

Comparison of Serum Mannan, Arabinitol, and Mannose in Experimental Disseminated Candidiasis

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Concentrations of arabinitol, mannose, and mannan in serum have independently been reported to be elevated in patients with invasive candidiasis. These three marker substances were compared in a rabbit model. Twelve rabbits, immunosuppressed with cortisone, were infected intravenously with *Candida albicans* 3181A. Six uninfected control animals also received cortisone, and four rabbits were neither infected nor immunosuppressed. Blood samples, drawn from 2 days before to 14 days after infection, were assayed for serum mannan by sandwich enzyme immunoassay, antibodies to mannan by indirect enzyme immunoassay, arabinitol and mannose by gas-liquid chromatography, and serum creatinine. Serum mannan, negative before infection, peaked in all infected animals 4 days after infection (mean, 18 ng/ml) and decreased thereafter. Significant increases (2 standard deviations greater than mean in normals) in arabinitol, the arabinitol/creatinine ratio, and mannose were found in 12, 8, and 12 of the infected rabbits, respectively, but also in all 6 uninfected animals receiving cortisone. Only serum mannan was specific in this immunosuppressed rabbit model.

Disseminated candidiasis is an important cause of morbidity and mortality in immunosuppressed patients, especially those with leukemia. Autopsy data have shown that the incidence can be as high as 34% (20). Increasingly aggressive chemotherapy results in prolonged neutropenia and increased susceptibility to infection. Because of immunosuppression, tests for antibody to *Candida* species are often falsely negative among leukemic patients (5, 7, 9, 19). However, colonization with *Candida* species often gives positive results in conventional serological tests. Because of these shortcomings, considerable attention has been directed to tests based on the direct detection of *Candida* antigens or metabolites in body fluids. Among these, three methods have independently been described; enzyme immunoassay (EIA) or radioimmunoassay for the detection of mannan (8, 13, 16, 21, 22, 24-26), gas-liquid chromatography (GLC) for the detection of arabinitol (1, 4, 11, 23, 28, 29), and GLC detection of mannose (15, 17, 18). Although all three techniques seem promising, their sensitivities, specificities, and predictive values have not been compared in immunosuppressed animals or humans. Furthermore, it has not been determined whether mannose accumulates in renal insufficiency, as is the case for arabinitol (28). Because invasive candidiasis results in renal insufficiency (29), it must be determined that increases in arabinitol and mannose are not an effect of decreased renal function rather than an effect of candidiasis. The goals of the present study were therefore as follows: (i) to quantitate and compare the in vitro production of mannan, arabinitol, and mannose by *Candida albicans* 3181A; (ii) to compare the appearance of these three substances in immunosuppressed rabbits experimentally infected with *C. albicans* 3181A; and (iii) to evaluate the effect of renal insufficiency and cortisone acetate administration on

arabinitol and mannose concentrations in the serum of uninfected rabbits.

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MATERIALS AND METHODS

In vitro production of mannan, arabinitol, and mannose. *C. albicans* 3181A, a serotype A human isolate obtained in 1971 and stored lyophilized at -40°C , was used in all experiments. Logarithmic-phase cells were added in appropriate numbers to both pooled normal rabbit serum (60 ml in a 250-ml flask) and to yeast nitrogen base (Difco Laboratories, Detroit, Mich.) supplemented with 3 g of glucose per liter (250 ml in a 1-liter flask) to yield an initial viable count of 1.0×10^4 CFU/ml. Uninoculated media served as controls. Cultures were incubated on a gyratory shaker (New Brunswick Scientific Co., Inc., Edison, N.J.) at 37°C and at 150 rpm, and 5-ml portions were removed at 0, 6, 12, 24, 48, and 72 h. Optical density was measured at 550 nm (Coleman Junior Spectrophotometer; Coleman Instruments Corp., Maywood, Ill.) by using uninoculated media as a blank, and viable counts were determined by serial dilutions in 0.9% NaCl and plating out on Sabouraud glucose medium. Before assay for mannan, arabinitol, and mannose, samples were centrifuged at $1,500 \times g$ for 10 min, and the supernatants were filtered through a $0.45\text{-}\mu\text{m}$ filter (Millipore Corp., Bedford, Mass.).

Effect of renal insufficiency on arabinitol and mannose concentrations in serum of uninfected rabbits. Eight female New Zealand white rabbits (2 to 3 kg) were obtained from the Animal Resources Branch, Centers for Disease Control, Atlanta, Ga. These animals are specific pathogen-free. After a base-line bleeding, bilateral nephrectomies were carried out with sterile precautions, using an anterior approach under halothane anesthesia. Blood samples were drawn at 24, 48, 72 and 96 h, and survivors were exsanguinated at 96

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h. Absence of postoperative sepsis was documented at autopsy. Serum samples were assayed for creatinine with an automated analyzer (Gilford Instrument Laboratories Inc., Oberlin, Ohio), and arabinitol and mannose concentrations were determined by GLC.

Disseminated candidiasis in the immunosuppressed rabbit. Twelve female New Zealand white rabbits (2 to 3 kg) were immunosuppressed with daily subcutaneous injections of cortisone acetate (10 mg/kg per day), beginning 2 days before and ending 7 days after infection. After two base-line bleedings from the central ear artery, these animals were infected intravenously with 1.0×10^7 blastoconidia of *C. albicans* 3181A. This inoculum was prepared by suspending a 24-h slant culture on Sabouraud glucose medium with 3 ml of 0.9% NaCl, counting the suspension in a hemacytometer, and diluting to yield 1.0×10^7 CFU/ml. The number of viable cells injected was confirmed by serially diluting the inoculum cell suspension and plating out on Sabouraud glucose medium. Blood samples were then drawn every 2 days until death, and survivors were sacrificed by exsanguination 14 days after infection. Autopsies were done on all animals, and both kidneys and a sample of liver were immediately removed and divided for quantitative culture and histopathology.

