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IN THE PERFUSED MOUSE LIVER

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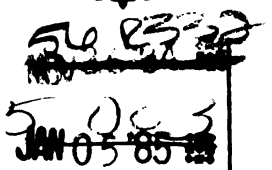

CLEARANCE AND KILLING OF *CANDIDA ALBICANS* IN THE PERFUSED MOUSE LIVER

by

Lee Robert Schwocho

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ABSTRACT

CLEARANCE AND KILLING OF CANDIDA ALBICANS
IN THE PERFUSED MOUSE LIVER

By

Lee Robert Schwocho

Hepatic interactions of two C. albicans isolates with perfused mouse livers were characterized and compared in normal and glucan-treated mice. Normal livers, in the absence of serum, trapped greater than 90% of both isolates and killed greater than 20% of isolate I and approximately 15% of isolate II. Silica treatment abolished killing and decreased trapping suggesting that candidicidal activity of the liver is mediated by Kupffer cells. Phenylbutazone had no effect. Immune serum, but not normal serum, enhanced trapping and killing of isolate I but not isolate II. Liver hypertrophy was evident in mice treated with glucan, but no enhanced candidicidal activity was observed in the absence of humoral factors. Specific immune serum and normal calf serum increased killing of both isolates in glucan stimulated livers suggesting a requirement for serum opsonin in facilitating glucan enhanced killing. Specific immune serum potentiated the greated increase in killing. Glucan treatment in conjunction with immune serum increased killing of isolate I to approximately 40% and isolate II to greater than 33%. D-mannose, but not D-glucose or D-mannitol, impaired trapping of both isolates in livers of normal mice. Together, the data suggest that

hepatic trapping of Candida albicans involves phagocytic events as well as interactions of the yeasts with surface receptors on sinusoidal cells. Cumulatively, the results support the role for the liver in restricting hematogenous dissemination of C. albicans in the infected host.

To Anne

for her constant encouragement

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INTRODUCTION

Knowledge of the candidicidal properties of the macrophage is scant. Some investigators have suggested that ingestion of Candida albicans by macrophages may facilitate dissemination of the organism rather than impede infection (88, 93, 150, 179).

Much of the recent work on the candidicidal ability of macrophages has centered on in vitro studies with peripheral, peritoneal, and alveolar macrophages, while little attention has focused on the liver Kupffer cell. Sawyer et al. (142), using a rat liver perfusion model demonstrated that normal livers, in the absence of serum, were capable of trapping, but not killing perfused C. albicans. Neither trapping nor killing was improved by the addition of humoral factors, but killing was enhanced by treating rats with Corynebacterium parvum vaccine. Trapping involved both phagocytic and non-phagocytic parameters.

The major objective of this thesis was to evaluate the trapping and candidicidal properties of the perfused mouse liver. Preliminary studies demonstrated that normal livers were capable of killing C. albicans with great efficiency. The role of the Kupffer cell in mediating killing was established by the use of the macrophage-specific toxin, crystalline silica (54). Silica-poisoned livers, depleted of Kupffer cells, were unable to kill perfused yeast. A second objective was to determine if non-immune and specific-immune serum could enhance

hepatic clearance and killing. Additionally, activation of the reticuloendothelial (RE) system by glucan, a potent macrophage stimulant (33-40, 126) was attempted to determine whether hepatic tissue could be non-specifically enhanced in its ability to kill cleared Candida. Final studies focused on C. albicans adherence in the perfused liver. Diamond and Krzesicki (31) and Warr (168) have described mannose (and related compounds) impaired adherence of Candida species to neutrophils (PMN) and macrophages in vitro. Additionally, Freidman and Moon (54) and Sawyer et al. (142), have described the trapping of bacteria and yeast in the perfused liver and noted that both microorganisms associated extensively with endothelial cells of the sinusoids. Consequently, preliminary studies were performed using mannose and related compounds in an attempt to block adherence of C. albicans in the liver and impair trapping.

LITERATURE REVIEW

Candida albicans and candidiasis

Candida albicans is frequently an indigenous human inhabitant often colonizing the mucous membrances of the alimentary tract, respiratory tract, and urogenital tract. It appears only transiently on the skin (26, 46). Rarely causing disease in healthy individuals, C. albicans is an opportunistic pathogen which can cause disease under adverse or abnormal conditions (1, 14). The most common manifestations of infection are superficial lesions of the mucous membranes, especially of the mouth and vagina. It may also manifest as an allergic phenomenon. Less common, but more severe forms of the disease include chronic mucocutaneous and systemic involvement (44, 114, 117).

As an opportunistic pathogen C. albicans causes disease in the compromised and debilitated host. A multitude of factors has been identified as contributing to man's susceptibility. Elevated glycogen and glucose concentrations in body secretions, associated with hormonal imbalances, are thought to specifically contribute to the incidence of C. albicans infections in people with diabetes (76) and during pregnancy (43). Trauma (106), drug addiction (88), and concomittant bacterial infection (75) are other, common potential causes underlying C. albicans infections.

Among hospitalized patients iatrogenic procedures often result in infection (3, 48). Surgery and broad spectrum antibiotic therapy

have been implicated in altering normal flora, thereby allowing C. albicans an access for colonization (48). Additionally, some antibiotics can inhibit the synthesis of anti-Candida antibodies (19) as well as, the phagocytic and metabolic activity of granulocytes (22, 79). Sulfonamides at pharmacologically active concentrations in vivo inhibit the myeloperoxidase (MPO)-dependent antimicrobial system of human PMN in vitro (79). Chan and Balish (22) have reported that phagocytosis of C. albicans by human PMN is retarded by 1 ug amphotericin B, a drug commonly used to treat systemic Candida infections (102). The use of broad spectrum antibiotics also causes an increase in the number and severity of lesions provoked by C. albicans in humans (146). Corticosteroid therapy decreases resistance to infection, persumably by inhibiting the release of lysosomal enzymes into C. albicans containing phagosomes (48).

The two major forms of severe human illness caused by C. albicans are chronic mucocutaneous candidiasis (CMCC) and severe disseminated candidiasis (44). Chronic mucocutaneous candidiasis is a rare disease characterized by persistent Candida infection of skin, hair, nails, mucous membranes and regularly associated with a cell-mediated immune system defect (42, 44, 139). Clinical and experimental evidence suggests that: (i) tolerance exists to a specific Candida antigen, (ii) a specific T-lymphocyte defect exists such that there is a basic failure in antigen recognition or mediator production, or (iii) monocyte dysfunction precludes the possibility of an effective immune response. The most common clinical and laboratory manifestations indicative of a cell-mediated immunodeficiency associated with CMCC are: (i) cutaneous anergy to Candida antigens, (ii) reduced, or absence of, lymphocyte

transformation in response to Candida antigens, and (iii) reduced, or complete suppression of, production of leukocyte migration inhibitory factor after stimulation with Candida antigens. Because other host defense mechanisms are intact, notably granulocyte function, the infection does not become systemic (44, 139).

People afflicted with chronic granulomatous disease (CGD) or MPO-deficiency are highly susceptible to the disseminated form of candidiasis (81, 82, 85). The MPO-H_2O_2-halide system of human PMN and monocytes is the predominate means by which these phagocytic cells kill ingested C. albicans (60, 80, 82). The exact mechanism by which the MPO-H_2O_2-halide system generates its anti-candidicidal effect is uncertain, but MPO and H_2O_2 are essential (8, 80, 81). Myeloperoxidase deficiency is characterized by a paucity of complete lack of the enzyme, whereas monocytes and PMN from individuals with CGD fail to generate H_2O_2 (80-82, 153-155). Lehrer and Cline (82) were the first to demonstrate that phagocytic leukocytes from patients with MPO-deficiency or CGD are capable of phagocytizing C. albicans normally, but cannot kill them.

Candida albicans infections complicating neoplastic and myeloproliferative disease have contributed significantly to the mortality of cancer patients in the past twenty years and the incidence of such infections in these patients continues to rise. Evas et al. (48), in a review of 2,517 autopsy reports, between the years 1960 to 1964, verified 109 demonstrable fungal infections of internal viscera by C. albicans. In a more extensive case review, between the years 1963 to 1975, Myerowitz et al. (114), reported that the incidence of disseminated candidiasis associated with leukemia rose from 1.1 cases/year

prior to 1971, to 6.8 cases/year between 1971 and 1975. These investigators and others (14, 129) attribute the increased occurrence of disseminated candidiasis to prolonged granulocytopenia; induced either by chemotherapeutic agents as part of the treatment regimen or caused by the disease itself.

In the severely immunosuppressed individual excess colonization of the gastrointestinal (GI) tract by C. albicans, facilitated by the use of antibiotics, is increasingly becoming a major portal of entry leading to dissemination (152). Injury to the mucosal epithelium of the GI tract, due to direct cytotoxicity of chemotherapeutic agents, direct penetration of the organism, or both results in invasion of submucosal vessels and eventual hematological dissemination throughout the body (113, 114). Severe histopathology and inflammation accompanying disseminated candidiasis in humans has been observed in the skeletal system (25), joints (111), myocardium (52), epidermis (15), cerebrum (87), meninges (9), eye (75, 103), urinary tract (61), kidneys (24, 114), liver and spleen (113, 114). Myerowitz et al. (114) have suggested that the increasing involvement of the liver and spleen may be complicated by depressed macrophage clearance in these two major RE organs. Klein and Watanakunkron (75) studied the fate of 85 episodes of hospital acquired candidemia in 77 patients and noted that the patients followed one of four clinical courses: (i) spontaneous resolution (42.8%), (ii) development of endophthalmitis following apparent resolution (5.1%), (iii) severe illness requiring appropriate therapy (31.4%), and (iv) no therapy resulting in death from candidemia (20.7%).

Pathogenicity and virulence of C. albicans

Despite the reports of extensive organ involvement the pathogenesis of systemic C. albicans infections is poorly understood. Causes which have been specifically proposed as contributing to mortality include embolization (138), toxemia (66), uremia (174), pancreatic damage (179), myocardial damage (116), and pulmonary hypersensitivity (175). Nevertheless, the kidney has generally been assumed to be the major target organ; mortality thus attributed to subsequent renal pathology (93, 131, 133). In a recent study of experimental candidiasis in mice, however, Leunk and Moon (86) observed fundamental differences in the pathogenesis of the disease following i.v. administration of different doses. A low dose challenge (1.0×10^6) of C. albicans resulted in a non-fatal or slowly progressive renal infection, whereas a high dose challenge (4.5×10^6) invariably resulted in a rapid toxemic-like death.

