

Modulating Effect of Dietary Carbohydrate Supplementation on *Candida albicans* Colonization and Invasion in a Neutropenic Mouse Model

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We studied the effect of dietary carbohydrate supplementation on *Candida albicans* colonization and invasion of the gastrointestinal tract in a neutropenic mouse model. Mice inoculated with *C. albicans* were allowed free access to standard chow and drinking water supplemented with either glucose or xylitol or no carbohydrates (control). On days 33 through 36 postinoculation, the mean \pm standard error \log_{10} CFU of *C. albicans* per gram on the mucosal surface, determined by quantitating CFU dislodged in the first wash of the gastric wall, was significantly higher in mice given the glucose supplement: 7.20 ± 0.09 (glucose) versus 5.38 ± 0.28 (xylitol) and 5.11 ± 0.33 (control) CFU/g ($P \leq 0.05$ for each comparison by Fisher's protected least-significant-difference test). Fecal cultures also yielded the highest quantities of *C. albicans* in the glucose group. Invasion of the gastric wall by *C. albicans* correlated well with surface colonization in glucose-supplemented animals. Eight of 10 mice in this group, all with $>10^6$ CFU/g, showed extensive invasive growth, as compared with only 2 of 26 mice in the remaining groups ($P = 0.00006$ by Fisher's exact test). These results indicate that dietary glucose intake is a key determinant of *C. albicans* growth in the gastrointestinal tract. The data provide an experimental rationale for clinical trials to decrease the intake of glucose or its utilization by *C. albicans* in immunocompromised patients.

Gastrointestinal (GI) candidiasis causes appreciable morbidity in cancer patients receiving chemotherapy and in recipients of bone marrow transplants. Disseminated candidiasis, a major contributor to mortality in this population (1, 30), is believed to arise primarily from colonization of the GI tract by *Candida albicans* (10, 38, 39). Thus, measures that would inhibit *C. albicans* GI growth and adherence to the mucosa might prevent disseminated candidiasis (38). Chemoprophylaxis with oral antifungal drugs (1, 27), although useful in theory, has been limited by the efficacy of currently available agents, the poor tolerance of chemotherapy by the GI tract, and noncompliance due to the unpalatable taste of compounds such as the polyenes (27).

Freter et al. (13) demonstrated that the growth of microbial species in the GI ecosystem is regulated by the efficiency with which they utilize available substrates. Highly efficient utilizers form larger colonies than do species less able to incorporate a particular substrate, both in vitro and in models of murine GI flora (13, 28). Glucose is a highly utilizable substrate for *C. albicans*, both in culture media (7, 18) and in human saliva (20). Candidiasis occurs more often when there is high availability of glucose, as in persons with diabetes, in patients receiving total parenteral nutrition, and in sugar cane workers, who may develop *Candida* paronychia (1, 7). Carbohydrate-rich diets also favor the oral carriage of *C. albicans* in rats and monkeys, whereas sucrose rinses initiate *Candida* stomatitis in human subjects (2, 16, 29, 32).

To test the idea that *C. albicans* growth in the GI tract might be influenced by the dietary intake of glucose, we selected infant mice as an experimental model and xylitol as

a substitute for glucose. The infant mouse model has been used successfully to study *Candida* colonization of the GI tract and invasion from that site (31). Infections in this host, including invasiveness under immunosuppression (15), closely resemble those in humans (42). Xylitol is not used as a substrate by *C. albicans* (24, 25) and does not appear to affect the growth of these organisms in vivo (23). As an oral or parenteral sugar substitute, xylitol provides 4.06 cal (ca. 17.0 J)/g and does not adversely affect the nutritional or metabolic activities of the host.

MATERIALS AND METHODS

The experimental plan is depicted in Fig. 1.

Animals. The litters of six pregnant CFW(SW) mice (Charles River Laboratories, Inc., Wilmington, Mass.) were used in all experiments. Mice were given free access to food and water throughout the study. The total carbohydrate content of the standard Purina chow (Purina Mills, Richmond, Ind.) was 49% (<0.5% fructose, <0.5% glucose, 3.8% sucrose, <1.25% maltose, <1.25% lactose, <1.5% maltotriose, 27.3% starch, 13% cellulose).