Quantitative culture was done by homogenizing the tissue in 5 ml of phosphate buffered saline, pH 7.2, and serially diluting and plating out the homogenate on Sabouraud glucose medium. A sample of each organ was also placed in 10% phosphate-buffered Formalin, pH 7.0, and stained by the hematoxylin and eosin, Gomori methenamine silver, and periodic acid-Schiff procedures. With each run of slides, tissue sections containing known noncarminophilic fungi were included as positive stain controls for the Gomori methenamine silver procedure.

Uninfected control animals were divided into two groups. Six rabbits received cortisone acetate but were not infected. An additional four animals were neither infected nor immunosuppressed. Quantitative stool cultures were carried out for all control animals. Stools were removed within the hour from urine-free areas of a clean pan, homogenized in phosphate-buffered saline, serially diluted, and then plated out on Sabouraud glucose medium supplemented with chloramphenicol. Quantitative culture and histopathology of both kidneys and liver in these control animals were also carried out.

Serum samples from all infected and control rabbits were assayed for creatinine, mannan, antibodies to mannan, arabinitol, and mannose.

GLC method for simultaneous determination of serum arabinitol and mannose, double-antibody sandwich EIA for serum mannan, and indirect EIA for antibodies to mannan. These assays were performed as previously described (2, 12, 22). In the GLC method, the minimum sensitivity was 0.1 $\mu\text{g/ml}$ and the mean coefficient of variation within the concentration range from 0.39 to 50 $\mu\text{g/ml}$ was 10.4%.

GLC-MS. GLC-mass spectrometry (GLC-MS) analyses were performed on a Finnigan 4000 GLC-MS system with an INCOS 2300 data system. The gas chromatograph was equipped with a 25-m SE-54 cross-linked fused silica capillary column (inner diameter, 0.32 mm; film thickness, 0.52 μm). Helium was used as the carrier gas at a linear velocity of 61 cm/s at 100°C. The column oven temperature was held at 150°C for 1 min and then increased at a rate of 4°C/min to 275°C. The ionizer and injector temperatures were maintained at 250°C, the separator temperature was at 270°C, and the manifold temperature was at 120 to 140°C. The electron

multiplier voltage was 1,200 V, the ionization voltage was 70 eV, and the source emission current was 0.5 mA. The mass range of *m/e* 50 to *m/e* 600 was scanned with a scan time of 2 s. Injections were made in the splitless mode.

Statistics. The concentrations in serum of mannose adjusted for renal function and the arabinitol/creatinine ratios were compared by the Mann-Whitney U test.

RESULTS

In vitro production of mannan, arabinitol, and mannose by *C. albicans* 3181A. The in vitro production of mannan, arabinitol, and mannose in yeast nitrogen base supplemented with 3 g of glucose per liter is shown in Fig. 1. Mannan increased in parallel with viable count and optical density in this culture medium. However, no significant production of arabinitol or mannose was found compared with that in uninoculated medium. Similar results were found with pooled normal rabbit serum, except that arabinitol increased to 76, 27, and 35 $\mu\text{g/ml}$, respectively, after 24, 48, and 72 h of incubation. Concentrations of mannose did not increase in pooled normal rabbit serum.

Effect of renal insufficiency on arabinitol and mannose concentrations in uninfected rabbit serum. Both mannose and arabinitol accumulated as a result of renal insufficiency after bilateral nephrectomy (Fig. 2). Linear regression showed a correlation coefficient of 0.82 for arabinitol and 0.81 for mannose ($P < 0.01$ for both regressions). Mannose concentrations in serum were adjusted for renal insufficiency by the equation: adjusted mannose concentration = measured mannose concentration - 2.41 (creatinine in serum) + 4.1. This equation subtracts from the measured mannose concentration that portion attributable to a creatinine amount in serum greater than 1.7 mg/dl, the mean concentration in normal animals. Adjustment for accumulation of arabinitol was made by calculating the arabinitol/creatinine ratio (28), expressed as arabinitol (micrograms per milliliter)/creatinine (milligrams per deciliter).

Disseminated candidiasis in the immunosuppressed rabbit. The survival of the 12 infected rabbits, as well as the results of our assays for serum mannan and mannan antibodies, are shown in Fig. 3. Serum mannan, uniformly undetectable before infection, peaked in all infected animals 4 days after infection and ranged from 4.2 to 66.3 ng/ml. Mannan antibodies, also negative before infection, began to rise 2 days after infection and coincided with decreasing serum mannan. All 12 animals showed antibodies to mannan. The range of peak reciprocal antibody titers was 64 to 1,024, and the mode was 256. Control animals had no detectable serum mannan or antibody to mannan.

Renal insufficiency (creatinine in serum greater than 2 mg/dl) appeared in 7 of the 12 infected animals (range, 2.2 to 8.1 mg/dl). Six of these seven animals died before the end of the experiment, whereas four of the five animals without renal insufficiency survived for 14 days after infection.