Montes and Wilbour (101) were the first to describe C. albicans as both an intracellular and extracellular parasite. In infected tissue it exists as a dimorphic fungus, growing in both yeast and mycelial forms (31, 47, 49, 65, 150, 179). Experimental animal studies have implicated both the yeast (47, 93, 165) and mycelial (65, 179) forms as being the more virulent. Iannini et al. (65) proposed that the enhanced pathogenicity of the mycelial form was related to its being preferentially trapped in peripheral capillary beds, thereby preventing delivery to phagocytic cells of the liver and spleen. In a more critical comparison of pathogenicity, however, Mardon et al. (93) reported that yeast phase C. albicans provoked a more lethal response in mice, as measured by mortality rates, than comparable numbers of mycelial

forms. Sprippoli and Simonetti (157) were able to enhance the virulence of both forms innoculated i.p. in mice, but not i.d. in rabbits by simultaneous administration of tetracycline. The effects of tetracycline were dose dependent. Vaughn and Weinberg (165) have correlated virulence of C. albicans with in vivo copper concentrations. Copper in physiological concentrations suppressed the filamentation of blastospores in vitro and potentiated the pathogenesis and growth of mycelial and yeast forms in mice. Evidence has also accumulated which suggests that aberrations in iron metabolism by the host may enhance the virulence of C. albicans. Elin and Wolff (45) have reported that high serum iron concentrations facilitates the growth of Candida and injections of iron enhance the lethality of the organism for mice. Iron unsaturated lactoferrin is fungastatic in human serum, but the effect is negated by the addition of iron (19).

Although C. albicans is primarily an opportunistic pathogen, it appears to be invasive in both the compromised and healthy host, capable of passing through mucous membrane barriers and gaining access to the vascular compartment. Candida albicans was found to pass unchanged through the GI wall of a healthy volunteer following oral administration, resulting in fungemia and funguria. Fortunately, the yeast was cleared in three hours by the host's defenses (48). C. albicans associated phospholipase activity has been implicated in aiding this invasive process, since epithelial cell membranes contain approximately 60% phospholipid (125).

Young (179), Stanley and Hurley (150), Mardon et al. (93), and Maisch and Calderone (88) have implicated phagocytic cells of the RE system in aiding the dissemination of C. albicans. These investigators

have observed germ tube and pseudomycelial formation by C. albicans following phagocytosis by mouse and rabbit macrophages. Ingested yeast remained dormant for approximately an hour, after which they began to germinate. By four hours macrophage integrity had been disrupted and macrophages were consumed by dense mycelial growth. Sasada and Johnston (140) have proposed that the ability of C. albicans to survive after being phagocytized related to its ability to limit macrophage oxidative metabolism. These authors found that phagocytized C. parapsillosis stimulated a more vigorous oxidative burst and superoxide (O_2^-) release than did phagocytized C. albicans. Macrophage metabolic changes correlated with enhanced killing of C. parapsillosis.

Cutler (28) has proposed that the degree of chemotactic activity elicited by C. albicans may have general relevance in regard to the invasiveness of different isolates. Candida albicans induced chemotaxis has been described for the guinea pig (28) and human PMN (126). Human PMN chemotaxis required complement activation. Viable C. albicans or twelve hour culture filtrates were chemotactic for guinea pig PMN by themselves. Cutler (28) ran exhaustive studies on the chemotactic ability of eight different strains of C. albicans and discovered that the only strain without demonstrable chemotactic activity was isolated from a case of severe disseminated candidiasis. The other isolates were from superficial infections or healthy volunteers.

Host defense mechanisms directed against C. albicans

Delineation of host defense mechanisms directed against C. albicans have relied on extrapolation from clinical observations in humans and laboratory experimentation in animals. A recent review of protective mechanisms against C. albicans has been published by Rogers and

Balish (135). Protective roles have been described for an intact humoral response (56, 89, 94, 106), an intact cell-mediated immune response (42, 44, 100, 139), and PMN (2, 31, 32, 60, 82, 133). Phagocytic cells of the RE system (distinct from thymus-dependent activated macrophages) have been ascribed both a protective role (49, 81, 85, 89, 173) and a detrimental role (already described) in combating C. albicans infections.

Moser and Domer (106) in assessing the role of antibody-mediated immunity during systemic candidiasis in mice selectively eliminated the B-cell arm of immunity by cyclophosphamide (CY) treatment. Following cutaneous immunization with viable C. albicans, CY-treated mice, though retaining an intact T-cell arm of immunity possessed depressed levels of circulating antibodies and were markedly more susceptible to a subsequent i.v. challenge than untreated mice. Additionally, CY-treated mice were unable to confine the spread and multiplication of C. albicans from the cutaneous innoculation. Giger et al. (56) reported similar results in an earlier study. Maita et al. (89) have proposed that antibodies are important only as an adjunct to phagocytosis and killing. However, what role if any, antibodies play in conferring protection in humans remains to be determined. Patients with Candida disease typically possess normal or elevated levels of circulating and secretory antibodies against Candida antigens (94, 139).

The relationship between thymus-dependent cell-mediated immunity and CMCC has already been discussed, but the role of thymus-dependent cell-mediated immunity against systemic candidiasis remains controversial. Miyake et al. (100) have reported that protective immunity against systemic cnadidiasis can be transferred from subcutaneously

vaccinated mice to normal recipients with lymphoid cells, but not serum. However, this protective effect appeared to be only partially effective and was not evident until late in infection. Pearsall et al. (122) have reported on the existence of a lymphokine capable of reducing the number of viable C. albicans in vitro. In contrast, Rogers and Balish (133) have proposed that an intact cell-mediated immune system is not required for defense against disseminated candidiasis and may actually be associated with decreased murine resistance (132). Rogers et al. (132) reported that congenitally athymic nude mice possessed a greater capacity to prevent the growth of i.v. administered C. albicans in the kidney and clear the microbe from the liver than their phenotypically normal litter mates. Furthermore, thymus reconstituted mice were just as susceptible as normal mice to systemic infection.

The role of the PMN in combating systemic Candida infections has been suggested by clinical observations that patients with MPO-deficiency or CGD are readily susceptible to infection. Lehrer and Cline (82) compared the phagocytic and intracellular killing abilities of PMN from normal individuals and patients suffering from MPO-deficiency and CGD. Neutrophils from normal volunteers phagocytized 100% of a 10^7 dose of C. albicans after ten minutes and killed 29% after one hour. Although phagocytosis of a similar inoculum was complete after ten minutes, PMN from patients with MPO-deficiency or CGD failed to kill the ingested yeast after one hour. Subsequent investigations by others have substantiated these findings (32, 80, 85, 95).

The high incidence of disseminated candidiasis in patients suffering from neoplastic diseases, rendered neutropenic by the disease or therapy, has provided additional evidence in support of the PMN as a

primary defense against systemic candidiasis. Laboratory confirmation has come from Johnson et al. (71). They demonstrated that L1210 leukemia cells rendered mice neutropenic, suppressed the inflammatory reaction normally elicited by C. albicans, and enhanced the susceptibility of leukemic mice to i.v. challenge.

Histological evidence in support of the PMN has been provided by Rogers and Balish (133). These authors compared renal histology in normal and BCG activated mice over a 21 day period, following i.v. challenge with viable C. albicans. They found the predominate cellular infiltrate in both groups of mice to be PMN. Neutrophil accumulation was strikingly evident by day seven and persisted throughout the period of observation. Pearsall and Lagunoff (121) described similar results for a thigh muscle Candida induced lesion in mice. A modest infiltration of other cell types, notably macrophages and a few lymphocytes, preceded the inital PMN response.

In assessing the role of the macrophage in compating C. ablicans infections it is necessary, and often difficult, to differentiate between 'inate' macrophages and macrophages which have been 'sensitized' through the T-cell mediated arm of immunity. This is especially true, since experimental animals and humans are routinely exposed to Candida antigens or similar antigens very early in life (135). Presently, controversy exists over whether the macrophage can effectively kill C. albicans and what role it may play in containing infection.

Arai et al. (2), similar to the results obtained by others (93, 150, 179), reported that rabbit alveolar macrophages from normal and immunized animals were incapable of killing ingested C. albicans in vitro. Phagocytizing macrophages were eventually destroyed by

proliferation and pseudomycelial formation. Maisch and Calderone (88) also reported similar results for rabbit blood monocytes in vivo. In contrast, Lehrer et al. (83) reported just the opposite. They found that alveolar macrophages from both normal and Mycobacterium butrycium stimulated rabbits were capable of killing ingested C. albicans effectively. Resident macrophages killed 28% of the ingested yeast after one hour incubation and stimulated macrophages killed 33% under similar conditions. Additionally, killing was not confined to alveolar macrophages. Resident and stimulated peritoneal macrophages were also capable of killing ingested yeast, but not as efficiently as alveolar macrophages. Some resident peritoneal macrophages were disrupted by mycelial formation after prolonged incubation. Evron (49) reported that peritoneal macrophages from mice sensitized with viable C. albicans, but not from mice sensitized with heat killed cells in incomplete Freunds adjuvant killed ingested C. albicans and limited to a degree germination and myceliam formation. Killing and inhibition were not 100% effective. Maita et al. (89) used resident, BCG activated, and PHA-induced lymphokine-stimulated mouse peritoneal macrophages to study the candidicidal properties of these cell populations in vitro. Resident macrophages were unable to kill ingested C. albicans in the absence of homologous immune serum. Bacille Calmette Guerin and lymphokine-stimulated macrophages were candidicidal by themselves. Lymphokine-stimulated macrophages possessed the greatest degree of phagocytic and candidicidal activity. Sasada and Johnston (140) compared the candidicidal activity of BCG and LPS elicited mouse peritoneal macrophages to resident peritoneal macrophages and reported results similar to Maita et al. (89).

The results of these investigations support the concept that macrophages are capable of killing C. albicans and point to a possible role for the macrophage in controlling infection. They also suggest that macrophage stimulation by the cell-mediated arm of immunity and possibly antibody may potentiate the process.

Lehrer (81) and Leijh et al. (85) have reported on the ability of human periperal blood monocytes to ingest and kill C. albicans. After 60 minutes incubation in vitro human monocytes ingested 96% of an inoculum of C. albicans and killed 50% of the ingested yeast (85). Human monocytes, like PMN, from patients with MPO-deficiency and CGD are impaired in their ability to kill C. albicans. Phagocytosis is normal, but the yeast are not killed (21, 81).

Fundamental to the anti-Candida properties of phagocytic cells are recognition, phagocytosis, and intracellular killing. Recognition is an energy independent event. Phagocytosis and intracellular killing are energy dependent events (20). The energy for phagocytosis is derived primarily from glycolosis. The energy for intracellular killing is derived primarily from oxidative metabolism (153-156).

Recognition and phagocytosis of Candida species by granulocytes can be potentiated by specific antibody directed against the cell wall (2, 18, 44, 60, 85, 89). The cell wall of C. albicans is a multilayered structure (41, 124) composed of glucans, mannans, N-acetylglucosamine, and some glycoproteins (23, 180, 181). The outer layer is composed almost exclusively of mannans; the primary antigenic determinats of the cell wall being different alpha-1,2-glycosidic linkages of the mannan hexose moeities (96). Accordingly, antibodies induced by intact cells are directed against the mannans of the cell wall.

