_., 1981). It is important to note that mannan does not inhibit the phagolysosome fusion within macrophages thus inhibition of this fusion is not an aid in establishing an infection in candidiasis. An avenue of investigation that may help in the understanding of how mannan may be an aid in establishing an infection is to examine the effects of long term exposure of low doses of mannan on lymphocytes and phagocytic cells, especially macrophages.

LITERATURE CITED

Amano, F. and D. Mizuno. 1981. Carbohydrates may play an important role in the interaction between phagocytic vesicles and lysosomes. J. Biochem. 89: 1149-1154

Arai, T., Y. Mikami and K. Yokoyama. 1977. Phagocytosis of Candida albicans by rabbit alveolar macrophages and guinea pigs neutrophils. Sabouraudia 15: 171-189

Baccarini, M., A. Vecchiarelli, A. Cassone and F. Bistoni. 1985. Killing of yeast, germ tube and mycelial forms of Candida albicans by murine effectors as measured by a radiolabel release microassay. J. Gen. Microbiol 131: 505-513

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

Bartnicki-Garcia, S. 1968. Cell wall chemistry, morphogenesis, and taxonomy of fungi. Ann. Rev. Microbiol. 22: 87-108

Berg, R.D. and D. Savage. 1972. Immunolgical responses and microorganisms indigenous to the gastrointestinal tract. Amer. J. Clin. Nutr. 25: 1364-1371

Bishop, C.T., F. Blank and P.E. Gardner. 1960. The cell wall polysaccharides of Candida albicans: glucan, mannan, and chitin. Canad. J. Chem. 38: 869-881

Bodey, G.P. and V. Fainstein, eds. 1985. Candidiasis. Raven Press, New York 281 pp.

Bulychev, A., A. Trouet and P. Tulkens. 1978. Uptake and intracellular distribution of neutral red in cultured fibroblasts. Exp. Cell Res. 115: 343-355

Burgaleta, C., M.C. Territo, S.G. Quan and D.W. Golde. 1978. Glucan-activated macrophages: functional characteristics and surface morphology. J. Reticuloendothel. Soc. 23: 195-204

Burges, G., H.P. Holley and G. Virella. 1983. Circulating immune complexes in patients with Candida albicans infections. Clin. Exp. Immunol. 53: 165-174

Cassone, A., D. Kerridge and E.F. Gale. 1979. Ultrastructural changes in the cell wall of *Candida albicans* following cessation of growth and their possible relationship to the development of polyene resistance. J. Gen. Microbiol. 110: 339-349

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

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

Chew, W.H. and T.L. Theus. 1967. *Candida* precipitins. J. Immunol, 98: 220-224

Cline, M.J. 1981. Leukocyte Function. Churchill, Livingstone. 149 pp.

Cohn, Z.A. and E. Wiener. 1961. The particulate hydrolases of macrophages. I. Comparative enyzmology, isolation and properties. J. Exp. Med. 118: 991-1008

Cockayne, A. and F.C. Odds. 1984. Interactions of *Candida albicans* yeast cell, germ tubes and hyphae with human polymorphonuclear leukocytes *in vitro*. J. Gen. Microbiol. 130: 465-471

Culter, J.E. and A.H. Poor. 1981. Effect of mouse phagocytes on *Candida albicans* in *in vivo* chambers. Infect. Immun. 31: 1110-1116

Danley, D.L. and A.E. Hilger. 1981. Stimulation of oxidative metabolism in murine polymorphonuclear leukocyte by unopsonized fungal cells: evidence for a mannose specific mechanism. J. Immunol. 127: 551-556

Danley, D.L., A.E. Hilger and C.A. Winkel. 1983. Generation of hydrogen peroxide by *Candida albicans* and influence on murine polymophonuclear leukocyte activity. Infect. Immun. 40: 97-102

D'Arcy Hart, P. 1981. Macrophage antimicrobial activity: evidence for participation by lysosomes in the killing of *Saccharomyces cerevisiae* by normal resident macrophages. Infect. Immun. 31: 828-830

