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Physiological and Metabolic Alterations Accompanying Systemic Candidiasis in Mice†

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Mice challenged intravenously with 10^6 viable *Candida albicans* died between 1 and 16 days after infection. Near the time of death, over 98% of the recoverable fungi came from the kidneys. Physiologically, animals were in renal failure near the time of death as evidenced by elevated blood urea nitrogen (BUN) and blood creatinine levels and a creatinine clearance rate which was about one-half normal. No abnormalities in liver glycogen and blood glucose levels were detectable. When mice were challenged with 4.5×10^6 viable *C. albicans*, they all died within 12 h. Near the time of death they had normal BUN values and were hyperglycemic. In mice receiving 4.5×10^6 heat-killed *C. albicans*, no deaths occurred and liver glycogen, blood glucose, and BUN levels all remained within a normal range and were different from responses to bacterial endotoxin. Cumulatively, the results demonstrate two distinct syndromes for the pathogenesis of experimental *C. albicans* infections. At the lower dose, mice were in renal failure associated with progressive renal infection. At the higher dose, renal failure was not observed. If a toxin was associated with death from the latter dose, it was not similar to bacterial endotoxin.

Serious gaps still remain in understanding the pathogenesis of systemic *Candida albicans* infections. Experimental studies in mice have demonstrated extensive histopathology and inflammation in a variety of host organs (1, 2, 19, 20, 30), most notably the kidneys (7, 19, 20, 26). Although it has been generally assumed that renal pathology is a primary factor in this disease (10, 21, 23), clear experimental data aside from histology are lacking. The absence of such data coupled with demonstrations of frequent and severe involvement of other organs (1, 4, 10, 19, 20, 23-25, 30) has led various authors to suggest that embolization (27, 28), toxemia (9, 15, 27), uremia (31, 32), pancreatic damage (34), myocardial damage (1, 24), or hypersensitivity reactions in the lungs (33) are responsible for death.

From both the literature (14, 22, 31, 33) and our own preliminary experiments, it appears that the type of host response to *C. albicans* depends on the number of yeasts injected. The objective of this study was to evaluate selected parameters of renal function and carbohydrate metabolism during systemic candidiasis in an effort to identify and compare significant physiological and metabolic alterations contributing to pathogenesis at both doses. Carbohydrate metabolism is of particular interest, since the pathogenesis of *C. albicans* infection may have effects similar to those of endotoxin (3, 6, 11, 12, 17, 22, 27).

MATERIALS AND METHODS

Mice. Female mice (HA/ICR) weighing 24 to 28 g were obtained from Spartan Research Animals, Haslett, Mich. They were housed six animals per cage, with water and food (Wayne Lab-Blox, Allied Mills, Inc., Chicago, Ill.) available ad libitum. Fasting animals had no food but had access to water. Bilaterally nephrectomized mice were given 0.85% saline instead of water.

Cultivation of fungi. A strain of *C. albicans* isolated from a case of vaginal candidiasis at Olin Health Center, Michigan State University, and identified by A. L. Rogers was used. Stock cultures were maintained on Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) slants at room temperature. To prepare inocula, a transfer was made from a slant into 100 ml of tryptic soy broth (Difco) supplemented with 4% D-glucose (Fisher Scientific Co., Fair Lawn, N.J.) in a 250-ml culture flask. This was incubated for 12 to 14 h with agitation at 37°C. Cells were harvested by centrifugation at 3,000 rpm for 10 min and then washed three times with sterile saline. A cell count was obtained with a hemocytometer and confirmed by pour plates. Dilutions for injection were made with saline.

Heat-killed *C. albicans* were prepared by incubation in a 56°C water bath for 1 h. Sterility checks were made by using pour plates.

Tissue distribution studies. To determine the number of viable organisms in various tissues of in-

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fected mice, the organs were aseptically removed, weighed, and placed in separate, sterile glass homogenizing tubes. Sterile saline was added to bring the total volume to 10 ml. Organs were homogenized with Teflon homogenizing pestles attached to a Tri-R Stir-R tissue homogenizer (model S63C, Tri-R Instruments, Inc., Rockville Centre, N.Y.), and pour plates of appropriate dilutions were made.