Hasenclever and Mitchell (58) used these agglutinating antibodies to divide C. albicans into two serological groups; group A and group B. Group A contains all the antigenic structures of group B, plus an additional antigen or antigens. Considerable cross-reactivity also exists among different Candida species.

Human IgG is an effective C. albicans opsonin. Normal serum contains substantial titers of 'natural' Candida specific antibodies (44). Diamond et al. (32) have reported that direct interactions between human PMN and C. albicans pseudohyphae occurs in the absence of serum, but serum factors (IgG) facilitate pseudohyphae damage. Arai et al. (2) concluded that the opsonization potential of immune serum was species specific. Rabbit Candida specific-immune serum enhanced the phagocytic indices of rabbit alveolar macrophages for C. albicans, but not guinea pig PMN. Mouse Candida specific-immune serum enhanced phagocytosis of C. albicans by resident and activated mouse peritoneal macrophages and in contrast to the results of Arai et al. also enhanced the candidicidal potential of resident macrophages (89).

Several investigators (50, 82, 85) have reported the necessity for a heat-labile serum factor for maximum phagocytosis of C. albicans by human PMN and monocytes. In the absence of fresh serum phagocytosis was essentially absent. Opsonic activity was abolished by heating at 56°C for 30 minutes, suggesting a role for complement in the process. Serum opsonins have also been reported to facilitate the phagocytosis of other pathogenic fungi besides Candida species (10, 18, 161).

Recent evidence has accumulated for the existence of a carbohydrate(s) specific surface receptor capable of promoting adherence and phagocytosis of Candida species by direct surface-surface interactions

with phagocytic leukocytes. Binding of an organic glycoprotein and synthetic glycoconjugate to murine alveolar macrophages was inhibited by yeast mannan (149). Because the nature of the synthetic glycoconjugate was known, Stahl (149) proposed that the surface receptor recognized either glucose or mannose moieties. In a later study, Warr (168) demonstrated that resident mouse alveolar macrophages bound and ingested C. kurzei in vitro with a high degree of efficiency in the absence of serum. Binding was inhibited by D-mannose, D-glucosamine, horse-radish peroxidase, and beta-glucouronidase. It was not effected by D-mannitol, D-glucose, D-galactose, or L-fucose. Pretrypsinization of alveolar macrophages also prevented binding of yeast cells. Further evidence in support of a receptor was reported by Diamond and Krzesicki (31). These investigators demonstrated that C. albicans pseudohyphae were capable of adhering to human PMN in the absence of serum. Binding was inhibited by Candida mannans, but not D-mannose, dextran, chitin, Con A, or highly charged amino acids. Pretreatment of pseudohyphae with chymotrypsin or PMN with trypsin also impaired binding. Additionally, UV light induced damage to pseudohyphae promoted the release of a protein-polysaccharide complex which also blocked adherence to human PMN. Warr (168) and Diamond and Krzesicki (31) have postulated that the surface receptor specifically recognized mannose moieties. Slight biochemical or antigenic differences between C. kruzei and C. albicans pseudohyphae have not been ruled out in explaining the differences in mannose-sensitive adherence between the two.

Phagocytic and microbicidal mechanisms of phagocytic leukocytes have been reviewed by Stossel (153-155) and Barboir (8). The primary microbicidal (and candidicidal) mechanism employed by phagocytes is the

oxygen-dependent MPO-H_2O_2-halide system originally described by Klebanoff (74). Following activation by the appropriate stimulus two cellular events important in the microbicidal process occur: degranulation and initiation of the respiratory burst (8). Degranulation involves the fusion of the phagosome with cytoplasmic granules. The granules contain lytic enzymes and other materials associated in degradation and killing. Granular contents are discharged into the vesicle containing the microorganism (153-155). Respiratory burst describes a metabolic pathway responsible for generating microbicidal agents, via the reduction of oxygen (8). The metabolic events incorporated in the respiratory burst include augmented oxygen consumption, enhanced hexose-monophosphate-shunt activity, and increased production of O_2^- and H_2O_2 (8, 156, 164). The exact mechanism(s) whereby the MPO-H_2O_2-halide system exerts its microbicidal effect is uncertain, but thought to proceed by one of three mechanisms (8, 80, 81). In the presence of H_2O_2 MPO catalyzes the halogenation of the microbial cell wall with death resulting from the loss of integrity of the halogenated surface (74). Cl^-, I^-, and Br^- have been used to halogenate different microbes in vitro, but it is believed that Cl^- is the physiological substance, since it is the most abundant halide in the cell (8). Lehrer (81) has challenged the validity of this hypothesis, however. Methimazode, isoniazid, and aminotriazode can inhibit the halogenation of C. albicans by normal monocytes without impairing killing.

A second mechanism proposes that MPO and H_2O_2 catalyze the decarboxylation and deamination of microbial cell wall amino acids, resulting in disruption of the cell surface and death (21). A correlate

to this hypothesis is that free amino acids are decarboxylated generating microbicidal free-aldehydes (153-155).

The most recent hypothesis proposes that the MPO-H_2O_2-halide system kills by means of singlet oxygen, the reactive species being $OH^{\cdot}$ and/or O_2^- (8). Presently, this hypothesis appears the most likely, but evidence remains inconclusive.

MPO is present in, and therefore considered, the primary candidicidal mechanism of the mouse (140), rat (81), and guinea pig (2) granulocyte but not the rabbit macrophage (83). Lehrer et al. (83) discovered that the candidicidal activity of rabbit macrophages was not impaired by agents inhibitory to the MPO-system of human monocytes, but was inhibited by other agents which were ineffective in those cells. Their conclusion was that the candidicidal mechanism of the rabbit macrophage was uniquely different from the MPO-H_2O_2-halide system of human granulocytes.

Human PMN (80) and monocytes (81) in addition to the MPO-system possess a second MPO-independent candidicidal mechanism. This second mechanism is ineffective against C. albicans, but lethal for C. parapsillosis and C. pseudotropicalis. It is functional in normal granulocytes under anaerobic conditions and MPO-deficient and CGD impaired granulocytes also. Several cationic-like proteins have been isolated from human, rabbit, and guinea pig granulocytes which may account for the nature of this second mechanism (84).

The reticuloendothelial system and Kupffer cells

Much of the information regarding the candidicidal properties of macrophages has come from in vitro studies. Little attention has been focused on the functional role or the candidicidal activity of the

intact RE system during systemic candidiasis. Nevertheless, some information has accumulated suggesting a beneficial role for the RE system in combating deep Candida infections. Rogers and Balish (134) were able to confer protection in germ-free rats, from i.v. challenge with viable C. albicans, by stimulating the RE system with incomplete Freunds adjuvant. Bird and Sheagren (12) reported enhanced RE function in mice systemically infected with C. albicans, but failed to correlate enhanced function with protection. Trnovec et al. (163) also provoked enhanced RE function in mice by treating with various C. albicans fractions and concluded that properties of the RE system played a significant role in limiting the spread of C. albicans. However, they too failed to prove it.

Cells of the RE system originate from mesenchymatous tissue and include fixed tissue macrophages of the spleen, lymph nodes, bone marrow, alveolar macrophages of the lung, microglial cells of the central nervous system, liver Kupffer cells, and blood monocytes (72, 158, 170). Macrophages of the RE system are not end cells. Under normal conditions they proliferate with low frequency (98, 166), however, it is generally believed that different tissue macrophages, at one time or another, are derived from bone marrow promonocytes via the blood monocyte (33, 115, 169).

Macrophages of the RE system represent one of the most important short term defenses against invading microorganisms, comprising the major cellular barrier against microorganisms in the bloodstream (29, 99, 151). Liver Kupffer cells represent the largest group of fixed tissue microphages in mammals, and on the basis of functional and numerical superiority play a major role in the physiological events of

the RE system (4, 35, 123). Kupffer cells account for 38% of total liver cells and 50% of total RE cells (62).

The cytochemistry of Kupffer cells is typical of macrophages. Unlike monocytes, endogenous peroxidase activity is located in the nuclear envelope and endoplasmic reticulum and not cytoplasmic granules (33). Acid phosphatase and other lytic enzymes are contained in lysosomes (169).

Kupffer cells reside in hepatic sinusoids where they are anchored to the fenestrated endothelium by cytoplasmic lamellopodia and filopodia (54, 107, 142). They protrude well into the sinusoidal lumen and are covered with numerous invaginations, blebs, and ruffles which contribute significantly to the surface area exposed to the blood (107, 141). Such extensive surface morphology aids the Kupffer cell in scavenging particulate matter from the blood (20, 130).

Blood enters the liver via the portal vein and to a lesser extent the hepatic artery. Portal venules branch out from the portal vein and empty into liver sinusoids at the periphery of liver lobules. Blood perculates through the sinusoids, which radiate at right angles from the portal venules; generally running parallel to each other. Sinusoids empty into central venules near the center of the lobules. Arterioles of the hepatic artery empty into sinusoids distal from the sinusoidal-central venule junction. Central venules converge on the central vein, which eventually empties into the inferior vena cava via the hepatic vein. Particulate matter is first deposited in the sinusoids along the periphery and later towards the center of the lobules (13, 158). Certain cells in the sinusoidal wall exert a sphincter control over the lumen, which acts to regulate blood flow through the

sinusoids (13). Altered flow rates influence the uptake of inert and viable particulate matter by the liver (5, 6, 63).

Vascular clearance of C. albicans

Bloodstream clearance of C. albicans has been characterized by various investigators. Sawyer et al. (142) and Iannini et al. (65) categorized the bloodstream clearance of C. albicans blastospores into two phases: an initial rapid clearance followed by a slower decrement phase. Greater than 90% of the injected yeast were cleared in the first five minutes. The entire inoculum was recovered in RE organs as viable cells thirty minutes after administration. Hepatic recovery accounted for the highest percentage of recovered organisms (142).

Iannini et al. (65) and Evans and Mardon (47) studied the vascular clearance of both blastospores and mycelial phase C. albicans. Both groups reported that blastospores were preferentially sequestered in the liver, but noted different locations of preferential trapping of mycelial forms. Iannini and his group reported that mycelial forms were preferentially cleared from the bloodstream of rabbits in peripheral capillary beds. Evans and Mardon observed that mycelial forms were preferentially cleared from the blood of mice by the lungs. Whether the differences reflect the use of different experimental animals or different routes of injection is not known. Baine et al. (7) have reported different tissue localization of yeast phase C. albicans in rabbits, depending on the route of injection. Prior immunization with heat killed cells had no effect on trapping of either form in rabbits (65). Viability of both forms decreased in the liver and the lungs of mice twelve days after injection. Disappearance from the lungs was faster

than in the liver (47). Spores, cell walls, and glucan of C. albicans preferentially localized in the liver after i.v. injection into mice (97). Electron micrographs of liver tissue revealed that viable C. albicans were phagocytized by Kupffer cells within fifteen minutes after injection. Phagocytosis was proceeded by step-wise degradation of the cell wall and eventual death of the yeast. Glucan of the cell wall persisted, however, and induced granuloma formation four days after injection. Granulomas were characterized by large numbers of macrophages and lymphocytes, but few PMN.