Densen, P. and G.L. Mandell. 1980. Phagocytic strategy vs. microbial tactics. Rev. Infect. Dis. 2: 817-838

Diamond, R.D. 1981. Antifungal properties of granulocytes. Microbiology 1981. American Society for Microbiology, Washington, D.C. pp. 184-187

Diamond, R.D., R.A. Clark and C.C. Haudenschild. 1980a. Damage to

Candida albicans hyphae and pseudohyphae by the myeloperoxidase system and oxidative products of the neutrophil metabolism in vitro. J. Clin. Invest. 66: 908-917

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

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

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

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

DiLuzio, N.R. 1975. Pharmacology of the RES--accent on glucan. in Reichard, S.M., M.R. Escobar and H. Friedman, eds. The RES in health and disease. Advances in Experimental Medicine and Biology. vol. 73A Plenum Press, New York pp. 412-421

Djaczenko, W. and A. Cassone. 1971. Visualization of new ultrastructural components in the cell wall of Candida albicans with fixatives containing TAPO. J. Cell Biol. 52: 186-190

Edwards, J.E., R.I. Lehrer, E.R. Stiehm, T.J. Fischer and L.S. Young. 1978. Severe candidal infections. Clinical prespective, immune defense mechanisms, and current concepts of therapy. Ann. Internal Med. 89: 91-106

Elinov, N.P. 1984. Carbohydrases and their role in the biology of pathogenic fungi. Zbl. Bakt. Hyg. A 257: 246-256

Elorza, M.V., H.Rico, D.Gozalbo and R. Sentandreu. 1983. Cell wall composition and protoplast regeneration in Candida albicans. Antonie van Leeuwenhoek 49: 457-469

Eng, R.H.K., R. Drehmel, S.M. Smith and E.J.C. Goldstein. 1984. Saccharomyces cerevisiae infections in man. Sabouraudia 22: 403-407

Evans, Z.A. 1980. Tissue responses to the blastospores and hyphae of Candida albicans in the mouse. J. Med. Microbiol. 14: 307-319

Evans, Z.A. and D.N. Mardon. 1977. Organ localization in mice challenged with a typical Candida albicans strain and a pseudohyphal variant. Proc. Soc. Exp. Biol. Med155: 234-238

Evron, R. and J.A. Drewe. 1984. Demonstration of the polysaccharides in the cell wall of Candida albicans blastospores, using silver methenamine staining and a sequence of extraction procedures. Mycopathologia 84: 141-149

Fearon, D.T. and W.W. Wong. 1983. Complement ligand-receptor interactions that mediate biological responses. Ann. Rev. Immunol. 1: 243-271

Ferrante, A. and Y.H. Thong. 1979. Requirement of heat-liable opsonins for maximal phagocytosis of Candida albicans. Sabouraudia 17: 293-297

Fischer, A., J.J. Ballet, and C. Griscelli. 1978. Specific inhibition of in vitro Candida-induced lymphocyte proliferation by polysaccharide antigens present in the serum of patients with chronic mucocutaneous candidiasis. J. Clin. Invest. 62: 1005-1013

Fontenla de Petrino, S.E. and A. Sirena. 1984. Release of lysosomal enzymes in Candida albicans phagocytosis by rat peritoneal macrophages. Mycopathologia 84: 103-106

Foo, M.D. and A. Lee. 1972. Immunological reposne of mice to members of the autochthonous intestinal flora. Infect. Immun 6: 525-532

Gotschlich, E.C. 1983. Thoughts on the evolution of strategies used by bacteria for the evasion of host defenses. Rev. Infect. Dis. 5(suppl. 4): S778-S783

Gough, P.M., D.W. Warnock, M.D. Richardson, N.J. Mansell and J.M. King. 1984. IgA and IgG antibodies to Candida albicans in the genital tract secretions of women with or without vaginal candidosis. Sabouraudia 22: 265-271

Gresham, G.H. and C.H. White. 1961. Studies of the invasive, mycelial form of Candida albicans. Sabouraudia 1: 30-33

Hammond, M.E., J. Goodwin and H.F. Dvorak. 1980. Quantitative measurements of neutral red uptake and excretion by mammalian cells. J. Reticuloendothel. Soc. 27: 337-346