The results of the assays for mannose in serum, adjusted for renal function, are shown in Fig. 4. The infected animals had significantly increased ($P < 0.05$) concentrations compared with the nonimmunosuppressed, uninfected rabbits from 2 to 14 days after infection. However, noninfected animals receiving cortisone acetate also had significantly increased concentrations of mannose ($P < 0.05$). Ten days after infection, infected animals had greater concentrations ($P < 0.05$) of this substance than did uninfected animals receiving cortisone.

The arabinitol/creatinine ratios are shown in Fig. 5. As for the adjusted mannose, both the infected and uninfected

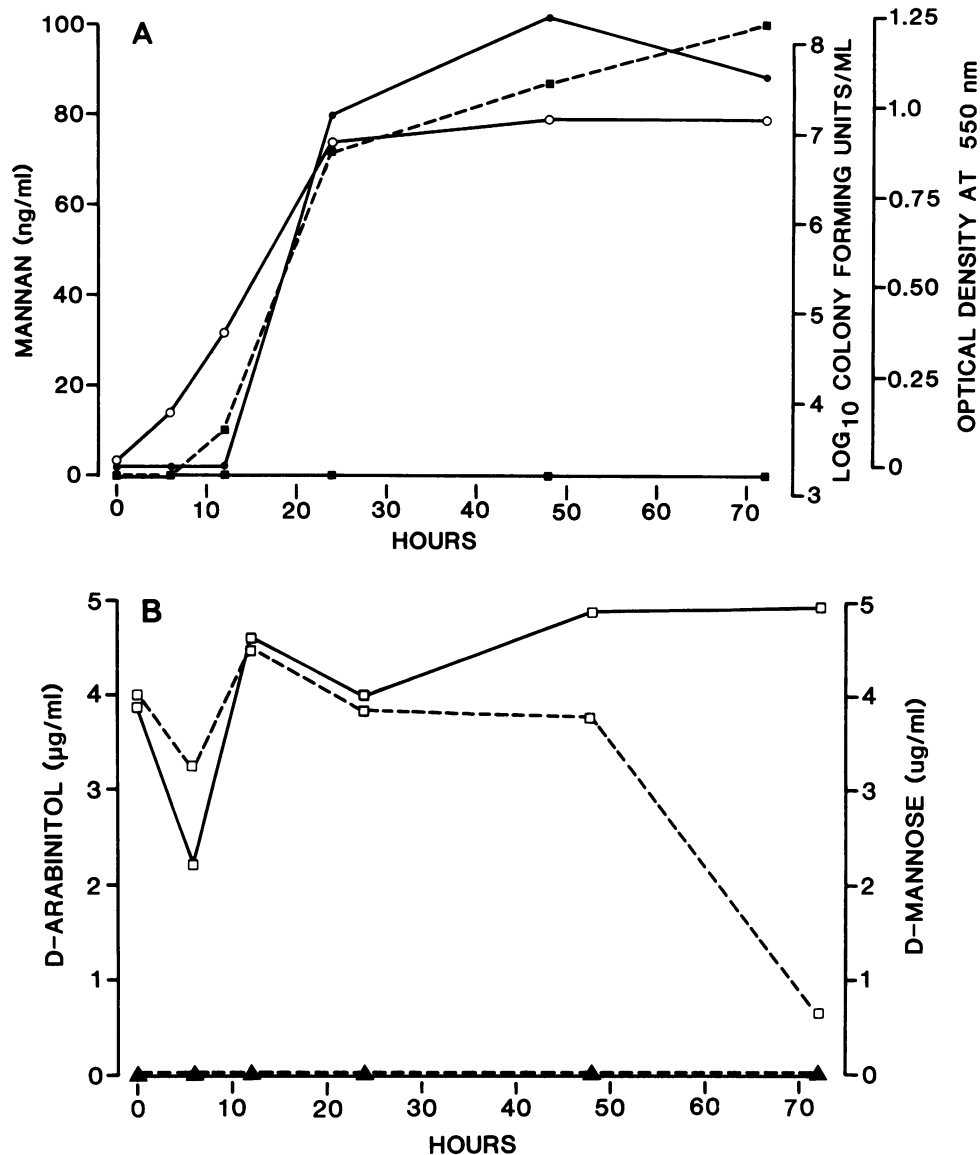


FIG. 1. In vitro production of mannan (A) and arabinitol and mannose (B) in yeast nitrogen base supplemented with 3 g of glucose per liter. Symbols: ○, log₁₀ CFU/ml; ●, optical density at 550 nm; ■, mannan in uninoculated (—) and inoculated (---) media; □, arabinitol in uninoculated (—) and inoculated (---) media; ▲, mannose in uninoculated (—) and inoculated (---) media.

animals receiving cortisone had significantly increased ratios ($P < 0.05$) compared with the uninfected, nonimmunosuppressed rabbits.

Overall, sensitivities (percentage of infected animals having a positive test on any day of the experiment) were mannan, 100%; arabinitol/creatinine ratio, 67%; adjusted mannose, 100%. Specificities, taking into account false-positives on any day of the experiment, in uninfected animals receiving cortisone were mannan, 100%; arabinitol/creatinine ratio, 0%; adjusted mannose, 0%.

Cultural and histopathological evidence of disseminated candidiasis was obtained at autopsy in all infected animals. Infected kidneys were greatly enlarged with multiple microabscesses. The average kidney weight was 27.0 g, compared with 11.1 g in uninfected animals receiving cortisone. Kidneys yielded 5.2×10^4 to 3.2×10^6 CFU of *C. albicans*

per g, whereas the livers contained 1.1×10^2 to 4.8×10^4 CFU/g. A progressive decrease in renal viable counts was observed between 5 and 14 days after infection.