Liver perfusions: microbicidal and trapping mechanisms

The perfused liver provides an excellent opportunity to study factors involved in hepatic clearance and killing of microorganisms. Jeunet et al. (67-70) have used the perfused liver to study phagocytosis and the RE blockade. Moon et al. (104) have demonstrated that the perfused liver approximates in vivo events and is capable of distinguishing between microbial trapping and microbial killing functions of the liver.

Manwaring and Coe (91) did liver perfusions using rabbits and pneumococci. Pneumococci suspended in Ringers-Locke solution were not removed by normal livers. Media supplemented with 1 to 10% normal rabbit serum had no effect, but 1% immune serum potentiated 100% removal, after multiple passes. The activity in immune serum was heat stable, 60°C for thirty minutes suggesting the removal was mediated by antibody. Manwaring and Fritschen (92) performed similar studies in dogs using E. coli, Staph aureus, and B. anthracis and got similar results, however, some trapping was evident in the absence of humoral

factors. Additionally, different bacteria manifested different degrees of retention. In the absence of serum E. coli and Staph aureus were trapped with greater efficiency than B. anthracis. Wardlaw and Howard (167) and Jeunet et al. (67) reported similar results using a number of different bacteria in rats. B. meletensis and S. typhosa were readily removed by the perfused liver in the absence of specific antibody or plasma opsonin. Clearance was not improved by specific immune serum. In contrast, B. abortus was removed by the perfused liver only in the presence of specific antibody (67). Humoral factors were not necessary for hepatic trapping of E. coli, P. pyocyana, P. mirabilis, Staph aureus, C. murium, B. cereus, and S. pyogenes. Conversely, two Clostridia species and S. gallimurium suspended in buffered medium passed straight through the liver. Trapping of all bacteria species was enhanced by the addition of normal human serum to the perfusion medium, except for Staph aureus, C. murium, B. cereus, and S. pyogenes, which was actually reduced (167). In a parallel study, Howard and Wardlaw (63) attributed the opsonic activity of normal serum to specific antibody, complement, and possibly properidin. The opsonic activity of the sera was destroyed by heating (56°C, 30 mins.) or absorption with homologous bacteria, antibody-antigen immune complex, and zymosan. They attributed the removal of bacteria from the perfusion medium to phagocytosis, however Moon et al. (104) have demonstrated that hepatic trapping is not synonomous with phagocytosis.

Although trapping does not imply phagocytosis, Friedman and Moon (54) have demonstrated that Kupffer cells are necessary for maximum trapping. Normal nurine livers trapped an average of 63% of a 1×10^{10} to 2×10^{10} dose of S. typhimurium suspended in M-199. Trapping of a

similar inoculum in Kupffer cell depleted livers was reduced over 30%. From these results they suggested that bacterial trapping is a physical event mediated by surface receptors on endothelial cells or simply due to mechanical restriction of the sinusoids. Mechanical restriction seems unlikely though, since some strains of bacteria, both cocci and bacillus shaped, are capable of passing entirely through the liver without being retained (67, 91, 167).

None of the studies reported herein have dealt with the relative bactericidal properties of hepatic and humoral factors. Bonventre and Oxman (16) studied the cellular and humoral factors associated with hepatic clearance and killing in rats. The immunological status of the animal had no effect on phagocytosis or killing of Staph aureus, but did effect killing of S. enteritidis. Immune humoral or cellular factors by themselves provoked a limited degree of destruction. Together, they accounted for a 95% reduction in viability of the bacteria. Moon et al. (104) demonstrated that non-immune humoral factors were capable and necessary for killing of S. typhimurium in perfused rat livers. Normal livers killed only 7% of an inoculum suspended in M-199. In the presence of 10% whole rat blood or plasma killing was increased to over 50%. Friedman and Moon (55) further characterized the blood components responsible for hepatic killing of S. typhimurium. Their results showed that viable Kupffer cells and complement, via the alternate pathway, were necessary for killing. They also concluded that specific antibody only enhanced trapping in normal animals. Normal livers in the presence of 5% plasma killed more than 37% of an inoculum of S. typhimurium. Killing of a similar inoculum in livers depleted of Kupffer cells was only 9%. To determine the importance of complement

in killing, complement activity was depleted by heating (57° and 50°, 30 mins.), zymosan absorption, chelation with EGTA, and immunoabsorption of C3. All treatments significantly reduced bactericidal activity in the perfused liver. Chelation with EDTA had no effect, which suggested a role for the alternate complement pathway, since EDTA specifically impairs the classical pathway. When immune plasma was treated to destroy complement activity bactericidal activity in the liver was reduced, but trapping was increased, emphasizing the role of specific antibody in trapping only.

Contradictory to the results of Friedman and Moon, Ruggiero et al. (137) reported a requirement for both classical complement activation and specific antibody in effecting killing of E. coli in perfused rat livers of endotoxin tolerant animals. Whether this difference reflects the use of endotoxin tolerant animals or different bacteria is not known. Endotoxin tolerant animals, however, may possess high levels of antibody to E. coli which could mask the surface of the bacteria and prevent direct interaction with the alternate complement pathway.

Hepatic trapping by the perfused liver is not unique to bacteria, since tumor cells (136) and C. albicans (7, 142) are avidly trapped as well. Mouse liver Kupffer cells manifested a preferential phagocytosis and degradation of different tumor cells in vitro. Adenocarcinoma and melanoma cells were avidly trapped and degraded within 90 minutes of infusion, but lymphosarcoma and leukemia cells were rarely effected.

Perfused rabbit livers manifest a strong avidity for C. albicans blastospores (7). Normal rabbit livers trapped an average of 90% of a dose of C. albicans suspended in buffered medium. Addition of 5% normal rabbit serum increased trapping, slightly but significantly, to

98%. Trapping in the presence of heated 5% normal rabbit serum was intermediate between the high and low values. Sawyer et al. (142) did C. albicans liver perfusions in rats. Normal livers cleared greater than 85% of the infused inoculum without any killing. Neither trapping nor killing were enhanced by the addition of 10% homologous whole blood to the perfusion medium. Scanning electron micrographs revealed that C. albicans spores were trapped in liver sinusoids adhering to fenestrated endothelial cells. Rarely were trapped yeast associated with Kupffer cells. These results differ from those of Meister et al. (97) previously described for whole animals. Enhanced trapping and significant killing was induced in rats by treatment with C. parvum vaccine (143). Corynebacterium parvum-treated, perfused livers killed only 5% of an inoculum of C. albicans suspended in M-199, after thirty minutes. Extending the perfusion time to 60 minutes increased killing to over 20%. In the presence of 5% homologous plasma, perfused livers killed greater than 30% of a similar inoculum after 60 minutes. Hepatic trapping in C. parvum-treated animals involved both phagocytic and non-phagocytic parameters.

Kupffer cells possess complement (C3b) and Ig Fc surface receptors (64, 144) which may facilitate phagocytic trapping in the presence of humoral factors. In the absence of humoral factors trapping of C. albicans by Kupffer cells may be mediated by the proposed granulocyte, mannose-sensitive surface receptor described by Diamond and Krzesicki (31) and Warr (168). It remains to be determined if non-phagocytic trapping of C. albicans involves mechanical factors or chemical interactions of yeast cell walls with sinusoidal membranes. Day et al. (30) recently reported that hepatic clearance of i.v. administered, iodine-

labelled IgM:BSA immune complexes in rats was impaired by pre- or coinjection of mannan. The report emphasized that hepatic endothelial cell surface receptors responsible for immune clearance may recognize and bind mannose oligosaccharides associated with circulating antibodies. These same receptors may mediate non-phagocytic sinusoidal trapping of bacteria and C. albicans as emphasized by Moon et al. (104), Friedman and Moon (54), and Sawyer et al. (142).

Glucan and phenylbutazone

The RE system can be activated by innumerable different agents including zymosan (127). Riggi and DiLuzio (127) identified glucan as the RE stimulatory agent in zymosan. The glucan of zymosan was first physically characterized by Hassid et al. (59) and found to consist predominately of linear glucopyranose units joined by beta-1,3-glucosidic linkages. The molecular weight of glucan extracted by the method of Hassid et al. is approximately 6,500 daltons.

The biological properties of glucan have been reviewed by DiLuzio (34, 35). Glucan is a potent stimulant in mice (177), rats (128), humans (90), and even crayfish (147), but not rabbits or dogs (40). Glucan administration in susceptible animals potentiates profound hyperplasia and hypertrophy of RE organs (6, 105, 128), significantly enhances primary and secondary immune responses (105, 177, 37), and increases serum lysozyme levels (77). Glucan administration is proceeded by marked, reversible hypertrophy and hyperfunction in the liver, lung, and spleen (5, 177) in a time and dose dependent manner (178). Liver weights of mice treated with three daily consecutive i.v. injections of glucan (4 mg/Kg body wt.) increased 11% 24 hours after the last injection and peaked after ten days. Enhanced phagocytosis

coincided with increases in liver weight. Carbon clearance was enhanced 24 hours after the last glucan injection and peaked after ten days. Twenty-five days after treatment ceased depressed phagocytic activity associated with reduced liver weights was pronounced. By 30 days liver weights and phagocytic activity returned to normal.

The pharmacological effects of glucan are specific for cells of the RE system (39, 128). Sulphobromophthalein clearance from the bloodstream, a function of hepatic parenchymal cells, is not altered following glucan administration (6), though vascular clearance of colloidal carbon (177), RE test lipid emulsion (5), and sheep red blood cells (105) is accelerated. The enhanced vascular clearance associated with glucan administration is due primarily to enhanced Kupffer cell activity (105). The hepatic uptake of foreign RBC by glucan treated mice, in relation to controls, was increased 140% 30 minutes after injection. After one hour, hepatic activity was still 63% greater than the glucan-treated group. In contrast to enhanced liver uptake, lung removal of foreign RBC in RE hyperfunctional mice decreased 60% at 30 minutes and one hour. Spleen uptake in the glucan-treated group was unaltered from controls at any time period.

The hypertrophy and hyperfunction stimulated by glucan treatment reflects the formation of new RE cells and enhanced activity of pre-existing cells, especially Kupffer cells (38, 127, 128). Glucan has no direct effect on circulating opsonin levels, nor are opsonic factors involved in glucan induced alterations of the RE system, though opsonins are required for maximum phagocytosis by isolated RE cells (37). Deimann and Fahimi (33) reported a large influx and differential localization of monocytes and macrophages into rat livers 18 to 48 hours

after a single i.v. injection of glucan (30 mg/Kg body wt.). Monocytes preferentially adhered to the endothelial lining of portal venules, whereas macrophages typically congregated to central venules and adhered. Transmission electron micrographs and cytochemical techniques suggested the existence of transition forms between peripheral blood monocytes and Kupffer cells in the sinusoids. No PMN were evident in any of the liver preps.