Hattori, M., K. Yoshiura, M. Negi and J. Ogawa. 1984. Keratinolytic proteinase produced by Candida albicans. Sabouraudia 22: 175-183

Hilger, A.E. and D.L. Danley. 1980. Alteration of polymorphonuclear leukocyte activity by viable Candida albicans. Infect. Immun. 27: 714-720

Hopfer, R.L. 1985. Mycology of Candida infections. in Bodey, G.P. and

V. Fainstein, eds. Candidiasis. Raven Press, New York pp. 1-12

Horwitz, M.A. 1982. Phagoyctosis of microorganisms. Rev. Infect. Dis. 4: 104-123

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

Hurley, R. 1980. The pathogenic Candida species and disease caused by Candidas in man. in F.A. Skinner, S.M. Passmore and R.R. Davenport, eds. Biology and Activities of Yeasts. Academic Press, New York pp. 231-248

Hurley, R. and V.C. Stanley. 1969. Cytopathic effects of pathogenic and nonpathogenic species of Candida on cultured mouse epithelial cells: relation to the growth and morphology of the fungus. J. Med Microbiol. 2: 63-74

Iannini, P.B., G.D. Arai and F.M. LaForce. 1975. Vascular clearance of blastospore and pseudomycelial phase of Candida albicans. Sabouraudia 15: 201-206

Kasckin, P.N. 1974. Some aspects of the candidosis problem. Mycopathol. Mycol. appl. 53: 173-181

Kielian, M.C. and Z.A. Cohn. 1980. Phagosome-lysosome fusion. Characterization of intracellular membrane fusion in mouse macrophages. J. Cell Biol. 85: 754-765

Klebanoff. S.J. 1968. Myeloperoxidase-halide-hydrogen peroxide antibacterial system. J. Bacteriol. 95: 2131-2138

LaForce, F.M., D.M. Mills, K. Iverson, R. Cousins and E.D. Everett. 1975. Inhibition of leukocyte candidacidal activity by serum from patients with disseminated candidiasis. J. Lab. Clin. Invest. 86: 657-666

Lehnmann, P.F. and E. Reiss. 1980. Comparison ELISA of serum anti-Candida albicans mannan IgG levels of a normal population and in diseased patients. Mycopathologia 70: 89-94

Lehrer, R.I. 1981. Ingestion and destruction of Candida albicans. in Adams, D.O., P.J. Edelson and H.S. Koren, eds. Methods for Studying Mononuclear Phagocytes. Academic Press, New York. pp. 693-708

Lehrer, R.I. 1975. The fungicidal mechanisms of human monocytes. I. Evidence for myeloperoxidase-linked and myeloperoxidase-independent candidacidal mechanisms. J. Clin. Invest. 55: 338-346

Lehrer, R.I. 1969. Antifungal effects of peroxidase systems. J.

Bacteriol. 99: 361-365

Lehrer, R.I. and M.J. Cline. 1969. Leukocyte myeloperoxidase deficiency and disseminated candidiasis: the role of myeloperoxidase in resistance to Candida infections. J. Clin. Invest. 48: 1478-1488

Lehrer, R.I. and J. Fleischmann. 1982. Antifungal defense by macrophages. Microbiology 1982. American Society for Microbiology, Washington D.C. pp. 385-387

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

Louria, D.B, R.G. Brayton and G. Finkel. 1963. Studies of the pathogenesis of experimental Candida albicans infection in mice. Sabouraudia 2: 271-283

McCann, J., E. Choi, E. Yamasaki and B.N. Ames. 1975. Detection of carcinogens as mutagens in the Salmonella/microsome test: assay of 300 chemicals. PNAS (USA) 72: 5135-5139

Macdonald, F. and F.C. Odds. 1983. Virulence for mice of a proteinase-secreting strain of Candida albicans and a proteinase-deficient mutant. J. Gen. Microbiol. 129: 431-438

Macdonald, F. and F.C. Odds. 1980. Inducible proteinase of Candida albicans in dagnostic serology and in the pathogenesis of systemic candidosis. J. Med. Microbiol. 13: 423-435