Preparation and administration of inoculum. A *C. albicans* isolate from the spinal fluid of a patient who died of disseminated candidiasis at St. Jude Children's Research Hospital was used to establish stock cultures, which were kept at room temperature and passaged in brain heart infusion agar (Difco Laboratories, Detroit, Mich.). This isolate was examined for growth characteristics in yeast nitrogen base broth (Difco) supplemented with equimolar (277 mM) concentrations of glucose or xylitol; non-carbohydrate-supplemented media were used as controls. Glucose allowed enhanced growth of this *C. albicans* isolate as compared with that in the xylitol- or non-carbohydrate-supplemented broth. Colo-

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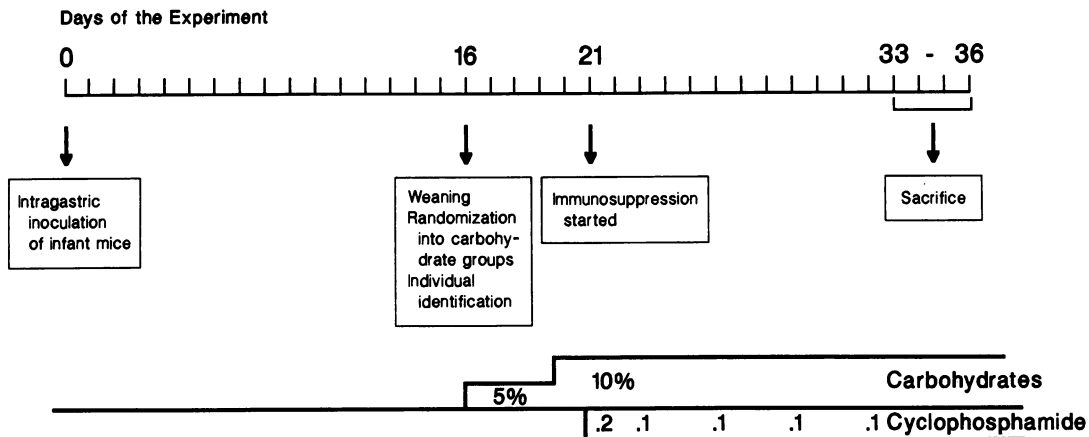


FIG. 1. Diagram of experimental protocol: day 0, inoculation; day 16, weaning, identification, fecal culture for colonization, and randomization of mice into glucose, xylitol, and control groups; day 21, cyclophosphamide started at 0.2 mg/g with a decrease to 0.1 mg/g; days 33 through 36, sacrifice and culture of feces, gastric wall first washing solution (surface), and tissue homogenates from gastric wall and kidney.

nies taken from an agar plate after 48 h of growth were inoculated into 500 ml of tryptic soy broth (Difco) in a 1-liter Erlenmeyer flask and grown overnight in a shaker-incubator at 150 rpm (New Brunswick Scientific) at 37°C. Aliquots of 50 ml were centrifuged for 15 min at $225 \times g$, washed three times in sterile distilled water, and then resuspended to a final concentration of 2×10^8 CFU/0.05 ml of water, as determined with a hemacytometer (31). The 0.05-ml suspension was administered intragastrically with a 21-gauge Teflon catheter (Quick-cath; Baxter Diagnostics, Inc., Chicago, Ill.) and a tuberculin syringe to 6-day-old mice as previously described (31). Offspring were separated from their mothers for 5 h before and 30 min after inoculation; day 0 began as soon as mice were returned to their mothers.

Carbohydrate trial. Of the 63 inoculated mice, 46 survived and were weaned on day 16 after inoculation. Ten were later discarded because they weighed less than 5 g; the remaining 36 were earmarked and assigned to one of three experimental groups. In two of these, the mice were allowed free access to drinking water that contained either D-(+)-glucose (Sigma Chemical Co., St. Louis, Mo.) ($n = 10$) or xylitol (Sigma) ($n = 12$) each at 5% (wt/vol) concentration from day 16 to day 21 and at 10% thereafter until sacrifice (22, 44). The control group ($n = 14$) received water without carbohydrates. To calculate the carbohydrate intake, we determined the water intake for each experimental group by weighing their water bottles every other day. The levels of glucose in plasma were determined with a clinical chemistry analyzer (Synchron CX4CE-CX7; Beckman Instruments, Inc., Brea, Calif.) in five mice per group on day 30.