Blood urea nitrogen (BUN) assay. A heparinized syringe with a blunted 18-gauge needle was used to collect 0.1 ml of whole blood from the retroorbital plexus of each mouse. BUN was determined on whole blood by the method of Coulombe and Favreau (5). The red color which developed was quantitated spectrophotometrically at 530 nm (Gilford 240 spectrophotometer, Gilford Instrument Laboratories, Inc., Oberlin, Ohio). Concentrations of urea (Mallinckrodt, Inc., St. Louis, Mo.) standards ranging from 10 to 500 mg/100 ml were used to prepare a standard curve, from which BUN was read in milligrams per 100 ml.

Creatinine assay and creatinine clearance. Endogenous creatinine clearance was determined by measuring the creatinine concentration in urine and blood collected from the same animal. For urine collection, mice were housed individually in metabolism cages for 12 h (10 p.m. to 10 a.m.). Blood (0.5 ml) was collected at the end of the 12-h period.

Blood and urine creatinine were assayed by the method of Faulkner and King (8) with slight modification. For blood, 0.5 ml of 5% sodium tungstate, 0.5 ml of 0.66 N sulfuric acid, and 0.5 ml of deionized water were added to 0.5 ml of heparinized whole blood. The mixture was centrifuged at 3,000 rpm for 10 min. For urine, fecal material was removed by centrifugation, and the total urine volume was measured and diluted 1:40 with deionized water. Creatinine standards were prepared by appropriate dilution of a stock solution containing 0.143 g of creatinine sulfate in 100 ml of 0.1 N HCl (1 mg of creatinine per ml). For creatinine measurements, to 1 ml of sample or reagent blank (deionized water) was added 1.0 ml of deionized water, 0.5 ml of 0.04 M picric acid, and 0.5 ml of 0.75 N NaOH, with mixing after each addition. The mixtures were incubated for 20 min at room temperature, and the optical density was measured at 500 nm.

Creatinine clearance was calculated in milliliters of plasma cleared per minute by the formula UV/P, where U is urine creatinine concentration (in milligrams per 100 ml), V is urine volume (in milliliters), and P is blood creatinine concentration (in milligrams per 100 ml) (8).

Liver glycogen assay. Liver glycogen was assayed by the method of Kemp and Kits van Heijningen (16). Mice were killed by cervical dislocation, and a piece of liver tissue weighing 30 to 50 mg was excised and homogenized in 5 ml of trichloroacetic acid deproteinizing solution. The liver homogenates were placed in a boiling water bath for 15 min, cooled in tap water, and centrifuged at 3,000 rpm for 5 min. One milliliter of the supernatant was added to 3 ml of concentrated sulfuric acid. This was mixed vigorously and then heated in a boiling water bath for 6.5 min. The red color, reflecting development of 5-hydroxymethyl furfural, was read spectrophotometrically at 520 nm. A standard curve was prepared by using appropriate dilutions of glucose. The percentage of liver glycogen, expressed as glucose equivalents, was determined by the following formulation: milligrams of glycogen/milligrams of liver (wet weight) × 100.

Blood glucose assay. A modification of the Glucostat method (Worthington Biochemicals Corp., Freehold, N.J.) was used to determine blood glucose. A 0.1-ml sample of heparinized whole blood was added to 0.9 ml of deionized water and deproteinized with 1.0 ml of 1.8% $Ba(OH)_2$ and 1.0 ml of 2.0% $ZnSO_4$. Samples were centrifuged, and 1 ml of supernatant was added to 1 ml of the reconstituted Glucostat reagent prewarmed in a 37°C water bath. After 30 min at 37°C, the tubes were centrifuged for 5 min at 3,000 rpm, and the optical density was determined at 500 nm. An appropriate standard curve was prepared from various dilutions of glucose, from which the glucose concentration (milligrams of glucose/100 ml of whole blood) was determined.

Nephrectomy procedures. Mice were anesthetized by subcutaneous injection of 0.25 mg of sodium pentobarbital (Butler Co., Columbus, Ohio) followed by light etherization. They were restrained, and their backs were shaved and swabbed with a 2% solution of tincture of iodine. An incision was made through the skin over the backbone, followed by a smaller incision through the muscle layer over the left kidney. The organ was located, the renal artery, renal vein, and ureter were ligated with 000 silk suture, and the kidney was excised. For a bilateral nephrectomy, the same procedure was followed on the right side. The muscle layers were not sutured. The back was sutured with 000 catgut. Unilaterally nephrectomized mice were kept for 1 month for experimentation. Studies on bilaterally nephrectomized mice were performed near the time of their death (24 to 36 h after surgery).