Mackowiak, P.A. 1984. Microbial lactency. Rev. Infect. Dis. 6: 649-668

Mardon, D.N., J.L. Gunn and E. Robinette, Jr. 1975. Variation in the lethal response in mice ot yeast-like and pseudohyphal forms of Candida albicans. Canad. J. Microbiol. 21: 1681-1687

Marrie, T.J. and J.W. Costerton. 1981. The ultrastructure of Candida albicans infections. Canad. J. Microbiol. 27: 1156-1164

Martin, M.V., G.T. Craig and D.J. Lamb. 1984. An investigation of the role of true hyphae producton in the pathogenesis of experimental oral candidiasis. Sabouraudia 22: 471-476

McGinnis, M.R., L. Ajello, E.S. Beneke, E. Drouhet, N.L. Goodman, C.J. Halde, L.D. Haley, J. Kane, G.A. Land, A.A. Padhye, D.H. Pincus, M.G. Rinaldi, A.L. Rogers, I.F. Salkin, W.A. Schell and I. Weitzman. 1984. Taxonomic and nomenclatural evaluation of the genera Candida and Torulopsis. J. Clin. Microbiol. 20: 813-814

Meister, H., B. Heymer, H. Schafer and O. Haferkamp. 1977. Role of

Candida albicans in granulomatous tissue reactions. I. In vitro degradation of C. albicans and immunospecificity of split products. J. Infect. Dis. 135: 224-234

Metzger, Z., J.T. Hoffeld and J.J. Oppenheim. 1980. Macrophage-mediated suppression. I. Evidence for participation of both hydrogen peroxide and prostaglandins in suppression of murine lymphocyte proliferation. J. Immunol. 124: 983-988

Mitchell, T.G. 1980. Opportunistic Mycosis. in Joklik, W.K., H.P. Willet and D.B. Amos, eds. Zinsser Microbiology, 17th edition. Appleton-Century-Crofts, New York. pp. 1408-1429

Mills, D.R. 1941. Differential staining of living and dead yeast. Food Res. 6: 361-371

Mishell, B.B., S.M. Shiigi, C. Henry, E.L. Chan, J. North, R. Gallily, M. Slomich, K. Miller, J. Marbrook, D. Parks and A.H. Good. 1980. Preparation of mouse cell suspension. in Mishell, B.B. and S.M. Shiigi, eds. Selected Methods in Cellular Immunology. W.H. Freeman and Company, San Francisco pp. 3-27

Montes, L.F. and W.H. Wilborn. 1968. Ultrastructural features of host-parasite relationships in oral candidiasis. J. Bacteriol. 96: 1349-1356

Morrison, R.P. and J.E. Culter. 1981. In vitro studies of the interaction of murine phagocytic cells with Candida albicans. J. Reticuloendothel. Soc. 29: 23-34

Mundkur, B. 1960. Electron microscopical studies of frozen dried yeast. Localization of polysaccharides. Exp. Cell Res. 20: 28-42

Murray, H.W. 1984. Macrophage activation: enhanced oxidative and antiprotozoal activity. in Adams, D.O. and M.G. Hannan, Jr., eds. Comtemporary Topics in Immunolobiology. Vol. 13 Macrophage Activation. Plenum Press, New York pp. 97-116

Murray, H.W. 1982. Macrophage activity against intracellular protozoa. Microbiology 1982. American Society for Microbiology Washington, D.C. pp. 382-384

Nelson, R.D., M.J. Herron, R.T. McCormack and R.C. Gehrz. 1984. Two mechanisms of inhibition of human lymphocyte proliferation by soluble yeast mannan polysaccharide. Infect. Immun. 43: 1041-1046

Oda, L.M., X.D. Kubelka, C.S. Alviano and L.R. Travassos. 1983. Ingestion of yeast forms of Sporothrix schenckii by mouse peritoneal macrophages. Infect. Immun. 39: 497-504

Odds, F.C. 1979. Candida and Candidosis. Leicester University Press, Great Britain. 382 pp.