Immunosuppression. Mice were immunosuppressed by the method of Guentzel et al. (15) with cyclophosphamide (Cytosan; Bristol Myers, Evansville, Ind.) administered intraperitoneally on days 21, 23, 26, 29, and 32. The initial dose was 0.2 mg/g with subsequent doses decreased to 0.1 mg/g. Hematologic profiles, determined two times in the same three mice in each of the groups, showed a mean total leukocyte count of 1.33×10^9 /liter on day 25 with a mean absolute neutrophil count of 0.24×10^9 (range, 0.02×10^9 to 0.58×10^9)/liter, decreasing to 1.23×10^9 /liter on day 30 with an absolute neutrophil count of 0.42×10^9 (range, 0.14×10^9 to 0.67×10^9)/liter. The mean total leukocyte count in

healthy mice is $7.17 \times 10^9 \pm 0.09 \times 10^9$ (standard deviation) per liter (5).

Fecal cultures. On day 16, before carbohydrate supplementation was begun, a fresh fecal pellet was collected from each mouse and placed in a preweighed 15-ml plastic tube containing 1 ml of sterile distilled water. The same procedure was repeated on day 21, before administration of cyclophosphamide. The pellets were vigorously agitated, vortexed for approximately 1 min, and then serially diluted 10-fold in sterile distilled water. A 100- μ l sample of each dilution was spread with a glass hockey stick onto brain heart infusion agar (Difco) containing penicillin (100 mg/ml) and gentamicin (100 μ g/ml). Colonies were counted after 48 h of incubation at room temperature, and the average number of CFU per gram of feces was calculated. Colonies were verified by characteristic morphology and Gram staining characteristics. No bacterial or other fungal contamination was detected, in accord with previous studies (5, 9). At autopsy, one or two fecal pellets were squeezed from the distally sectioned rectum and processed as described above for routinely collected specimens.

C. albicans in tissue samples. Three or four mice per group were sacrificed daily on days 33 through 36 in a CO₂ chamber. Kidneys were removed first and placed in 2 ml of sterile distilled water at 4°C until they were homogenized; then they were cultured as described above for fecal pellets. A portion of the GI tract from the middle third of the esophagus to the pylorus was also clamped and sectioned. The cardioesophageal junction was stored in 10% Formalin for histologic studies. The stomach was opened along its internal curvature, and the gross contents were removed gently with a spatula until the mucosa appeared clean. The opened gastric wall was placed in a preweighed 15-ml plastic tube (Fisher Scientific, Pittsburgh, Pa.) containing 4 ml of distilled water at 4°C. The stomach was vigorously agitated and vortexed, washed in 5 ml of sterile distilled water three times to remove nonadherent candidas, and finally homogenized in 5 ml of water with a biohomogenizer (Biospec Products, Bartlesville, Okla.) (31). Tenfold dilutions of the first gastric-wall washing solution (GI surface) and the gastric wall homogenate (GI wall) were plated, and counts of C.

albicans were made as described above. The results are reported as CFU per gram of GI wall.

Histologic examination. GI specimens were fixed in Formalin, embedded in paraffin, serially sectioned, and stained with Gomori methenamine silver nitrate. Invasion by *C. albicans* was defined as the presence of one or more yeast cells or hyphae penetrating the stratified squamous epithelium of the esophageal mucosa within the cardiac antrum (4). The extent of mucosal invasion was graded by the method of Cantorna and Balish (3) (Fig. 2). Six histologic sections per mouse were interpreted by investigators without knowing the origin of the samples.

Body and cecum weight determinations. Mice were weighed with an electronic scale (sensitivity, ± 10 mg; Sunbeam, Milwaukee, Wis.) on days 0 and 21 and at sacrifice. Cecum specimens fixed in 10% Formalin were separated from the small and large bowels and then weighed with their contents intact. The cecum percentage of total body weight, an indicator of the complexity of the GI flora (35, 45), was then calculated for each mouse.

Statistical analysis. The Wilcoxon test was used to compare glucose levels in plasma among the three treatment groups. Analysis of variance, performed on \log_{10} -transformed data, was used to assess *C. albicans* colonization and invasion as well as the cecum percentage of total body weight. Pretreatment measurements of *C. albicans* colonization were used as a covariate in the analysis of variance because of the wide variability of this end point, as noted in previous studies (17) and demonstrated in our own analysis (Fig. 3). Mean \pm standard error values for fecal and gastric wall colonization were compared with Fisher's protected least-significant-difference test. Fisher's exact test was used to compare the proportions of mice that had invasion of the gastric wall. Statistical significance was defined as $P < 0.05$.