Lesions in the kidneys of infected rabbits were similar to those described previously by others (10, 14, 27). Because the histological findings in the kidneys of each rabbit were similar, they will be described together.

At 7 to 14 days after infection, there was partial effacement of normal renal architecture by multifocal and confluent abscesses that contained abundant neutrophils mixed with typical blastoconidia, pseudohyphae, and hyphae of *C. albicans*. Abscesses appeared to begin in the interstitium of the cortices from fungal elements originating in intertubular blood vessels and capillary tufts of glomeruli. Renal abscesses in rabbits that survived for 14 days after infection were commonly rimmed by loose aggregates of epithelioid cells,

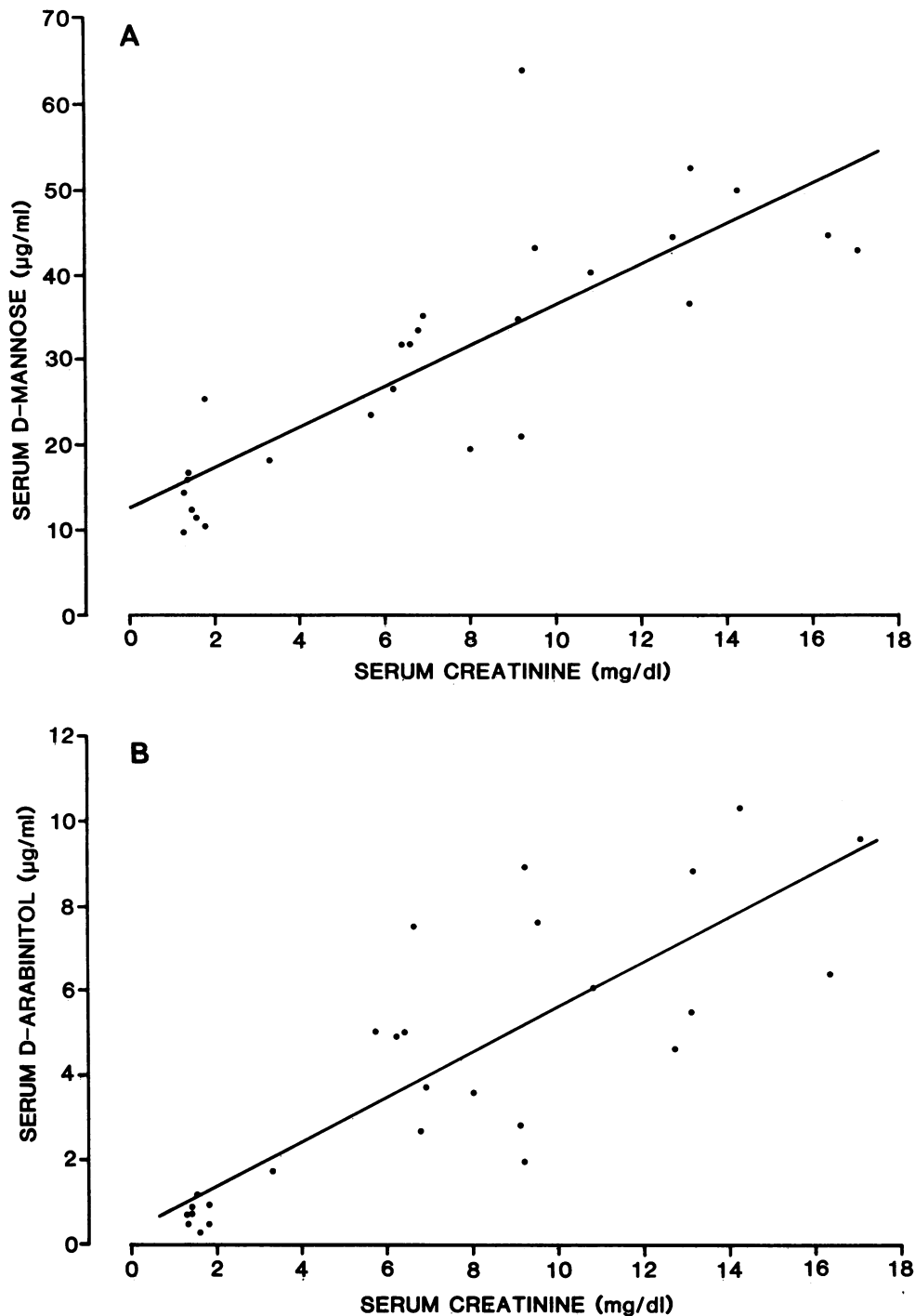


FIG. 2. Effect of renal insufficiency on serum mannose (A) and on serum arabinitol (B) in the bilaterally nephrectomized uninfected rabbit.

fibroblasts, and multinucleated giant cells of both Langhans and foreign body types. Compact aggregates of fungal elements were clustered within the huge giant cells, and renal tubules contiguous to abscesses were often distended by inflammatory cell debris and proliferating blastoconidia. Large numbers of candidal cells partially filled the renal pelvis in most 14-day-old infections. All fungal cells stained intensely by Gomori methenamine silver but were poorly

stained by hematoxylin eosin. With the latter, fungi were so weakly hematoxylinophilic that they were easily overlooked.