Glucan administered i.v. is preferentially sequestered in the liver and the most pronounced effects associated with RE organs occur in the liver (5, 35) which may partially explain why enhanced vascular clearance is attributable primarily to increased hepatic uptake. Twenty-four to 48 hours after glucan administration monocytic granulomas appear around the periphery of liver lobules. These monocytes are phagocytic and account for a large proportion of particulate uptake. Kupffer cells not only increase in number, but also in appearance. Ribonucleo-protein and endoplasmic reticulum increase in number. Mitochondria are more numerous and larger. Cytoplasmic vesicles are also more numerous and many contain clearly discernable glucan particles. Kupffer cells as a whole are also larger and swollen, displaying prominent cytoplasmic bulging into sinusoidal lumens. The net effect is to markedly restrict blood flow through the sinusoids (5). Measurement of total hepatic perfusion flow remains normal in glucan-treated animals, but when expressed as perfusion rate per unit of liver mass the flow rate is decreased. Burgaleta et al. (17) have studied the cellular effects of glucan on mouse peritoneal macrophages and have reported similar effects. Additionally, glucan treatment increased

spreading and adherence to glass, as well as augmenting chemotactic activity by 20 to 50%.

Treatment of experimental animals and human volunteers with glucan has conferred protection against a number of different infectious and cancerous agents. DiLuzio (34) and DiLuzio et al. (36) have reported protection in mice against Shays myelogenous leukemia. Protection in animals has also been reported against ascite sarcoma tumors, L1210 leukemia, and adenosarcoma tumors (119). In clinical trials with humans Mansell et al. (90) have reported necrosis and tumor cell destruction of metastatic lesions in patients with malignant melanoma and pulmonary seated adenocarcinoma.

Glucan treatment in animals has conferred protection against experimental infections with Staph aureus (78), Sporotrichum schenkii, Cryptococcus neoformans, Plasmodium bergheii, Mycobacterium leprae (35), and C. albicans (173). Pretreatment of mice with glucan was necessary to confer protection against systemic C. albicans challenge. Glucan pretreatment enhanced survival time and postponed initial mortality compared to control mice or mice receiving treatment after challenge. Glucan pretreatment also reduced the severity and duration of a subcutaneously induced C. albicans lesion. Glucan had no effect on experimental Toxoplasma gondii infection in mice (35). Glucan treatment rendered rats hyperactive to endotoxin shock and enhanced mortality (27, 162), but reduced tourniquet shock induced mortality (162).

Glucan mediated macrophage activation appears to be a non-specific thymus-independent phenomenon (35), however, the exact chemical or physical nature of glucan responsible for activation remains obscure. Fitzpatrick et al. (51) reported that thermal degradation and

oxidation of glucan resulted in total loss of RE stimulatory activity. These same authors reported formylation increased and acetylation did not alter the effects of glucan. According to DiLuzio and Riggi (40) sulfation effects the activity of glucan depending on the degree of sulfation (by weight). A low degree of sulfation (0.4%) did not alter the RE stimulatory ability or the degree of liver, lung, and spleen hypertrophy induced by glucan. Eleven percent sulfation decreased the hyperphagocytic state compared to native glucan, but was still stimulatory compared to saline controls. Additionally, pulmonary and liver hypertrophy was not induced by the 11 percent sulfated glucan, but spleen hypertrophy to a lesser extent than native glucan was.

DiLuzio et al. (36) contend that the pharmacologically active component of glucan is related to the beta-1,3-glucosidic linked backbone of the molecule. Minor, beta-1,6-glucosidic linked components have been discovered, but are unable to induce macrophage activation or hypertrophy. Fred and Zaremba (53), however, have reported that the RE response to glucan is more dependent on particle size than on unique chemical structure. Their conclusion is supported by the work of Suzuki et al. (160) who reported that water-soluble glucan had minor effects on macrophage mediated tumor destruction in mice. By comparison, water-insoluble glucan promoted greater than 88% inhibition and destruction of tumor growth. At variance with the results of Fred and Zaremba and Suzuki et al. is the hypothesis of DiLuzio and Riggi (40) that a specific metabolite of glucan rather than its particulate nature is responsible for RE stimulation. DiLuzio and Riggi reported that water-soluble di- and oligosaccharides of glucan were capable of stimulating phagocytic activity without inducing hypertrophy in experimental

animals. The results of Suzuki et al. and Riggi and DiLuzio should be compared with caution, however. Lack of tumor destruction by water-soluble glucan-treated macrophages does not preclude the possibility of enhanced phagocytosis. It may be that hyperphagocytosis is inducible by water-soluble glucan, but the full complement of activated macrophage activities requires treatment with particulate glucan.

Phenylbutazone (PB), an antiinflammatory drug which inhibits phagocytosis and intracellular killing by different leukocytes (118, 148, 156) is commonly used to evaluate granulocyte-microbe interactions. Leijh et al. (85) demonstrated that 1 mM PB inhibited the intracellular killing of C. albicans by human PMN and blood monocytes, but had no effect on phagocytosis. Lehrer et al. (83) reported that PB had no effect on phagocytosis or intracellular killing of C. albicans by rabbit macrophages and concluded the candidicidal mechanism of rabbit macrophages was different than the MPO-system of human granulocytes. In metabolic studies, Whitehouse (171) observed that PB uncoupled oxidative phosphorylation in isolated rat mitochondria without impairing electron transport. Strauss et al. (156) noted that the drug inhibited oxidation of glucose-1-C^{14} and ^{14}C-formate in both resting and phagocytizing PMN, suggesting an effect on the hexose-monophophate-shunt. The study also reported that PB inhibited glucose-6-phosphate and 6-phosphogluconate dehydrogenase activity. Kjosen et al. (73) verified that PB inhibits intracellular killing primarily by blocking the hexose-monophosphate-shunt. Odegaard et al. (118) have mused that PB may also block intracellular killing by preventing fusion of phagosomes with lysosomal granules.

MATERIALS AND METHODS

Animals

Male and female CD-1 mice were obtained from the Carworth Farms Division of Charles River Laboratories; Portage Michigan and housed in our laboratory. Animals were supplied with standard lab chow and water ad libitum. Both male and female mice, 25 to 30 g were used without regard to sex.

Candida albicans

Two different isolates of C. albicans were generously supplied by Drs. Everett Beneke and Alvin Rogers of Michigan State University. For purposes of clarification they have been designated simply as isolate I and isolate II. Isolate I was a fecal isolate from a woman experiencing recurrent vaginitis. Isolate II was originally from Hasenclever's lab and known to be serotype B. Identification was confirmed by assimilation of glucose, maltose, and sucrose, but not lactose or cellobiose, germ tube formation in fetal calf serum, and chlamydospore production on corn meal agar plus 1% Tween 80.

Stock cultures of Candida were maintained on Sabouraouds dextrose agar (Difco, Detroit, Michigan) at 4°C and trasnferred to fresh agar slants every two weeks. Yeast used for perfusions were grown in 100 ml tryptic soy broth (Difco, Detroit, Michigan) plus 4% dextrose (Fisher Scientific Co., Fair Lawn, N.J.) for 18 to 20 hr at 37°C with constant stirring. Cells were harvested by centrifugation at 850 x g for 10 min

and washed three times in 0.85% sterile saline. After the third wash, cells were suspended in 50 ml sterile saline and blended in a Waring blender. Haemocytometer counts were done and the yeast adjusted to 10^7 cells/ml in sterile M-199 (GIBCO, Grand Island, N.Y.) for use in liver perfusions.

Glucan preparation

Glucan was prepared by the method of Hassid et al. (59). Basically, bakers yeast (Sacchromyces cervisae, MSU Stores, Annheiser Busch, St. Louis, Mo.) was digested in 2L of 3% NaOH in a warm water bath for 4 hr then allowed to stand at room temperature overnight. The supernatant was decanted and 2L of fresh 3% NaOH was added to the residue. The mixture was boiled in a water bath for 2 hr then allowed to cool at room temperature overnight. The supernatant was decanted and the residue acidified with approximately 800 ml concentrated HCl. An additional 2L of 3% HCl was added to the residue and heated again for several hours on a warm water bath. The supernatant was decanted, the residue washed several times with distilled water, and finally washed with boiling distilled water. After washing, the residue was centrifuged, decanted and mixed with 1L of alcohol. After standing at room temperature for several days the residue was resuspended in fresh alcohol, centrifuged, washed with ether, and dried under vacuum. The final product (glucan) was collected and stored at room temperature.

In preparation for injection, glucan was suspended in saline (10 mg/ml) and sonicated (MSE 100 watt Ultrasonic Disintegrator, the Dann Co., Cleveland, Ohio) at 22,000 cycles/sec for 30 minutes. After sonication the glucan was sterilized by autoclaving and stored at 4°C.

The biological activity of glucan was confirmed by carbon clearance studies (Appendix A).

Glucan treatment

Two different regimens of glucan treatment were employed. Regimen one consisted of a single i.v. injection of 0.5 mg glucan 2 to 4 days prior to perfusions. Regimen two consisted of the same injection schedule, but waiting 7 to 8 days before perfusions.

Chemicals and reagents

Phenylbutazone (10 mM) (PB, Sigma Chemical Co., Columbus, Ohio) dissolved in 95% ethanol was added to M-199 to yield a final concentration of 1 mM or 5 mM (pH adjusted to 7.3 by addition of 1N NaOH). The solution was filter sterilized (Millipore Corp., Bedford, Mass., pore size 0.22 um) and stored at 4°C.

For perfusion studies, livers were infused with 1 ml of an appropriate concentration of PB, washed for 20 min with M-199 and infused with yeast.

For silica studies, Dorenturp crystalline silica (DQ12), particle size 5 um or less, was supplied by Dr. Ing M. Reisner, Steinkohlenbergbauereiw, 43 Essen-Kray, Frillendolfer Strabe 351, W. Germany. Silica prepared by autoclaving in powder form was suspended in sterile saline at a concentration of 20 mg/ml. Prior to injection the suspension was sonicated (Bronsonic Ultrasonic Cleansor, no. B220, Branson Instruments Co., Sketon, Conn.) and a total of 10 mg were administered i.v. over a three day period. Mice received 3 mg on days one and two and 4 mg on day three. Perfusions were done on day four.