Ofek, I. and F.J. Silverblatt. 1982. Bacterial surface structures involved in adhesion to phagocytic and epithelial cells. Microbiology 1982. American Society for Microbiology, Washington, D.C. pp. 296-300

Ogawa, K., N. Mizuno and M. Okamoto. 1961. Lysosomes in cultured cells. J. Histochem. Cytochem. 9: 202

Oppenheim, J.J. and B. Schechter. 1974. Lymphocyte Transformation. in N.R. Rose and H. Friedman, eds. Manual of Clinical Immunology. American Society for Microbiology, Washington, D.C. pp. 81-94

Ozato, K. and I. Uesaka. 1974. The role of macrophages in _Candida albicans_ infection _in vitro_. Jap. J. Microbiol. 18: 29-35

Pantzis, C.B. and W.T. Kniker. 1979. Assessment of blood leukocytes microbial killing by using a new fluorochrome microassay. J. Reticuloendothel. Soc. 26: 155-170

Patterson-Delafield, J., R.J. Martinez and R.I. Lehrer. 1980. Microbicidal cationic proteins in rabbit alveolar macrophages: a potential host defense mechanism. Infect. Immun. 30: 180-192

Pereira, H.A. and C.S. Hosking. 1984. The role of complement and antibody in the opsonization and intracellular killing of _Candida albicans_. Clin. exp. Immunol. 57: 307-314

Piccolella, E., G. Lombardi and R. Morelli. 1981a. Generation of suppressor cells in the response of human lymphocytes to a polysaccharide from _Candida albicans_. J. Immunol. 126: 2151-2155

Piccolella, E., G. Lombardi and R. Morelli. 1981b. Mitogenic response of human peripheral blood lymphocytes to a purified _C. albicans_ polysaccharide fraction: lack of helper activities is responsible for the _in vitro_ unresponsiveness to a second antigenic challenge. J. Immunol. 126: 2156-2160

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

Price, M.D. and R.A. Cawson. 1975. Phospholipase activity in _Candida albicans_. Sabouraudia 15: 179-186

Pugh, D. and R.A. Cawson. 1977. The cytochemical localization of phospholipase in _Candida albicans_ infecting the chick chorio-allantoic membrane. Sabouraudia 15: 29-36

Reiss, E., L. Stockman, F.J. Kuykendall and S.J. Smith. 1981. Dissociation of mannan-antibody complexes and detection of *Candida albicans* by enzyme immunoassay variations. Clin. Chem. 28: 306-310

Remington, J.S. 1982. Role of the activated macrophage in resistance to infection. Microbiology 1982. American Society for Microbiology, Washington, D.C. pp. 374-377

Rippon, J.W. 1982. Medical Mycology. The Pathogenic Fungi and The Pathogenic Actinomycetes. W.B. Sunders Company, Philadelphia 842 pp.

Rodman, J.S., P. Schlesinger and P. Stahl. 1978. Rat plasma clearance of horseradish peroxidase and yeast invertase is mediated by specific recognition. FEBS Letters 85: 345-348

Rogers, T.J. and E. Balish. 1980. Immunity to *Candida albicans*. Microbiol. Rev. 44: 660-682

Rogers, T. and E. Balish. 1978. Experimental *Candida albicans* infection in conventional mice and germfree rats. Infect. Immun. 14: 37-38

Roos, D., A.A.M. Bot, M.L.J. van Schaik, M. deBoer and M.R. Daha. 1981. Interaction between human neutrophils and zymosan particles: the role of opsonins and divalent cations. J. Immunol. 126: 433-440

Ross, G.D., J.A. Cain and P.J. Lachmann. 1985. Membrane complement receptor type three (CR3) has lectin-like properties analogous to bovine conglutinin and functions as a receptor for zymosan and rabbit erythrocytes as well as a receptor iC3b. J. Immunol. 134: 3307-3315

Ruchel, R. 1984. A variety of *Candida* proteinases and their possible targets of proteolytic attack in the host. Zbl. Bakt. Hyg. A 257: 266-274