RESULTS

Carbohydrate intake and plasma glucose levels. The mean intakes of supplemental carbohydrate per gram of body weight per day were 0.085 g for the glucose group and 0.019 g for the xylitol group. This discrepancy can be explained, in part, by the longer time that xylitol remains in the stomach (34). Mice in all groups appeared healthy throughout the study; their mean weight increases were 8.44 g (xylitol), 8.20 g (glucose), and 9.60 g (control). Mean glucose levels in plasma \pm standard errors, measured 15 days after dietary supplementation, were significantly lower in mice receiving xylitol: 155 ± 19.3 mg/dl versus 272 ± 27.3 mg/dl and 241 ± 22.0 mg/dl for the glucose and control groups, respectively ($P = 0.04$ for each comparison by the Wilcoxon test).

Colonization of the GI tract. Fecal cultures were uniformly positive for *C. albicans* by 16 days after inoculation (Fig. 3). By day 21, the concentration of organisms in the feces of glucose-supplemented animals had risen to $6.71 \pm 0.14 \log_{10}$ CFU/g and was significantly different from counts in the xylitol and control groups ($P \leq 0.05$ for each comparison by Fisher's protected least significant difference test). Under cyclophosphamide-induced immunosuppression, the number of organisms in feces continued to increase in the glucose group but was relatively stable in the remaining groups: means of $7.58 \pm 0.20 \log_{10}$ CFU/g compared with 5.22 ± 0.25 (xylitol; $P \leq 0.05$), and 5.09 ± 0.23 (control; $P \leq 0.05$) \log_{10} CFU/g. Definitive results were obtained when concentrations of *C. albicans* dislodged from the gastric wall surface were determined: mean of $7.20 \pm 0.09 \log_{10}$ CFU/g of tissue for glucose-supplemented animals, compared with $5.38 \pm$

0.28 (xylitol; $P \leq 0.05$) and 5.11 ± 0.33 (control; $P \leq 0.05$) \log_{10} CFU/g.

Invasion of the gastric wall. The majority (80%) of mice given the glucose supplement showed histologic evidence of *C. albicans* invasion of the gastric wall, ranging from numerous yeast cells and hyphae to confluent growth (histologic score, 2 to 4; Fig. 2 and 4). In the xylitol and control groups, by contrast, $>90\%$ of the mice had either no invasion or only sporadic infiltration ($P = 0.00006$ by Fisher's exact test). The extent of mucosal invasion correlated well with colonization of the GI surface ($P = 0.00008$). For example, all 10 mice with histologic scores of 2 or higher had $>10^6$ CFU/g of washed gastric wall.

Results obtained with gastric wall homogenates were consistent with the histologic evaluation. The mean \pm standard error concentrations were $5.23 \pm 0.50 \log_{10}$ CFU/g of homogenate in the glucose group, $3.56 \pm 0.42 \log_{10}$ CFU/g in the xylitol group ($P \leq 0.05$), and $3.21 \pm 0.37 \log_{10}$ CFU/g in controls ($P \leq 0.05$). *C. albicans* counts of $>10^5$ CFU/g of washed gastric wall correlated with histologic scores of 2 to 4 ($P = 0.002$; data not shown). Cultures of kidney homogenates were sterile for all mice.

Body and cecum weight determinations. Because of concern that xylitol might adversely affect the GI flora (35), we examined the ceca of all mice. Generally, an enlarged cecum (5 to 10% of the total body weight) indicates the presence of relatively simple intestinal microflora (12, 19). The mean \pm SE percentage of total body weight in the xylitol group ($5.2\% \pm 0.62\%$) was significantly higher than that in either the control ($2.8\% \pm 0.11\%$) or glucose ($1.7\% \pm 0.11\%$) group ($P \leq 0.05$ for each comparison by Fisher's protected least-significant-difference test).

DISCUSSION

In this study, glucose added to the drinking water of infant mice stimulated the growth of *C. albicans* in the GI tract. Substitution of xylitol, a naturally occurring carbohydrate approved for dietary use in the United States, yielded a *C. albicans* growth pattern that was essentially the same as that in controls. The simplest explanation for these results is that glucose acts as a preferred growth substrate for *C. albicans* (13, 46), whereas xylitol is metabolized poorly if at all (24). The slight metabolism of xylitol occurs by an NAD-linked dehydrogenase system; the K_m for the reaction is 3.3×10^{-3} M at pH 8.9 (14), attesting to the very low affinity for this carbohydrate (40).