For carbohydrate studies mannose and glucose (Sigma Chemical Co., St. Louis, Mo.) were dissolved in deionized distilled water at a concentration of 0.5 g/ml (50% wt./vol.), filter sterilized, and added to sterile M-199 to a 1% or 5% final concentration. Mannitol (Sigma Chemical Co., St. Louis, Mo.) was dissolved at a concentration of 0.25 g/ml (25% wt./vol.), filter sterilized, and added to M-199 to a 1% final concentration. All solutions were stored at 4°C. Experimentally, a cell suspension containing 10^7 C. albicans/ml was made in M-199 plus the appropriate carbohydrate and stored in an ice bath immediately prior to use. Livers were washed with 30 ml M-199 and immediately prior to infusion of yeast, perfusion of M-199 plus the appropriate carbohydrate was begun.

For studies using serum, normal calf serum (NCS, Flow Laboratories, Rockville, Md.) was added to a concentration of 5% in M-199, filter sterilized, and stored at -20°C. Bovine immune serum (BIS) specific for C. albicans I was obtained as a gift from Mr. James Veselenak. Immune serum was added to NCS to a 20% concentration. This mixture was added to M-199 to yield a final 1% concentration of immune serum, filter sterilized, and stored at -20°C prior to use. Experiments with serum were performed by the same methods used for carbohydrates.

Bovine immune serum and NCS were titered against each C. albicans isolate using a tube agglutination technique. Normal calf serum was prediluted 1/10 and serial 2-fold dilutions were made in saline. Bovine immune serum was prediluted 1/1000 and serially 2-fold diluted in saline. C. albicans was killed (1% formaldehyde, 60°C, 1 hr.), adjusted to 2×10^7 cells/ml by haemocytometer count, and added to diluted serum to yield 10^7 cells/test tube. Qualitative pour plates of

the killed yeast were done to assure none remained viable. Agglutination titers were read after 24 hr incubation in a 37°C water bath.

Normal calf serum showed no agglutination activity against either isolate. Bovine immune serum had an agglutination titer of 1:8000 to C. albicans I and 1:2000 to C. albicans II.

In situ liver perfusions

Procedures for liver perfusions have been described elsewhere (104). Briefly, a midline abdominal incision was made and skin and viscera reflected to expose the portal vein and inferior vena cava. Ligatures were placed beneath the portal vein and inferior vena cava above the renal vein. The portal vein was cannulated and secured. The inferior vena cava was snipped below the ligature and perfusion of M-199 begun. Next, a medial midline thoracic incision was made and the rib cage reflected to expose the heart and superior vena cava. The left auricle was snipped and an efferent cannula inserted into the incision, through the superior vena cava, and secured by ligature. The inferior vena cava was tied off above the renal vein and 30 ml of M-199 perfused through the liver to flush it of blood. After washing, 1 ml of 10^7 C. albicans was slowly and steadily perfused through the liver. Perfusions were done at room temperature and lasted approximately 30 min until 50 ml of effluent were collected. After perfusions were complete the liver was removed, homogenized (Tri-R-Stir-R homogenizer, Model no. S63C, Tri-R Instruments, Rockville Center, N.Y.) in 5 ml saline and adjusted to a final volume of 10 ml.

Quantitative pour plates of the liver homogenate were performed to determine the number of yeast trapped in the liver. The effluent

was blended and quantitative pour plates were carried out to determine the number of yeast that passed through the liver. Quantitative pour plates of the original inoculum were used to determine the total number of yeast perfused.

Recovery of yeast was expressed as a percentage of the total number perfused (100%). Those yeast not recovered in the liver homogenate or perfusate (total recovery) were presumed killed in the liver (Appendix B). They were determined by subtracting the percent total recovery from the percent perfused. The percent trapped represents those yeast not recovered in the effluent and is determined by subtracting the percent recovered in the effluent from the percent perfused.

Statistical analysis

Data was evaluated by the White rank order test (172).

RESULTS

Trapping and killing of C. albicans I and II by normal perfused mouse livers

Normal livers trapped an average of 93.5% of a 10^7 dose of C. albicans I on a single pass (Table 1). An average of 70.6% of the yeast were recovered in the liver and 6.5% in the perfusate for a total recovery of 77.1%. It is presumed that the 22.9% of the yeast not recovered were killed by the liver. Normal livers trapped an average of 98.7% of a 10^7 dose of C. albicans II on a single pass (Table 1). An average of 83.6% of the yeast were recovered in the liver and 1.2% in the perfusate for a total recovery of 85.9%. The liver killed 15.1% of the yeast. The data indicates there is a slight, but significant difference in the behavior of these two isolates in normal livers.

Effects of silica treatment and phenylbutazone on trapping and killing of C. albicans I and II by perfused mouse livers

Depleting livers of Kupffer cells, by silica poisoning, produced marked effects on trapping and killing of both C. albicans I and II. Total recovery of both isolates was greater than 100% indicating no yeast were killed (Table 2). The most striking effects occurred with C. albicans I. Recovery from the liver decreased 31.5% and increased in the perfusate 57.3%. Total trapping decreased 54.4%. In all cases the differences are significant.

Table 1. Trapping and killing of C. albicans I and II by normal perfused mouse livers.[a]

	Recovery %	
Experimental	C. albicans I	C. albicans II
Liver	70.6 ± 4.6[b]	83.6 ± 8.9
Perfusate	6.5 ± 4.6[b]	1.2 ± 1.8
Total	77.1 ± 4.6[c]	84.9 ± 9.2
Killing	22.9 ± 4.6[b]	15.1 ± 9.2
Trapping	93.5 ± 4.6[b]	98.7 ± 1.9

[a]Mean ± standard deviation of at least twelve separate experimental determinations.

[b]$P = .001$

[c]$P = .01$

The results with C. albicans II, though not as striking, are also significant. The precent recovery from the liver was similar in normal and silica-treated animals, but recovery in the perfusate from silica-treated mice increased 12.4%, an amount approximately equal to killing in normal animals. Trapping in this group decreased 9.7%.

Phenylbutazone had no effect on trapping or killing of C. albicans I by perfused livers (Table 2). Total recovery in the presence of 1 mM PB averaged 89.9%. Slightly more than 20% of the yeast were killed. In the presence of 5 mM PB total recovery averaged 95.5% and 22.2% of the yeast were killed. The results are similar to normal values. Incubation of C. albicans with PB and perfused through the perfusion apparatus in the absence of livers had no effect on yeast viability (data not shown).

Table 2. Effects of silica treatment and phenylbutazone on trapping and killing of C. albicans I and II by perfused mouse livers.[a]

	Recovery %					
	C. albicans I				C. albicans II	
Experimental	Normal	Silica-treated[b]	1 mM PB[c]	5 mM PB[c]	Normal	Silica-treated[b]
Liver	70.6 ± 4.6	39.1 ± 12.5[d]	69.3 ± 7.8	73.3 ± 4.3	83.6 ± 8.9	87.5 ± 3.3
Perfusate	6.5 ± 4.6	63.8 ± 11.9[d]	10.2 ± 9.3	4.5 ± 3.1	1.2 ± 1.8	13.6 ± 4.6[e]
Total	77.1 ± 4.6	102.9 ± 5.3[d]	79.6 ± 8.8	77.8 ± 4.0	84.9 ± 9.2	101.1 ± 5.2[d]
Killing	22.9 ± 4.6	---	20.4 ± 8.8	22.2 ± 4.0	15.1 ± 9.2	---
Trapping	93.5 ± 4.6	36.2 ± 12.5	89.9 ± 9.3	95.5 ± 3.1	98.7 ± 1.8	86.4 ± 3.3

[a]Mean ± standard deviation of at least six separate experimental determinations.

[b]Mice treated with a total of 10 mg crystalline silica, administered i.v. over a three day period, prior to perfusions.

[c]Livers perfused with 1 ml of the appropriate concentration of phenylbutazone prior to perfusion of yeast.

[d]P = 0.001

[e]P = 0.01

Effects of normal calf serum and Candida-specific bovine immune serum on trapping and killing of C. albicans I and II by normal livers

Both Candida isolates were perfused through normal livers in the presence of 5% NCS or 1% BIS. Normal calf serum had no effect on trapping or killing by normal livers perfused with either isolate (Table 3).

One percent BIS significantly enhanced trapping and killing of C. albicans I perfused through normal livers, even though the actual differences are slight (Table 3). Bovine immune serum increased trapping by 5.3% and killing by 8%. Only 1.2% of the dose was recovered in the perfusate. The increase in killing was not artifactual due to immune aggregation of the yeast, since when yeast were incubated in the presence of BIS and perfused in the absence of livers an average of 105.7% of a 10^7 dose was recovered (data not shown).

Bovine immune serum had no effect on trapping or killing of C. albicans II by normal livers, even though the immune serum did show considerable cross-reactivity (Table 3).

Comparison of liver weights from normal and glucan-treated mice

Glucan treatment produced a marked hypertrophy of mouse livers (Table 4). Two to four days following treatment liver weights increased 45% over controls. By seven to eight days livers weighed 58% more than controls. Increased liver weights are significantly greater than controls.

Table 3. Effects of normal calf serum and *Candida* specific bovine immune serum on trapping and killing of *C. albicans* I and II by normal mouse livers.[a]

	Recovery %					
	C. albicans I			*C. albicans* II		
Experimental	Normal	NCS[b]	BIS[c,d]	Normal	NCS[b]	BIS[c]
Liver	70.6 ± 4.6	70.5 ± 8.2	67.8 ± 3.9	83.6 ± 8.9	78.6 ± 4.1	80.7 ± 5.3
Perfusate	6.5 ± 4.6	9.2 ± 4.8	1.2 ± 0.6[e]	1.2 ± 1.8	1.9 ± 1.4	0.5 ± 0.5
Total	77.1 ± 4.6	79.6 ± 4.9	69.1 ± 4.4[f]	84.9 ± 9.2	80.5 ± 3.6	81.2 ± 5.6
Killing	22.0 ± 4.6	20.4 ± 4.9	30.9 ± 4.4[f]	15.1 ± 9.2	19.5 + 3.6	18.8 ± 5.6
Trapping	93.5 ± 4.6	90.9 ± 4.8	98.8 ± 0.6[e]	98.7 ± 1.8	98.1 ± 1.4	99.4 ± 0.5

[a]Mean ± standard deviation of at least six separate experimental determinations.

[b]5% normal calf serum (5 ml NCS plus 95 ml M-199).

[c]1% bovine immune serum (1 ml BIS, 4 ml NCS plus 95 ml M-199).

[d]Mean ± standard deviation of five separate experimental determinations.

[e]P = .001 vs. normal livers.

[f]P = .01 vs. normal livers.

Table 4. Comparison of liver weights following glucan administration.

Liver weights[a] (grams)		
Normal	Glucan treated[b]	Glucan treated[c]
1.44 ± 0.2	2.09 ± 0.5[d]	2.27 ± 0.5[d]

[a]Mean ± standard deviation of at least six separate experimental determinations.

[b]0.5 mg glucan i.v., single injection, liver weights determined 2-4 days after treatment.

[c]0.5 mg glucan i.v., single injection, liver weights determined 7-8 days after treatment.