Ruchel, R., R. Tegeler and M. Trost. 1982. A comparison of secretory proteinases from different strains of *Candida albicans*. Sabouraudia 20: 233-244

Saltarelli, C.G., K.A. Gentile and S.C. Mancusco. 1975. Lethality of *Candida* strains as influenced by the host. Canad. J. Microbiol. 21: 648-654

Samaranayake, L.P., J.M. Raeside and T.w. Macfarlane. 1984. Factors affecting the phospholipase activity of *Candida* species *in vitro*. Sabouraudia 22: 201-207

San-Blas, G. 1982. The cell wall of fungal human pathogens: Its possible role in host-parasite relationships. A review. Mycopathologia 79: 159-184

Sandin, R.L., A.L. Rogers, R.J. Patterson and E.S. Beneke. 1982. Evidence for mannose-mediated adherence of Candida albicans to human buccal cells in vitro. Infect. Immun. 35: 79-85

Sasada, M. and R.B. Johnson, Jr. 1980. Macrophage microbicidal activity. Correlation between phagocytosis-associated oxidative metabolism and killing of Candida by macrophages. J. Exp. Med. 152: 85-98

Schmid, L. and K. Brune. 1974. Assessment of phagocytic and antimicrobial activity of human granulocytes. Infect. Immun. 10: 1120-1126

Schuit, K.E. 1979. Phagocytosis and intracellular killing of pathogenic yeasts by human monocytes and neutrophils. Infect. Immun. 24: 932-938

Schwab, J.H. 1975. Suppression of the immune response by micro-organisms. Bacteriol. Rev. 39: 121-143

Segal, E., R.A. Berg, P.A. Pizzo and J.E. Bennett. 1979. Detection of Candida antigen in sera of patients with candidiasis by an enzyme-linked immunosorbent assay-inhibition technique. J. Clin. Microbiol. 10: 116-118

Segal, E., H. Sandovsky-Loscia and N. Vardinon. 1980. Suppressive action of cytoplasmic and metabolite extracts of Candida albicans on the immune response of guinea pigs. Mycopathologia 72: 121-130

Simpson, D.W., R. Roth and L.D. Loose. 1979. A rapid, inexpensive and easily quantified assay for phagocytosis and microbicidal activity of macrophages and neutrophils. J. Immunol. Methods 29: 221-226

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

Smith, C.B. 1985. Candidiasis: Pathogenesis, Host Resistance, and Predisposing Factors. in Bodey, G.P. and V. Fainstein, eds. Candidiasis. Raven Press, New York. pp. 53-70

Smith, M.C., J. Pensky and G.B. Naff. 1982. Inhibition of zymosan-induced alternative complement pathway activation by Concanavalin A. Infect. Immun. 38: 1279-1284

Spitler, L.E. 1976. Delayed Hypersensitivity Skin Testing. in N.R. Rose and J.Friedman, eds. Manual of Clincial Immunology. American Society for Microbiology, Washington, D.C. pp. 53-63

Spitznagel, J.K. 1983. Microbial interactions with neutrophils. Rev. Infect. Dis. 5(suppl. 4): S806-S822

Stahl, P. and S. Gordon. 1982. Expression of a mannosyl-fucosyl receptor for endocytosis on cultured primary macrophages and their hybrids. J. Cell Biol. 93: 49-56

Stanley, V.C. and R. Hurley. 1969. The growth of Candida species in cultures of mouse peritoneal macrophages. J. Pathol. 97: 357-366

Sullivan, P.A., C.Y. Yin, C. Molloy and M.D. Templeton. 1983. An analysis of the metabolism and cell wall composition of Candida albicans during germ tube formation. Canad. J. Microbiol. 29: 1514-1525

Summers, D.F., A.P. Grollman and H.F. Hasenclever. 1964. Polysaccharide antigens of Candida cell wall. J. Immunol. 92: 491-499

Sung, S.S.J., R.S. Nelson and S.C. Silverstein. 1985. Mouse peritoneal macrophages plated on mannan- and horseradish peroxidase-coated substrates lose the ability to phagocytose by their Fc receptors. J. Immunol. 134: 3712-3717