To relate our findings to the clinical setting, we first estimated the total amount of glucose ingested by an average mouse (0.0052 m^2 of body surface area in this study). The result was 2.24 g (1.19 g in drinking water and 1.05 g in Purina chow). This would be equivalent to 273 g if calculated for an average 6-year-old American child (body surface area, 0.8 m^2), whose actual daily glucose intake after carbohydrate breakdown by digestive enzymes is 190 g (6). Thus, based on this body surface area comparison (11), the highly concentrated glucose solutions used in our study resulted in total amounts of ingested glucose of about 1.5 times the average carbohydrate consumption by healthy children this age (6). We also considered that excessive glucose intake might induce hyperglycemia in mice. Results of glucose testing 3 days before sacrifice ruled out this possibility; the levels of serum glucose in the glucose-supplemented group were not significantly different from those in the controls.

Invasion of the gastric wall by *C. albicans* involves persorption (21, 38), the paracellular passage of large cor-

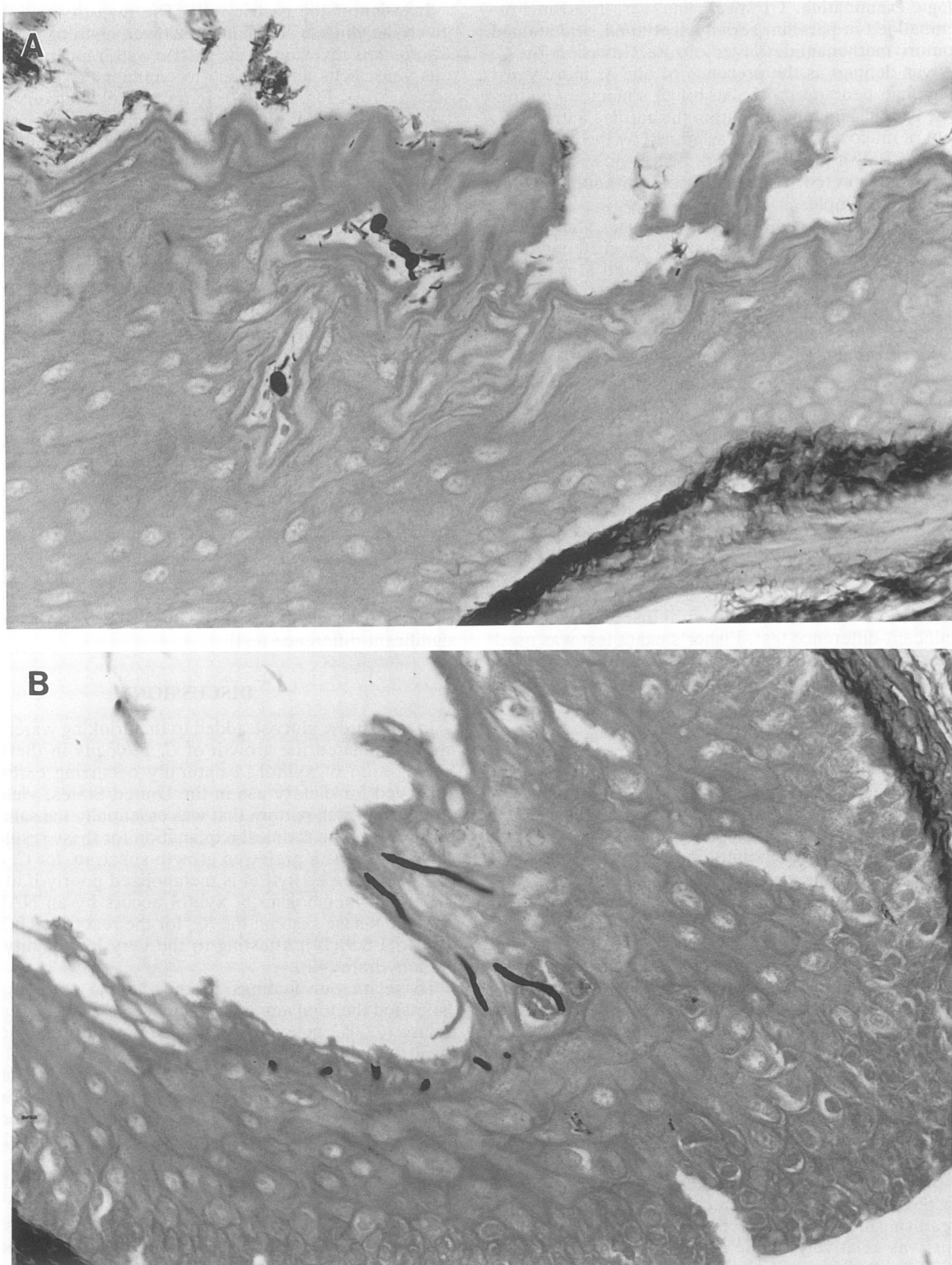


FIG. 2. Photomicrograph illustrating the histologic scoring system for mucosal invasion of the cardiac antrum mucosa (3). (A) Sporadic yeasts and hyphae, score 1; (B) numerous yeasts and hyphae, score 2; (C) abundant yeasts and hyphae but no confluence, score 3; (D) confluent invasion, score 4. Magnifications are the same ($\times 225$) for all prints. (A and B) Mice in the control group; (C and D) mice in the glucose group. Score 0, not shown.