Endotoxin. Lipopolysaccharide prepared by phenol-water extraction from *Salmonella typhimurium* was purchased from Difco Laboratories, Detroit, Mich. It was suspended in pyrogen-free saline to a concentration of 5.0 mg/ml, and 1.0-ml portions were stored at −20°C until used. For injection, a portion was thawed and diluted in saline to the desired concentration.

Statistics. Statistical significance was determined by the White rank order test (29).

RESULTS

Survival of mice infected with *C. albicans*. Groups of female mice were given either 1.0×10^6 or 4.5×10^6 viable *C. albicans* intravenously. Survival was recorded until all animals had died (Fig. 1). In mice receiving 1.0×10^6 cells, the first deaths occurred 1 day after infection, but some mice survived for as long as 16 days. Deaths occurred at almost regular intervals over the 17-day observation period, with the mean survival time being 8.8 days. All mice receiving 4.5×10^6 colony-forming units (CFU) died between 8 and 12 h after infection. The mean survival time was 9.0 h.

It is evident from the survival data that the two slightly different challenge doses resulted in

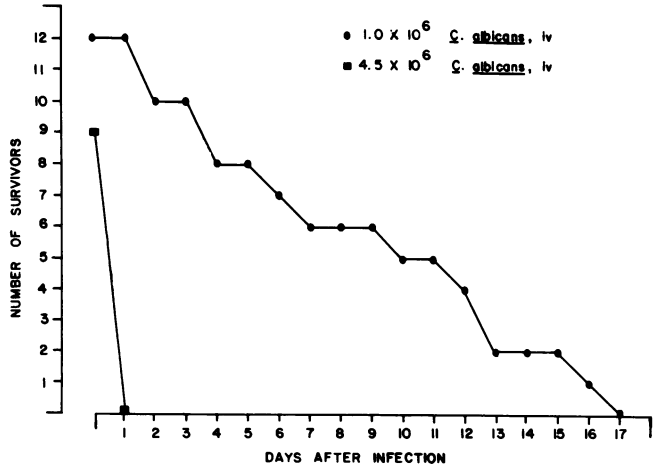

FIG. 1. *Survival of mice given C. albicans intravenously (iv).*

very different patterns of morbidity and mortality. Some pathophysiological differences in these two responses are described below.

Tissue population in infected mice injected with 10^6 *C. albicans*. The distribution of 10^6 *C. albicans* in liver, lungs, spleen, kidneys, heart, and brain was determined at 30 min after injection and when mice were moribund and assumed close to death (Table 1). In four of the six organs studied (liver, spleen, kidneys, and brain), the number of organisms increased from 30 min to near the time of death. The kidney showed over a 1,000-fold increase in CFU. Over 98% of the total organisms recovered just before death were present in the kidneys.

BUN and blood creatinine in normal, infected, and nephrectomized mice. For mice infected with 10^6 *C. albicans*, BUN was measured daily until all animals had died. As controls, normal mice were bled daily to determine whether multiple bleedings had any effect on BUN levels. Figure 2a shows the average BUN values versus days after infection. In normal mice, BUN remained relatively constant throughout the experimental period. In infected mice, the average BUN value was approximately twice normal from the 2nd day onward, but the data were extremely variable and showed large standard deviations.

Significant fluctuations occurred in the time of death within the infected group (Fig. 1). Hence, on any given day, the mean BUN value expressed in Fig. 2a was the average of only the surviving animals. Figure 2b shows the same data plotted as days before death rather than days after infection. When expressed in this manner, the data clearly establish that BUN began to rise in infected mice at about 4 days before death and increased to its highest concentration on the day of death. Standard deviations were much smaller than those seen in Fig. 2a.

TABLE 1. *Tissue populations in infected mice given 10^6 C. albicans*

Organ	CFU/organ ($\times 10^4$)[a]		% of total CFU recovered	
	30 min after challenge	At death	30 min after challenge	At death
Liver	7.5	14.0	21.0	0.5
Lungs	23.0	4.8	64.2	0.2
Spleen	0.13	0.27	0.4	0.0
Kidneys	1.8	2,700.0	5.0	98.2
Heart	2.4	0.23	6.7	0.0
Brain	1.0	31.0	2.8	1.1

[a] Mean of at least six mice.