Neither inflammatory lesions nor candidal cells were detected in liver sections of three infected rabbits. Sections of liver from all other infected animals had one or more microabscesses or small solid granulomas that usually contained occasional blastoconidia and pseudohyphae. In addi-

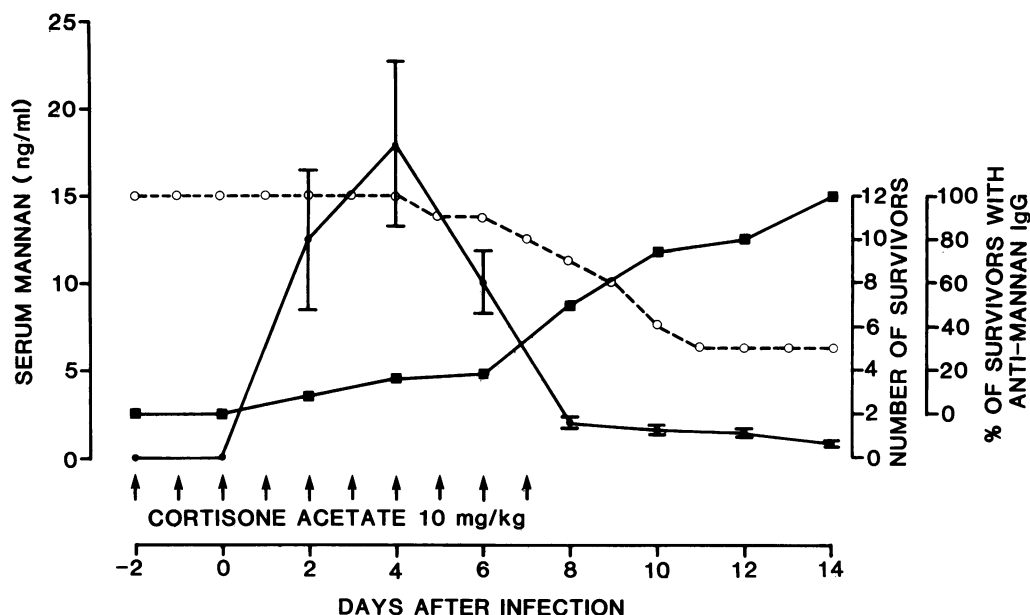


FIG. 3. Twelve rabbits infected intravenously with *C. albicans* 3181A on day 0. Symbols: ○, number of survivors; ●, mean mannan in serum \pm standard error of the mean; ■, percent of survivors with anti-mannan immunoglobulin G.

tion to these lesions, an interesting finding was the presence of blastoconidia and pseudohyphae in the hepatic blood vessels of one rabbit and blastoconidia within the Kupffer cells of two rabbits.

Culture and histopathology of the kidneys and livers in control animals showed no evidence of infection. However, the average kidney weight was greater in uninfected animals receiving cortisone (11.1 g) than in those that were not immunosuppressed (8.5 g). Stool cultures of uninfected rabbits receiving cortisone acetate or no immunosuppression showed an absence of growth of any *Candida* species or other yeasts.

GLC-MS. Selected sera were examined by GLC-MS to confirm the identity of the arabinitol and mannose peaks. This confirmation was especially important because ribitol and xylose could not be resolved from arabinitol on a column packed with OV-225 (2, 15). However, these three substances were separated on the GLC-MS system equipped with an SE-54 capillary column. Total ion monitoring of per-O-acetylated aldonitrile derivatives was done on sera from bilaterally nephrectomized uninfected rabbits, uninfected rabbits receiving cortisone, and infected rabbits receiving cortisone and on serum in which *C. albicans* had been grown in vitro. Xylose was not detected in any of these samples, and the concentration of ribitol was less than 5% of the arabinitol concentration in sera from nephrectomized uninfected rabbits, uninfected rabbits receiving cortisone, and infected rabbits receiving cortisone. When *C. albicans* was grown in pooled normal rabbit serum, however, both ribitol and arabinitol were produced, but the concentration of ribitol was only ca. 27% that of arabinitol. The identities of the arabinitol and ribitol peaks were confirmed by mass spectrometry. Confirmation of the mannose peaks was also obtained by GLC-MS.

DISCUSSION

Clinical diagnosis of disseminated candidiasis is often difficult (3), and tests that detect antibody often are negative

(5, 7, 9, 19) in profoundly immunosuppressed patients, who are most susceptible to invasion by this fungus. Efforts have therefore been made to develop tests that directly detect antigens or metabolites of *Candida* species. The three methods that have been described are mannan detection in serum by EIA or radioimmunoassay (8, 13, 16, 21, 22, 24-26), arabinitol detection by GLC (1, 4, 11, 23, 28, 29), and GLC detection of mannose (15, 17, 18). Although all three markers have independently been detected in experimental candidiasis (4, 8, 13, 15, 21, 25, 29), it has not been determined whether they appear in parallel. In the present report, we compared production of these three substances in growth media and in experimentally infected rabbits. An experimental model with rabbits, immunosuppressed with cortisone acetate, was used because of the difficulty in obtaining autopsy-proven human sera and to simulate the impaired immune response often found in patients with disseminated candidiasis. In addition, we evaluated the effect of renal insufficiency and cortisone acetate administration on the concentrations in serum of these three substances in uninfected control animals.