[d]P = .001

Effects of glucan treatment on trapping and killing of C. albicans I and II by perfused mouse livers

Livers from glucan-treated mice showed no enhancement in killing of C. albicans I either two to four days or seven to eight days post treatment, or C. albicans II at seven to eight days (Table 5). Trapping in glucan-treated animals was also similar to normal animals.

Effects of normal calf serum and Candida-specific bovine immune serum on trapping and killing of C. albicans I and II by livers from glucan-treated animals

Trapping of C. albicans I perfused with NCS through glucan-treated livers averaged 93.8% (Table 6). Total recovery declined to 70.2% suggesting a 6.9% increase in killing (22.9% vs. 29.8%; P = 0.05). Trapping of C. albicans II perfused with NCS through glucan-treated livers averaged 99.8% (Table 6), however, total recovery fell to 76.4% and killing increased by 8.5% (15.1% vs. 23.6%; P = 0.01).

Table 5. Trapping and killing of C. albicans I and II by glucan treated mouse livers.[a]

	Recovery %				
	C. albicans I			C. albicans II	
Experimental	Normal	Treatment I[b]	Treatment II[c,d]	Normal	Treatment II[b]
Liver	70.6 ± 4.6	71.5 ± 3.0	68.6 ± 7.2	83.6 ± 8.9	75.9 ± 7.7
Perfusate	6.5 ± 4.6	5.8 ± 5.5	5.7 ± 1.6	1.2 ± 1.8	3.7 ± 2.3
Total	77.1 ± 4.6	77.3 ± 4.4	74.3 ± 7.1	84.9 ± 9.2	79.6 ± 8.5
Killing	22.9 ± 4.6	22.7 ± 4.4	25.7 ± 7.1	15.1 ± 9.2	20.4 ± 8.5
Trapping	93.5 ± 4.6	94.2 ± 5.5	94.3 ± 1.6	98.7 ± 1.9	96.3 ± 2.3

[a]Mean ± standard deviation of at least six separate experimental determinations.

[b]0.5 mg glucan i.v., single injection, perfusions done two to four days after treatment.

[c]0.5 mg glucan i.v., single injection, perfusions done seven to eight days after treatment.

[d]Mean ± standard deviation of five separate experimental determinations.

Table 6. Effects of normal calf serum and *Candida* specific bovine immune serum on trapping and killing of *C. albicans* I and II by glucan treated mouse livers.[a]

	Recovery %					
	C. albicans I			*C. albicans* II		
Experimental	Normal	NCS	BIS	Normal	NCS	BIS
Liver	70.6 ± 4.6	64.0 ± 6.4[c]	58.3 ± 11.2[c]	83.6 ± 8.9	76.2 ± 9.1	65.4 ± 8.3[b]
Perfusate	6.5 ± 4.6	6.2 ± 5.2	0.9 ± 1.5[b]	0.9 ± 1.5[b]	0.2 ± 0.5[b]	0.3 ± 0.6[c]
Total	77.1 ± 4.6	70.2 ± 6.1[c]	59.2 ± 12.3[b]	84.9 ± 9.2	76.4 ± 9.1[c]	65.7 ± 8.4[b]
Killing	22.9 ± 4.6	29.8 ± 6.1[c]	40.8 ± 12.3[b]	15.1 ± 9.2	23.6 ± 9.1[c]	34.3 ± 8.4[b]
Trapping	93.5 ± 4.6	93.8 ± 5.2	99.1 ± 1.5[b]	98.7 ± 1.9	99.8 ± 0.5	99.7 ± 0.6

[a] 0.5 mg glucan i.v., single injection, perfusions done seven to eight days after treatment. Mean ± standard deviation of at least six separate experimental determinations.

[b] $P = .01$ vs. normal livers.

[c] $P = .05$ vs. normal livers.

Serum alone was not responsible for the increased killing. When either isolate was incubated with NCS and perfused in the absence of livers total recovery of a 10^7 dose averaged 109.8% and 118.7% for C. albicans I and II respectively (data not shown).

Glucan-treated livers perfused with BIS trapped 99.1% of C. albicans I and 99.7% of C. albicans II after a single pass. The increase in trapping is significant for isolate I, but not for isolate II (probably because of the already high percentage of C. albicans II trapped by normal livers (Table 6).

Comparing normal livers in the absence of serum to livers from glucan-treated animals perfused with BIS recovery of C. albicans I from the liver declined 12.3% and recovery from the perfusate declined 5.6% suggesting that killing increased 17.9% (Table 6). In all cases the observed differences are significant. The results for C. albicans II were similar. Compared to normal livers perfused with M-199, total recovery fell to 65.7% and killing increased 19.2% (Table 6). Recovery from the liver decreased 18.2% and from the perfusate 0.9%. All differences are significant.

Effects of mannose, glucose, and mannitol on trapping and killing of C. albicans I and II by normal perfused mouse livers

Glucose and mannitol had no effect on trapping of either C. albicans I or II (Tables 7 and 8). In contrast, livers perfused with 1% mannose trapped only 79.2% of C. albicans I, a significant decrease of 14.3% (Table 7). In the presence of 5% mannose the percent trapped declined 41.1% (Table 7). This represents an additional decrease of

Table 7. Effects of mannose, glucose, and mannitol on trapping and killing of C. albicans I by normal mouse livers.[a]

		Recovery %			
Experimental	Normal	1% D-mannose	5% D-mannose	1% D-glucose	1% D-mannitol
Liver	70.6 ± 4.6	53.1 ± 9.0[b]	33.8 ± 18.1[b,c]	72.0 ± 12.2	71.1 ± 2.2
Perfusate	6.5 ± 4.6	21.4 ± 7.2[b]	47.7 ± 19.3[b,c]	10.9 ± 5.1	6.6 ± 3.3
Total	77.1 ± 4.6	74.0 ± 7.4	81.4 ± 6.6	82.9 ± 11.4	77.6 ± 4.0
Killing	22.9 ± 4.6	26.0 ± 7.4	18.6 ± 6.6	17.2 ± 11.4	22.4 ± 4.0
Trapping	93.5 ± 4.6	79.2 ± 6.1[b]	52.5 ± 19.3[b,c]	89.2 ± 5.1	93.4 ± 3.3

[a]Mean ± standard deviation of at least six separate experimental determinations.

[b]P = .001 vs. normal livers.

[c]P = .05 vs. 1% mannose perfused livers.

Table 8. Effects of mannose, glucose, and mannitol on trapping and killing of C. albicans II by normal mouse livers.[a]

	Recovery %				
Experimental	Normal	1% D-mannose	5% D-mannose[c]	1% D-glucose	1% D-mannitol
Liver	83.6 ± 8.9	75.0 ± 11.1[b]	72.5 ± 13.7[b]	79.1 ± 7.5	84.2 ± 7.8
Perfusate	1.2 ± 1.8	14.7 ± 9.3[b]	8.3 ± 4.3[b]	2.4 ± 1.5	2.0 ± 2.7
Total	84.9 ± 9.2	89.7 ± 5.2	80.7 ± 12.0	81.5 ± 7.5	86.3 ± 7.3
Killing	15.1 ± 9.2	10.3 ± 5.2	19.3 ± 12.0	18.5 ± 7.5	13.7 ± 7.3
Trapping	98.7 ± 1.9	85.3 ± 9.3[b]	91.8 ± 4.3[b]	97.4 ± 1.5	97.5 ± 2.7

[a]Mean ± standard deviation of at least six separate experimental determinations.

[b]P = .05 vs. normal livers.

[c]NSS between 1% mannose and 5% mannose.

26.7% compared to livers perfused with 1% mannose. Both comparisons are significantly differnet, however, total recovery and killing are the same for mannose perfused and M-199 perfused livers. Declines in trapping were reflected in a decrease in the percent of yeast recovered in the liver and an increased recovery in the perfusate. Recovery from livers perfused with 1% mannose, compared to M-199, declined 17.5% and increased in the perfusate 14.9%. With 5% mannose recovery from the liver fell 36.8%, compared to M-199, and 19.3% compared to 1% mannose. Recovery from the perfusate increased 41.2% and 26.3% compared to M-199 and 1% mannose respectively.

A similar effect was observed with C. albicans II (Table 8), except that perfusion with 5% mannose did not have the same pronounced effects as was observed with isolate I. In the presence of 1% mannose normal livers trapped 13.4% less of a dose of C. albicans II. With 5% mannose a reduction of only 6.9% was observed, compared to M-199, but an increase of 6.5% was observed compared to 1% mannose. Recovery from livers perfused with 1% mannose declined 8.6% and from the perfusate increased 13.5%. These values are significantly different, although there was no difference in the percent total recovery or killed. With 5% mannose recovery from the liver fell 11.1%. Recovery from the perfusate increased 7.1%, but decreased 6.4% compared to M-199 and 1% mannose respectively. The differences between 5% mannose and M-199, but not those between 5% mannose and 1% mannose are significant.

DISCUSSION

While macrophages of the RE system represent the major, short term defense against invading microorganisms, their ability to contain C. albicans infection remains controversial. Evas et al. (48) and Myerowitz et al. (114) have reported increasing hepatic involvement in GI seeded cases of disseminated candidiasis. Liver Kupffer cells represent the largest group of fixed tissue macrophages in mammels (4, 35, 123), and therefore, could be postulated to play a major role in restricting hematogenous spread from the GI tract. There is general agreement in the literature that the liver is the major organ involved in bloodstream clearance of C. albicans (7, 47, 65, 114, 142).

The perfused liver model provides an opportunity to study hepatic-C. albicans interactions. Sawyer et al. (142) have shown that normal rat livers avidly trapped C. albicans, but no killing occurred even in the presence of homologous plasma or whole blood. In contrast, when C. albicans was perfused through normal mouse livers with only M-199 substantial killing of both isolates occurred (Table 1). Additionally, slight but significant differences in trapping and killing were observed. C. albicans I was less avidly trapped, but more efficiently killed than C. albicans II. Sasada and Johnson (140) recognized differential killing between C. albicans and C. parapsillosis by isolated mouse peritoneal macrophages. Enhanced C. albicans survivability correlated with its ability to limit macrophage oxidative metabolism.

Perhaps different C. albicans strains possess different abilities to limit macrophage oxidative metabolism and survive phagocytosis. Killing of C. albicans, presumably by Kupffer cells, is in agreement with the report of Lehrer et al. (83) which emphasized the candidicidal activity of isolated rabbit macrophages. Resident alveolar macrophages killed 28% and resident peritoneal macrophages 15% of a similar inoculum of C. albicans used in this study.