Table 2 compares BUN and blood creatinine levels in infected and surgically manipulated mice. Data from nephrectomized mice were obtained to provide a scale with which to compare the severity of the BUN and blood creatinine alterations observed in infected mice. Normal BUN levels of approximately 50 mg/100 ml were elevated to more than 450 mg/100 ml (greater than ninefold) at the time of death. Unilateral nephrectomy did not result in significantly increased BUN levels, and such animals survived on routine laboratory maintenance for at least 1 month. Bilaterally nephrectomized mice lived for no more than 36 h and when near death they had an average BUN value approximately the same as infected mice at the time of death. Similar changes were noted in blood creatinine levels. Normal and unilaterally nephrectomized mice had essentially the same levels of blood creatinine, whereas infected and bilaterally

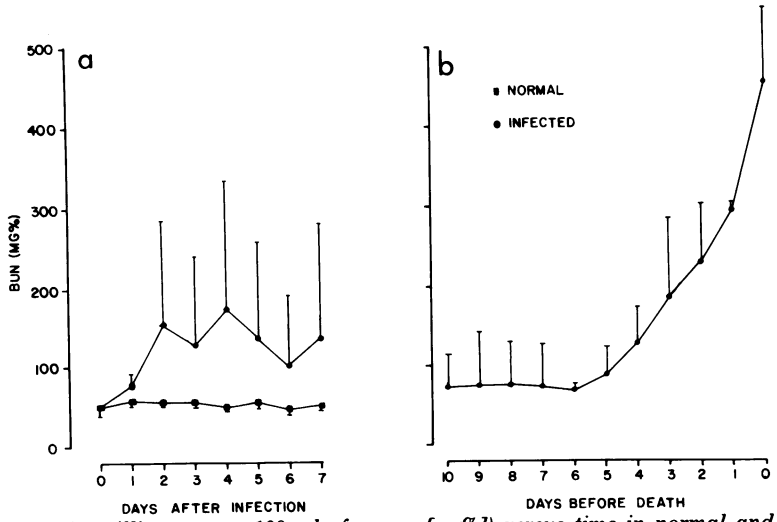

FIG. 2. *(a) BUN (in milligrams per 100 ml of serum [mg%]) versus time in normal and infected mice. Symbols: ■, normal mice; ●, infected mice (10^6 CFU of C. albicans given intravenously). (b) BUN in infected mice (10^6 CFU) on successive days before death. Day 0 is day on which death occurred.*

TABLE 2. *BUN and blood creatinine values for normal, infected, and nephrectomized mice*

Treatment	BUN (mg/100 ml)	Blood creatinine (mg/100 ml)
None	50.0 ± 8.7 (12)[a]	0.48 ± 0.12 (18)
Infected (10^6 CFU)[b]	463.8 ± 45.5 (7)	1.79 ± 0.84 (6)
Unilateral nephrectomy[c]	53.8 ± 12.2 (6)	0.46 ± 0.08 (5)
Bilateral nephrectomy[b]	427.6 ± 15.3 (3)	2.57 ± 0.29 (3)

[a] Mean ± standard deviation; number of mice in group is given in parentheses.
[b] Values obtained at the time of death.
[c] Mean of at least five determinations for each mouse made over 1 month after surgery.

nephrectomized mice had values three to six times higher than normal mice.

Creatinine clearance in normal and infected mice. The creatinine clearance rate for normal and infected mice was determined in three separate experiments with six animals in each group (Table 3). Normal mice had a creatinine clearance rate of 89.1 ml of plasma per min, whereas infected mice cleared only 44.1 ml of plasma per min, showing that by 4 days after infection, the creatinine clearance rate had decreased to approximately one-half that of normal mice.

Liver glycogen and blood glucose in normal and infected mice. Table 4 shows liver glycogen and blood glucose values for normal and infected mice after 48 h and close to the time of death. At both times, liver glycogen reserves were lower in infected mice than in normal mice, but even near death, liver glycogen was not depleted. Blood glucose levels of infected mice were not significantly different from normal values at any time points tested.

BUN, liver glycogen, and blood glucose levels in mice injected with 4.5×10^6 *C. albicans*. BUN, liver glycogen, and blood glucose levels were measured in mice receiving the higher (4.5×10^6) challenge dose of *C. albicans* (Table 5). Eight hours after challenge, the animals were close to death. The BUN level of infected mice near death was within the normal range. Liver glycogen steadily decreased with time after infection, yet at 8 h, the liver glycogen reserves of infected mice were not significantly different from those of saline control mice, which undergo a circadian decrease in liver glycogen at this time of day. Mice given saline at 10 a.m. showed percent liver glycogen values of 2.90 ± 0.38 after 4 h, 0.59 ± 0.59 after 8 h, 2.01 ± 1.32 after 12 h, and 3.60 ± 1.83 after 24 h. Blood

TABLE 3. *Creatinine clearance in normal and infected mice*

Mice	Creatinine clearance (ml of plasma cleared/min)	
Normal	89.1 ± 14.7 (18)[a]	$P < 0.001$
Infected[b]	44.1 ± 3.9 (18)	

[a] Mean ± standard error; total number of mice assayed is given in parentheses.
[b] Infected with 10^6 *C. albicans* given intravenously 4 days previously.

glucose increased after infection, and a slight hyperglycemia was evident as the time of death approached.