When *C. albicans* 3181A was grown in pooled normal rabbit serum and yeast nitrogen base supplemented with 3 g of glucose per liter, only mannan appeared in both media. The effect of morphology of *C. albicans* (blastoconidia or pseudohyphae) on the amount of solubilized mannan is unknown and would be interesting to determine in future studies. Arabinitol was produced in normal rabbit serum but not in the yeast nitrogen base medium, whereas mannose was not detected in either medium. These results are consistent with those of Wong et al. (29), who demonstrated production of arabinitol in pooled normal rat serum by *C. albicans* B311, and those of Bernard et al. (1), who showed that the range of arabinitol concentrations in yeast nitrogen base supplemented with 3 g of glucose per liter can be quite variable (0.36 to 51.9 $\mu\text{g/ml}$). However, the absence of detectable mannose production disagrees with the findings of Monson and Wilkinson (18) and may be due to differences

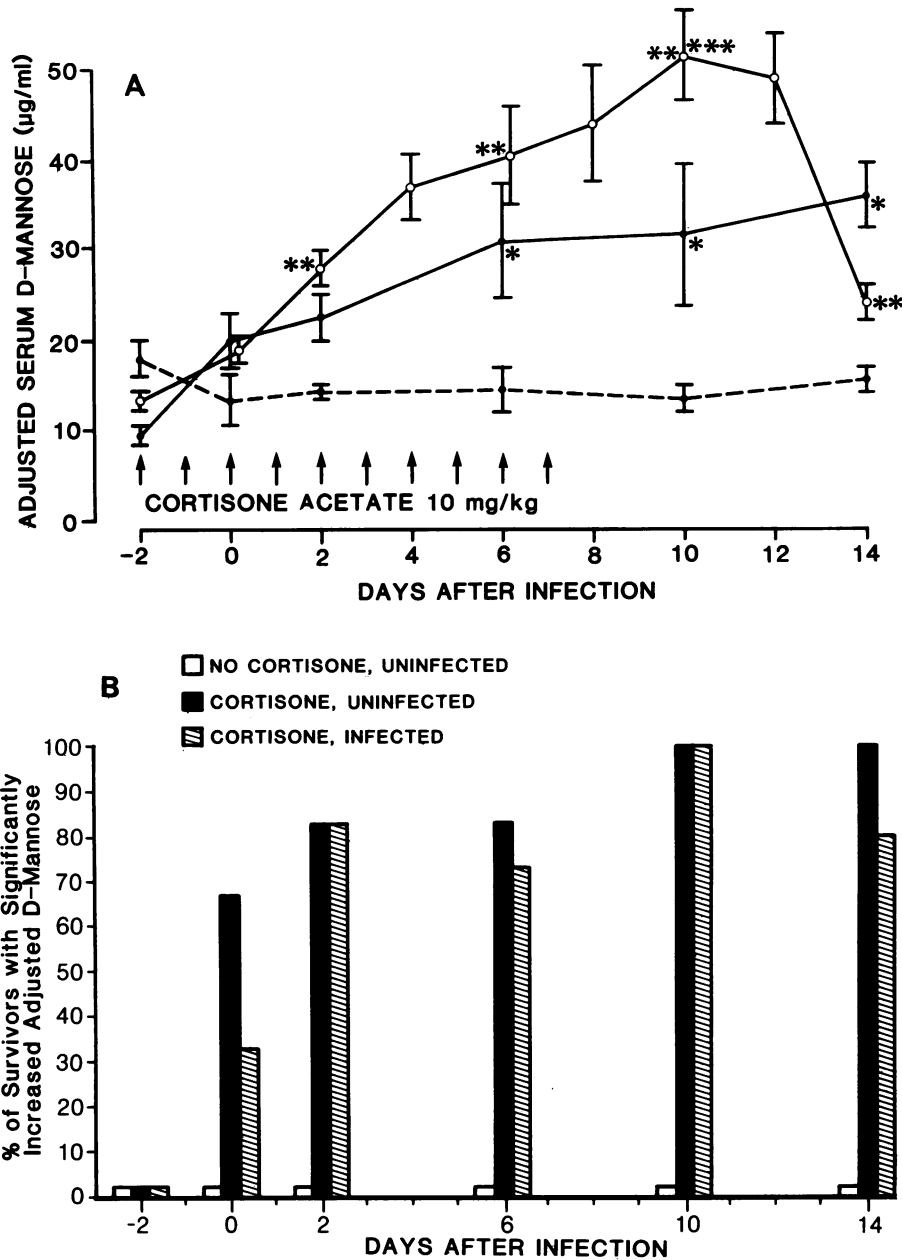


FIG. 4. (A) Mannose concentrations in sera, adjusted for renal function, in infected rabbits receiving cortisone (○—○), uninfected rabbits receiving cortisone (●—●), and nonimmunosuppressed, uninfected rabbits (●— — ●). Data are means ± standard error of the mean. Asterisks indicate significant difference ($P < 0.05$) between the following groups: *, between uninfected rabbits receiving cortisone and nonimmunosuppressed, uninfected rabbits; **, between infected rabbits receiving cortisone and nonimmunosuppressed, uninfected rabbits; ***, between infected and uninfected rabbits receiving cortisone. (B) Percentage of control and surviving infected animals with significantly increased (>2 standard deviations above mean value in normal animals) mannose concentrations, adjusted for renal function.

in growth media and in the strains of *C. albicans* used or may suggest that the detection of increased mannose concentrations in vivo depends on previous metabolism of mannan by the host.