Crystalline silica is a macrophage specific toxin (54). Friedman and Moon (54) used the compound to deplete mouse livers of Kupffer cells and subsequently reduced trapping and killing of S. typhimurium in perfused livers. Sawyer (143) used silica to inhibit killing of C. albicans in macrophage depleted livers of C. parvum-treated rats. In agreement with these results, silica treatment abolished killing and reduced trapping of C. albicans in perfused mouse livers (Table 2). Silica mediated abrogation of killing highlights two concepts critical to these perfusion studies. First, it strongly supports the assumption that non-recoverable yeast are killed by the liver. Second, it emphasizes a role for the Kupffer cell in mediating killing. It could be postulated that hepatic killing was mediated by blood-borne PMN. The fact that silica abolished killing and that livers were thoroughly washed free of blood prior to infusion of yeast argues strongly against this possibility. In vitro studies with isolated Kupffer cells would further clarify if these cells possess candidicidal activity.

Sawyer (143) demonstrated that PB completely abrogates killing of C. albicans by perfused rat livers. Killing in the perfused mouse liver was not inhibited by PB (Table 2). Kjosen et al. (73) demonstrated that PB inhibits killing through an effect on the MPO-system.

Lehrer et al. (83) could not block killing of C. albicans by rabbit macrophages with addition of PB to incubating cells and concluded the candidicidal activity of rabbit macrophages did not rely on the MPO-system. The same conclusion may be true for mouse Kupffer cells. Additional work using other agents (cyanide, azide, sulfadiazone) inhibitory to the MPO-system is needed, however.

Specific immune serum, but not normal serum enhanced trapping and killing of C. albicans I by normal livers (Table 3). Actual differences, though slight, are significant. Neither NCS nor BIS had an effect on hepatic interactions with C. albicans II (Table 3). The results for isolate I are similar to the reports of Maita et al. (89) and Bonventre and Oxman (16) which emphasized enhanced candidicidal (89) and bactericidal (16) activity of macrophages in the presence of immune serum. It can be postulated that increased trapping in the presence of immune serum is facilitated by enhanced adherence of opsonized yeast to Fc receptors on Kupffer cell surfaces (108-110). Perfusions with immune serum in livers treated with pronase or mercaptoethanol to cleave receptors and silica-poisoned livers depleted of Kupffer cells would help to delineate the mechanism.

Why BIS had no effect on killing of C. albicans II by normal livers is puzzling, especially since immune serum exhibited a strong degree of cross-reactivity and increased the candidicidal activity of glucan-treated livers (Table 6).

A single dose of glucan enhanced carbon clearance (Appendix A) and increased liver weights (Table 4). These results indicate that a single dose of glucan can stimulate at least some of the effects typically associated with multiple doses of the drug (5, 6, 105, 127,

177). Enhanced candidicidal activity was not evident in livers from glucan-treated animals in the absence of serum (Table 5). Increased killing was demonstrated in glucan-treated livers in the presence of serum (Table 6). The addition of either NCS or BIS to the perfusion medium potentiated killing of both isolates. Bovine immune serum produced the greatest increase in killing. Together, the results suggest that serum is required for manifestation of glucan enhanced killing of C. albicans by perfused livers. Sawyer (143) reported similar results accentuating a requirement for plasma opsonin for manifestation of increased candidicidal activity of C. parvum stimulated rat livers.

Two points critical to an explanation of glucan enhanced hepatic killing are necessary. Glucan administration is proceeded by a massive influx of mononuclear cells into the liver (33). It has been observed that this influx is largely responsible for the hypertrophy and hyperfunction associated with glucan treatment (38, 127, 128), however, on an individual cellular basis, mononuclear phagocytes isolated from glucan-treated livers manifest a decreased phagocytic potential (38). Additionally, although serum opsonins are not involved in glucan induced alterations of the RE system per se, they are required for maximum phagocytosis by isolated RE cells (37, 38, 105). It can be envisaged that the absence of enhanced C. albicans killing by glucan-treated livers in the absence of serum reflects an inability of new phagocytic cells to phagocytize the yeast. It should be noted that an hepatic PMN influx has never been described following glucan administration, therefore subsequent killing should not be attributed to them.

Contrary to the reports of Sawyer et al. (142) that hepatic trapping of C. albicans by normal rat livers involves predominately

non-phagocytic parameters, the high degree of killing in normal mouse livers (Table 1) and the reduction in trapping caused by silica treatment (Table 2) suggests that trapping in mice includes phagocytosis by Kupffer cells. This is consistent with the report of Meister et al. (97) which noted that viable C. albicans was avidly phagocytized and subsequently degraded by mouse Kupffer cells following injection into whole animals. Kupffer cell phagocytosis in perfused livers has also been described for cancer cells (136) and foreign RBC (112). Trapping does not appear to be exclusive of non-phagocytic events, however. Consistent with previous reports (54, 143) Kupffer cells were necessary for maximum trapping, but some trapping was still evident in Kupffer cell depleted livers.

Trapping, but not killing of C. albicans was also impaired by perfusion with D-mannose, but not D-glucose or D-mannitol (Tables 7 and 8). Previous reports have described the existence of a mannose sensitive receptor which facilitates the adherence of different Candida species to different granulocytes in vitro. Binding was impaired by yeast mannan (33) and mannose (168). If the adherence of C. albicans to mouse Kupffer cells is mediated by a similar receptor it would seem likely that killing in the presence of mannose would be reduced. However, it is premature to reject the idea of such a receptor. More conclusive results could probably be obtained by perfusion studies with mannan.

Sawyer et al. (142) have described the trapping of C. albicans in normal rat livers. Yeast became 'log jammed' in sinusoidal spaces and appeared to be adhering to endothelial cells of the sinusoidal wall. Another possible explanation for the mannose sensitive trapping of C.

albicans reported here is that there are endothelial cell surface receptors which promote adherence through interactions with the yeast surface. Evidence recently reported by Day et al. (30) support this concept. They observed that hepatic clearance of IgM:BSA immune complexes in rat livers was impaired by pre- or coinjection of mannan.

There are great disparities in the degree of trapping between C. albicans I and II in silica-treated livers (Table 2) and livers perfused with mannose (Tables 7 and 8). Further investigation is needed to explain this phenomenon. It may be that receptors, if present, have different affinities for the surface of the different isolates. Physical differences between the two surfaces may also present steric hindrances. Especially puzzling is why silica treatment reduced trapping of C. albicans I to a much greater extent than C. albicans II. Silica treatment reduced trapping and increased the percent recovery from the perfusate of isolate II by an amount approximately equal to normal killing. This is not true for isolate I. If, in addition to chemical and phagocytic interactions, phsycial restriction is involved in trapping, morphological features of C. albicans II may restrict its passage through the liver and account for a large proportion of trapping. Morphological differences between the two isolates suggests this may be true. Under the light microscope C. albicans I consisted primarily of discrete, individual, elliptical cells. Individual colony forming units of C. albicans II were composed of elongated, multi-budding units and therefore larger.

APPENDICES

APPENDIX A

EFFECTS OF C. PARVUM AND GLUCAN TREATMENTS ON CARBON CLEARANCE IN MICE

Carbon clearance studies were performed in Harlan ICR female mice weighing 20 to 25 g and done by the method of Biozzi et al. (11). Clearance rates were compared between normal, C. parvum-treated, and glucan-treated animals. C. parvum treatment consisted of a single i.v. injection of 0.35 mg C. parvum vaccine (Burroughs Wellcome Co., Research Triangle, N.C.) ten days before carbon clearance studies. Glucan treatment consisted of a single i.v. injection of 0.5 mg glucan two days before carbon clearance studies. Carbon clearance was done as follows: 5 mg colloidal carbon (Pelikan carbon suspension C/11/143/1, Gunther Wagner, Hanover, Germany) was injected i.v. Blood samples were drawn from the retro-orbital plexus after 2 and 15 min intervals and 0.05 ml samples were lysed in 4 ml of 0.1% Na_2CO_3. The concentration of carbon was determined photometrically using an Hitachi Perkin Elmer spectrophotometer with tungsten light at wavelength 650 nm. The equation, K (phagocytic index)=$(\log C_1-\log C_2)/(t_2-t_1)$, where C represents the blood colloidal carbon and t time, was used to determine the phagocytic index. To calculate the biological half-life ($t_{1/2}$), the equation $t_{1/2}=0.301/K$ was used.

In normal mice K equals 0.053 which is equivalent to a half-life of 6.32 min (Table 1). In C. parvum treated mice K equals 0.12 which

is equivalent to a half-life of 2.76 min. In glucan treated mice, K equals 0.14 which is equivalent to a half-life of 2.26 min. Both the phagocytic index (K) and the biological half-life ($t_{\frac{1}{2}}$) for C. parvum-treated and glucan-treated mice were significantly different compared to normal mice suggesting non-specific stimulation of the reticuloendothelial system.

Table A1. Effects of C. parvum and glucan treatments on carbon clearance in mice.[a]

Treatment	Phagocytic Index	Biological Half-life (mins.)	P
Normal	0.053	6.32	--
C. parvum	0.120	2.76	P=.05
Glucan	0.140	2.26	P=.05

[a]Mean value from at least six separate experimental determinations.

APPENDIX B

QUANTITATIVE COMPARISON OF THE PERCENT OF VIABLE C. ALBICANS RECOVERED FROM LIVER HOMOGENATES AND BLENDED LIVER HOMOGENATES

A major criticism of perfusion experiments is the presumption that non-recoverable organisms are killed, i.e. it is difficult to prove killing without a corpse. To help verify that the percent of C. albicans recovered from the liver homogenate actually reflects true values and the percent of Candida presumed killed is real and not artifactual due to aggregation of the yeast an additional control was performed. Normal CD-1 mice were perfused with both isolates of Candida. After homogenization the total liver homogenate was diluted 1:10 in 90 ml saline and blended (approximately 30 sec) in a Waring blender. Quantitative pour plates of the blended-homogenate were compared to values obtained by homogenization of livers only (Table 1). The results obtained from this procedure indicate that homogenization of livers was sufficient to disrupt any microaggregates of yeast, if present, and supports the presumption that killing is real and not artifactual. There is no significant difference in the calculated values for any parameter between the two methods. At first glance, there would appear to be marked differences in the percentages of C. albicans II recovered between the two methods. However, the discrepancy can be explained by the wide range of values derived from different animals.

Table B1. Quantitative comparison of the percent of viable C. albicans recovered from liver homogenates and blended liver homogenates.[a]

	Recovery %			
	C. albicans I		C. albicans II	
Experimental	Homogenize only	Homogenize & blend	Homogenize only	Homogenize & blend
Liver	70.6 ± 4.6	77.3 ± 4.3	83.6 ± 8.9	90.8 ± 4.4
Perfusate	6.5 ± 4.6	3.3 ± 0.9	1.2 ± 1.9	0.7 ± 1.4
Total	77.1 ± 4.6	80.5 ± 4.7	84.9 ± 9.2	91.5 ± 4.6
Killing	22.9 ± 4.6	19.5 ± 4.7	15.1 ± 9.2	8.5 ± 4.6
Trapping	93.5 ± 4.6	96.7 ± 0.9	98.7 ± 1.9	98.8 ± 1.4

[a]Mean ± standard deviation of at least six separate experimental determinations.

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