BUN, liver glycogen, and blood glucose in mice given saline, heat-killed *C. albicans*, or endotoxin. Mice were injected intraperitoneally either with 4.5×10^6 or 1.0×10^9 heat-killed *C. albicans* or with 150 μg of *S. typhimurium* endotoxin. Values for BUN, liver glycogen, and blood glucose are shown in Table 6. The lower dose of heat-killed *C. albicans* gave values similar to the saline controls. Treatment with 1.0×10^9 *C. albicans* depleted liver glycogen and caused hypoglycemia, but did not affect BUN. Endotoxin resulted in a decrease in liver glycogen to very low levels and also caused hypoglycemia. Unlike the finding at the higher yeast dose, endotoxin caused a marked elevation of BUN over saline controls.

TABLE 4. *Liver glycogen and blood glucose in normal and infected mice*

Mice	Liver glycogen (%)	Blood glucose (mg/100 ml)
Normal	4.99[a] ± 1.16[b]	103.0 ± 10.0[c]
Infected[d]		
48 hr after challenge	1.44 ± 1.31	79.8 ± 23.9[c]
At death	1.12 ± 1.43	82.5 ± 29.4[c]

[a] All values for this mean only assayed at 10 a.m.
[b] Mean ± standard deviation of at least six mice.
[c] Not significantly different.
[d] Infected with 10^6 *C. albicans* intravenously.

TABLE 5. *BUN, liver glycogen, and blood glucose in mice infected with 4.5×10^6 CFU of C. albicans*

Time post-challenge (h)	BUN (mg/100 ml)	Liver glycogen (%)	Blood glucose (mg/100 ml)
0[b]	50.0 ± 8.7[a,c]	4.99 ± 1.16	103.0 ± 10.0[d]
1	47.5 ± 8.1	3.68 ± 1.12	112.2 ± 10.5
4	39.5 ± 6.2	1.67 ± 0.96	116.7 ± 29.0
8	54.1 ± 14.0[c]	0.87 ± 0.68	132.6 ± 30.8[d]

[a] Mean ± standard deviation of at least six mice.
[b] Values for normal mice.
[c] Not significantly different.
[d] Significantly different at 0.05 level.

TABLE 6. *BUN, liver glycogen, and blood glucose in mice given saline, heat-killed C. albicans, or endotoxin*[a]

Treatment	BUN (mg/100 ml)	Liver glycogen (%)	Blood glucose (mg/100 ml)
Saline	52.1 ± 14.6	3.60 ± 1.83	90.6 ± 12.3
C. albicans (4.5×10^6 heat-killed cells, i.p.)	31.9 ± 5.3	4.11 ± 0.53	103.3 ± 7.1
C. albicans (1.0×10^9 heat-killed cells, i.p.)	31.1 ± 12.7	0.04 ± 0.05	34.3 ± 7.2
Endotoxin (150 μg, i.p.)	106.5 ± 44.9	0.22 ± 0.14	51.8 ± 14.8

[a] Values shown represent mean ± 1 standard deviation and were obtained 24 h after intraperitoneal (i.p.) injection.

DISCUSSION

The survival data in Fig. 1 confirm the previous results reported for mice (22), rabbits (31), and guinea pigs (14, 33) showing that a threshold dose for *C. albicans* infection exists below which a nonfatal or slowly progressing fatal infection occurs and above which rapid death ensues. The contrast in outcome of challenge with relatively small differences in the size of dose administered suggests that different mechanisms are likely responsible for death.

Death from lower doses of *C. albicans* probably results from progressive multiplication of the fungus. Although a relatively small percentage of the original inoculum localized in the kidneys, almost all of the organisms recovered from the major organs (approximately 98%) were found in the kidneys near the time of death (Table 1). This represents a 3-log increase over the initial number of organisms recovered from this organ. Previous studies have shown that yeast cells trapped in interstitial capillaries germinate hyphal forms which penetrate into the renal tubular lumen. Here they rapidly multiply away from the host's cellular defenses. Penetration back into renal tissue elicits significant tissue damage and an inflammatory response (19, 20).