Disseminated candidiasis results in renal insufficiency (29), and it must be ascertained that increases in arabinitol and mannose are not simply an effect of decreased renal function rather than an effect of candidiasis. It has been shown (28) that arabinitol clearance is identical to that of creatinine in serum and that the arabinitol/creatinine ratio

can be used to adjust arabinitol concentrations for renal function. However, it has not been determined experimentally whether mannose also accumulates in renal insufficiency. Monson and Wilkinson (18) studied four patients with renal insufficiency, all of whom had mannose concentrations of less than 20 µg/ml. In the present study, the results from the rabbits that had a bilateral nephrectomy confirm the earlier findings for arabinitol and demonstrate that mannose also accumulates in renal insufficiency. However, the mannose concentrations did not increase in direct proportion to

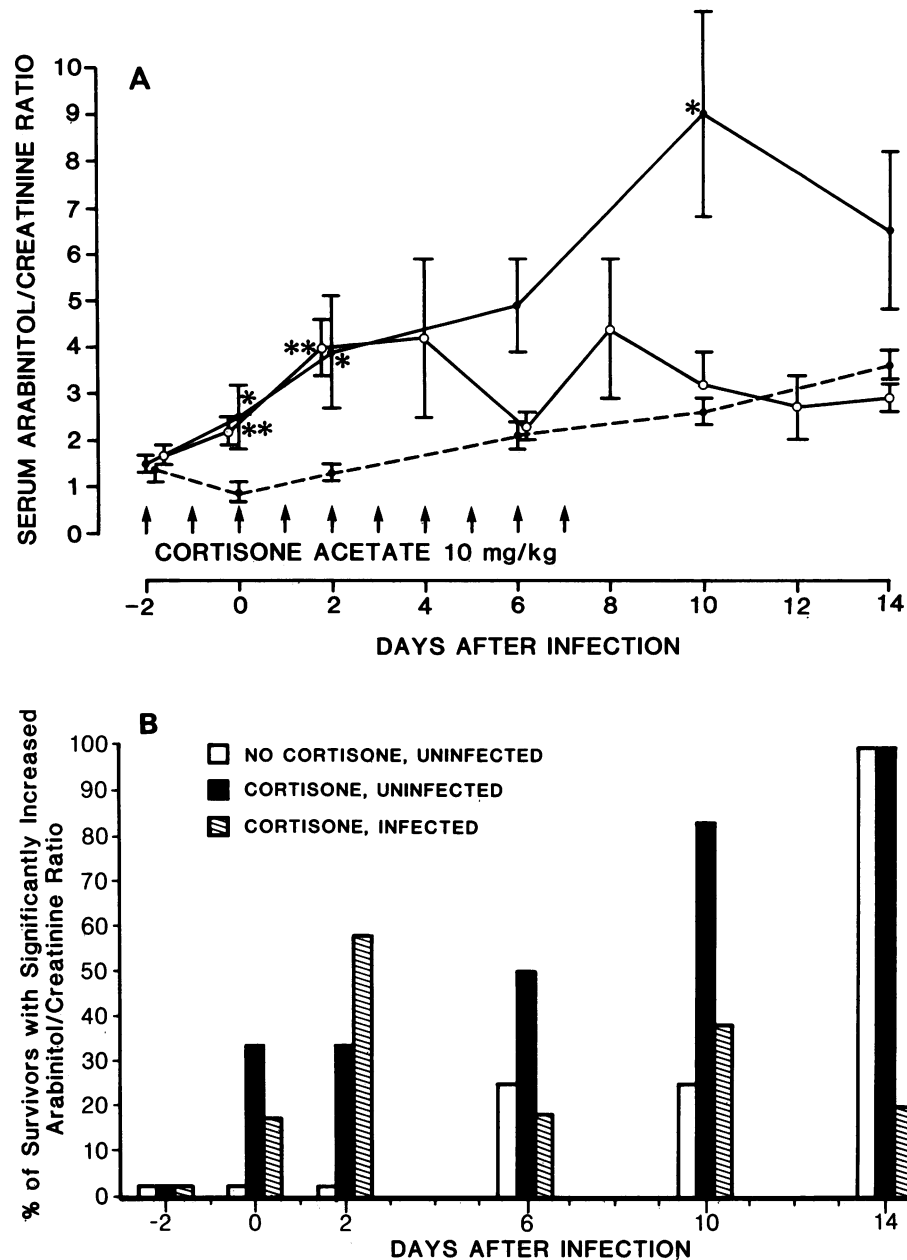


FIG. 5. (A) Arabinitol/creatinine ratios in sera of infected rabbits receiving cortisone (○—○), uninfected rabbits receiving cortisone (●—●), and nonimmunosuppressed, uninfected rabbits (●—●). Data are means \pm standard error of the mean. Asterisks indicate significant difference ($P < 0.05$) between the following groups: *, between uninfected rabbits receiving cortisone and nonimmunosuppressed, uninfected rabbits; **, between infected rabbits receiving cortisone and nonimmunosuppressed, uninfected rabbits. (B) Percentage of control and surviving infected animals with significantly increased (>2 standard deviations above mean value in normal animals) arabinitol/creatinine ratios.

creatinine in serum. Since mannose does not accumulate in a manner identical to arabinitol, a different means of adjusting concentrations was used, in which excess mannose attributable to renal insufficiency was subtracted from the measured mannose concentration.

Infection of *C. albicans* in rabbits immunosuppressed with cortisone uniformly resulted in disseminated candidiasis. All animals had either cultural or histological evidence, or both, of invasion by *C. albicans* in their kidneys and livers. The survival of the animals was prolonged compared with that

observed in most similar studies on detection of serum mannan (8, 13, 21, 25), arabinitol (4, 29), and mannose (15) in experimental candidiasis. Thus, this experimental model in the rabbit receiving cortisone may more closely resemble the often subacute course of candidiasis in humans. Furthermore, Weiner and Coats-Stephen (25) have previously shown that even nonlethal candidal infection can lead to increased mannan and anti-mannan antibodies. Thus, these experiments in the nonimmunosuppressed rabbit were not repeated.