Sung, S.S.J., R.S. Nelson and S.C. Silverstein. 1983. Yeast mannans inhibit binding and phagocytosis of zymosan by mouse peritoneal macrophages. J. Cell Biol. 96: 160-166

Suzuki, K., Y. Okawa, K. Hashimoto, S. Suzuki and M. Suzuki. 1984. Protecting effect of chitin and chitosan on experimentally induced murine candidiasis. Microbiol. Immunol. 28: 903-912

Thiery, J.P. 1967. Mise en evidence des polysaccharides sur coupes fines en microscopie electronique. J. Micro. (Paris) 6: 987-1018

Tronchin, G., D. Poulain and J. Biguet. 1979. Etudes cytochimiques et ultrastructurales de la paroi de Candida albicans. I. Localisation des mannanes par utilisation de Concanavaline A sur coupes ultrafines. Arch. Microbiol. 123: 245-249

Tronchin, G., D. Poulain, J. Herbaut and J. Biguet. 1981. Cytochemical and ultrastructural studies of Candida albicans. II. Evidence for a cell wall coat using Concanavalin A. J. Ultrastruct. Res. 75: 50-59

Uden, van N. and H.R. Buckley. 1970. Candida Berkhout, in Lodder, J., ed. The Yeasts--A Taxonomic Study 2nd edition. North-Holland, Amsterdam

Valdez, J.C., O.E. Meson, G.A. de Valdez and A. Sirena. 1984. Suppression of humoral response during the course of Candida albicans infection in mice. Mycopathologia 88: 61-68

Vardinon, N. and E. Segal. 1979. Suppressive action of Candida albicans on the immune response in mice. Exp. Cell Res. 47: 275-280

Vaughn, V. and E. Weinberg. 1978. Candida albicans dimorphism and virulence: role of copper. Mycopathologia 64: 39-42

Venezia, R.A. and R.C. Lachapelle. 1973. The use of ferritin-conjugated antibodies in the study of cell wall components of Candida albicans. Canad. J. Microbiol. 19: 1445-1448

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

Weiner, M.H. and M. Coats-Stephen. 1979. Immunodiagnosis of systemic candidiasis: mannan antigenemia detected by radioimmunoassay in experimental and human infections. J. Infect. Dis. 140: 989-993

Weiner, M.H. and W.J. Yount. 1976. Mannan antigenemia in the diagnosis of invasive Candida infection. J. Clin. Invest. 58: 1045-1053

Whittle, C.H. and G.A. Gresham. 1960. Candida in vitro and in vivo. Mycopathol. Mycol. appl. 12: 207-215

Williams, D., J.A. Cook, E.O. Hoffman and N.R. DiLuzio. 1978. Protectiver effect of glucan in experimentally induced candidiasis. J. Reticuloendothel. Soc. 23: 479-490

Wright, C.D., J.U. Bowie, G.R. Gray and R.D. Nelson. 1983. Candidacidal activity of myeloperoxidase: mechanism of inhibitory influence of soluble cell wall mannnan. Infect. Immun. 42: 76-80

Wright, C.D., J.U. Bowie and R.D. Nelson. 1984. Influence of yeast mannan on release of myeloperoxidase by human neutrophils: determination of structural features of mannan required for formation of myeloperoxidase-mannan-neutrophil complexes. Infect. Immun. 43: 467-471

Wright, C.D., M.J. Herron, G.R. Gray, B. Holmes and R.D. Nelson. 1981. Influence of yeast mannan on human neutrophil functions: inhibition of release of myeloperoxidase related to carbohydrate-binding property of the enzyme. Infect. Immun. 32: 731-738

Yamamura, M. and H. Valdimarson. 1977. Participation of C3 in intracellular killing of Candida albicans. Scand. J. Immunol. 6: 591-594

Yarrow, D. and S.A. Meyer. 1978. Proposal for admendment of the diagnosis of the genus Candida Berkhout nom. cons.. Intl. J. Sys. Bacteriol. 28: 611-615

Young. G. 1958. The process of invasion and the presistance of Candida albicans injected intraperitoneally into mice. J. Infect. Dis. 102: 114-120