The data in Table 1 do not prove that the organisms recovered near the time of death were directly descended from those recovered after 30 min. Previous studies suggest that the challenge dose is destroyed in all organs except the kidney, whereas the challenge dose multiplies in the kidneys (7, 19, 20). If this is true, then the organisms recovered from the liver, spleen, brain, lungs, and heart may have disseminated from the kidneys. Further, the data in Table 1 do not represent all fungi present in the host. Fungi are present in the carcass, etc. (unpublished data), but in quantities that do not substantially alter the patterns seen in Table 1. Artifacts in counting due to clumped organisms may artificially lower recovery values, but despite these concerns, it is clear that the kidneys carry a large infectious burden near the time of death.

Measurements of BUN (Fig. 2b), blood cre-

atinine (Table 2), and creatinine clearance (Table 3) establish that the infection in the kidneys resulted in altered renal function. The progressive elevation of BUN during the development of systemic candidiasis confirms and extends the observations by Winner (31), who found BUN values in infected rabbits ranging from 110 to 410 mg/100 ml at the time of death. By expressing the data with respect to time of death, we have removed much of the variability seen when assaying from the time after infection (Fig. 2a). The increases in BUN and blood creatinine levels were comparable to those in bilaterally nephrectomized mice and suggest that bilateral renal damage caused by the infection was extensive. Unilaterally nephrectomized mice had normal BUN and blood creatinine values (Table 2). Both BUN and blood creatinine values can be influenced by factors other than renal status (13, 18), and both parameters may be slow to indicate renal impairment. Creatinine clearance measures the glomerular filtration rate and is a very sensitive measure of kidney function (8). The data in Table 3, showing decreased creatinine clearance rates at 4 days after infection, indicate that the elevations in BUN and blood creatinine reflect impaired renal function. Four days postinfection was arbitrarily chosen for these assays as a time when large numbers of mice were visibly ill yet relatively few had died. Random assays at other times were consistent with the decreased creatine clearance observed after 4 days in infected mice.

The liver had a significant number of viable *C. albicans* at the time of death, although not as high as the number found in the kidney. It is presumed that had there been extensive hepatic damage, disruption of normal hepatic metabolism would have occurred. Since blood glucose measurements were not statistically different from normal values (Table 4) and the apparent decrease in liver glycogen only reflected the circadian fluxes of saline controls, no significant hepatotoxicity or metabolic damage occurred in mice infected with 10^6 yeast cells. The major damage seems to have been renal in nature.

Death that results from infection with 4.5×10^6 viable *C. albicans* resembles a toxic rather than an infectious process. It has been suggested that endotoxin-like material is produced by *C. albicans*, since various biological effects similar to those characteristic of endotoxemia have been demonstrated in animals receiving preparations or extracts of these yeast cells (3, 6, 11, 12, 17, 22, 27). For this reason, selected metabolic and physiological changes were compared in mice receiving either endotoxin, 4.5×10^6 viable or heat-killed cells, or 1.0×10^9 heat-killed cells. The latter dose was included because previous work has indicated that a pyrogenic response closely resembling that induced by endotoxin is associated with this number of cells (17). Both endotoxin and 10^9 heat-killed *C. albicans* produced severe hypoglycemia by 24 h, but, unlike endotoxin, heat-killed *C. albicans* cells did not elevate BUN (Table 6). It is doubtful that this large amount of fungal material would even exist in naturally acquired *C. albicans* infection in mice. By contrast, 4.5×10^6 heat-killed cells, which produced rapidly fatal disease when viable (Fig. 1), did not produce abnormalities in liver glycogen, blood glucose, or BUN. Although we did not determine whether fungal multiplication occurred before death in mice receiving 4.5×10^6 CFU of *C. albicans*, Louria et al. (20) reported that *C. albicans* populations in liver, spleen, heart, lungs, and brain did not change significantly in the first 24 h after infection with 5×10^6 viable cells. Because the values for the metabolic parameters studied were different in mice near death from the two *C. albicans* challenge doses (Tables 2 and 5), a different cause of death for each dose is suggested.

The events leading to death after challenge with 4.5×10^6 CFU are not well understood. It is not known whether a certain critical level of a toxic entity is present with 4.5×10^6 CFU which results in death. If a toxin is present in the *C. albicans* preparations, it does not closely resemble bacterial endotoxin in terms of metabolic toxicities. Cutler et al. (6) arrived at a similar conclusion by monitoring different biological parameters.

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