Serum mannan peaked in all infected animals 4 days after infection and was followed by a progressive rise in antibodies to mannan. Weiner and Yount (26) also observed an early period of mannan antigenemia, followed by a rapid rise in antibody to mannan in four patients with systemic candidiasis. These results suggest that the decrease in mannan concentrations results from the formation or host elimination of immune complexes and support the need to dissociate sera before assay for mannan (22). Serum mannan was specific for candidiasis and could not be detected in uninfected animals receiving cortisone or no immunosuppression. Antibodies against mannan, undetectable before infection, appeared in all infected animals but not in controls. In humans, however, this test is not useful because, unlike antibodies in rabbits, antibodies are detectable both in normal persons and in patients with candidiasis (12).

There was no clear relationship between total CFU in the right or left kidneys and mannan concentrations in serum on the day of death. This may reflect variation in the ability of the animals to form immune complexes which would result in a more rapid clearance of the antigen.

Elevated concentrations of mannose, adjusted for renal function, and elevated arabinitol/creatinine ratios were observed in both infected and uninfected animals receiving cortisone acetate. This effect of cortisone in uninfected rabbits could be caused either by an overgrowth of *Candida* organisms as the result of immunosuppression or by modifications of carbohydrate metabolism induced by the corticosteroid. Cultural and histopathological studies of kidneys and livers and cultures of stools showed no evidence of *C. albicans* or other yeasts; these are facts in favor of the latter hypothesis. Monson and Wilkinson (18) showed that poorly controlled diabetics can have elevated concentrations of mannose in the absence of candidiasis, and this may also be related to the disturbance in carbohydrate metabolism. Furthermore, nonspecific increases in arabinitol have been found in patients with sarcoidosis (G. H. Karam, A. M. Elliott, S. Polt, and C. G. Cobbs, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, Miami Beach, Fla., abstr. no. 486, 1982). Although data on oropharyngeal colonization would have been interesting, a positive culture might have been difficult to interpret because the degree of colonization would not have been quantitated. In rats with experimental candidiasis, the arabinitol/creatinine ratio became elevated only when the total renal CFU was greater than 2.8×10^7 (29). Mannose concentrations were only transiently increased in infected animals when compared with those in uninfected rabbits receiving cortisone. The detection of such minimal differences would be impractical because of the need for repeated blood samplings.

Gold et al. (6) did not find increased arabinitol/creatinine ratios in 28 cancer patients without invasive candidiasis who were under corticosteroid therapy. This may be due to different effects of corticosteroids on carbohydrate metabolism in humans and in rabbits, to the relative dose of corticosteroid given to each species, or to the proximity of corticosteroid administrations and blood samplings.

The arabinitol/creatinine ratios of control rabbit serum that received neither steroids nor yeasts increased during the course of the experiment (Fig. 5). It is unlikely that this was the result of variation in the analytical method, because no rabbit studied before the beginning of the experiment had such a high arabinitol/creatinine ratio (22 animals). An alternate explanation might derive from our observation that the arabinitol/creatinine ratio increases in uninfected rabbits receiving cortisone acetate. It may be that the trauma of

repeated bleedings from the central ear artery produces stress and increased levels of endogenous corticosteroids in these animals.

Some variation was observed in the arabinitol/creatinine ratio in normal rabbits. However, in no case did the ratios approach those found in the uninfected rabbits receiving cortisone. In addition, we have measured the arabinitol concentration in 50 normal human blood donors. The mean \pm standard deviation for serum arabinitol was 0.45 ± 0.37 $\mu\text{g/ml}$. These results closely agree with those of Roboz et al. (23), who found a mean arabinitol concentration of 0.52 $\mu\text{g/ml}$ (standard deviation, ± 0.34) in 39 normal subjects, and with those of Gold et al. (6), who reported a mean \pm standard deviation arabinitol concentration in serum of 0.56 ± 0.32 $\mu\text{g/ml}$ in 52 control patients with normal renal function.

GLC assay methods, using columns packed with OV-225, have not been capable of resolving ribitol and xylose from arabinitol (2, 15). However, when these sugars were separated on a fused silica capillary column, ribitol and xylose could not be detected in serum of infected or uninfected rabbits receiving cortisone or in serum of bilaterally nephrectomized uninfected rabbits. These results agree with the findings of Roboz et al. (23), who studied sera from humans with candidiasis. They provide assurance that ribitol and xylose did not contribute to the observed increases in arabinitol in animals belonging to these experimental groups. However, production of ribitol and arabinitol by *C. albicans* in vitro in our study contradicts these same authors, who could not demonstrate in vitro the appearance of ribitol when *C. albicans* was grown in human serum (23). In the future, it would be advantageous to routinely assay arabinitol and mannose on a capillary column, which eliminates possible interference from ribitol and xylose.

Only serum mannan was found to be both a completely sensitive and specific marker of candidiasis in rabbits immunosuppressed with cortisone. Serum mannan detection by EIA has the disadvantage that immunological reagents must be prepared to do the assay but has the advantage of immunological specificity. Serum arabinitol and mannose are directly detected by GLC and do not require the preparation of antisera and enzyme conjugates. However, in our animal model they lack the specificity of the immunological test. Further studies will be needed to compare these methods in humans.

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