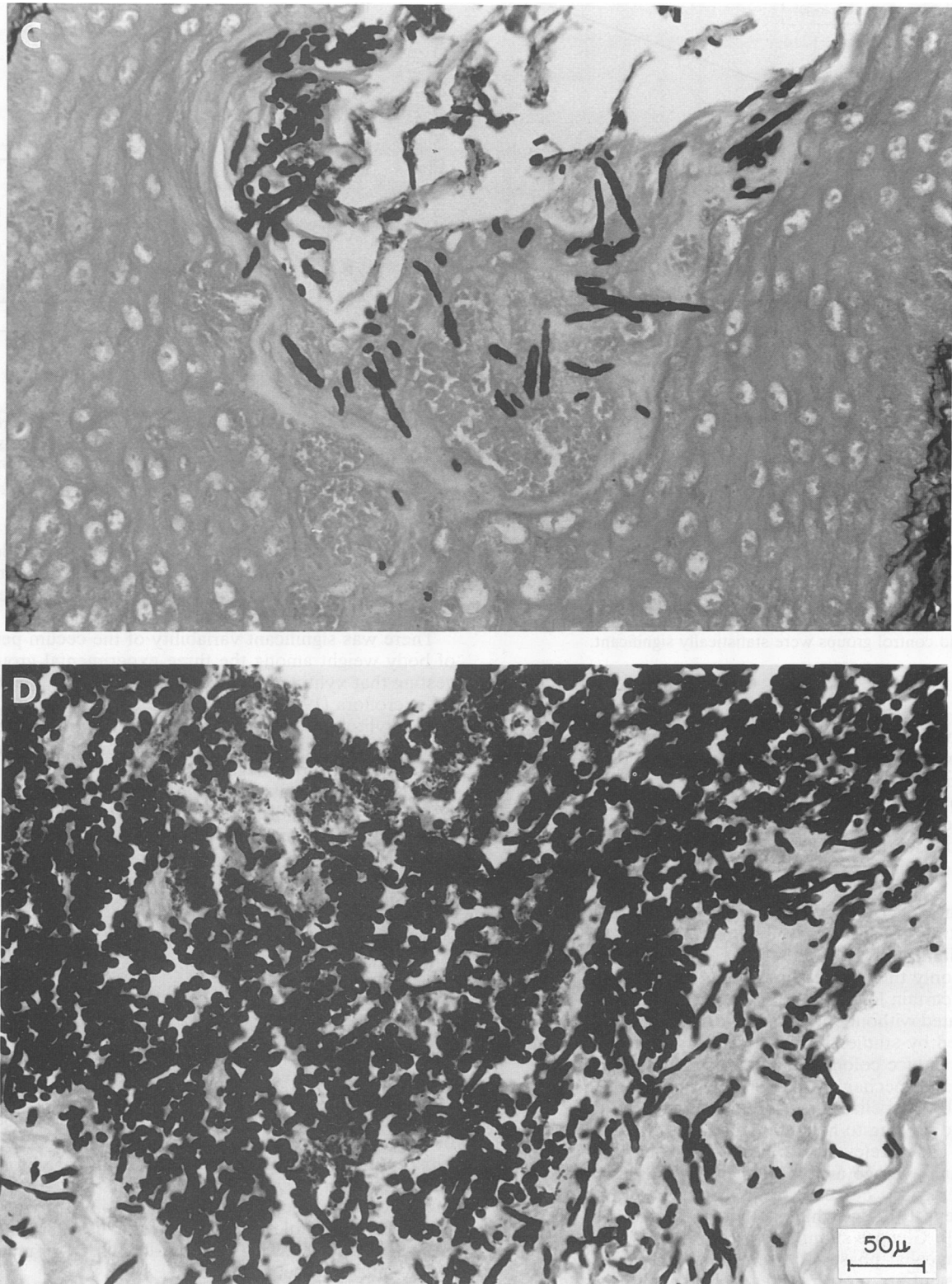


FIG. 2—Continued

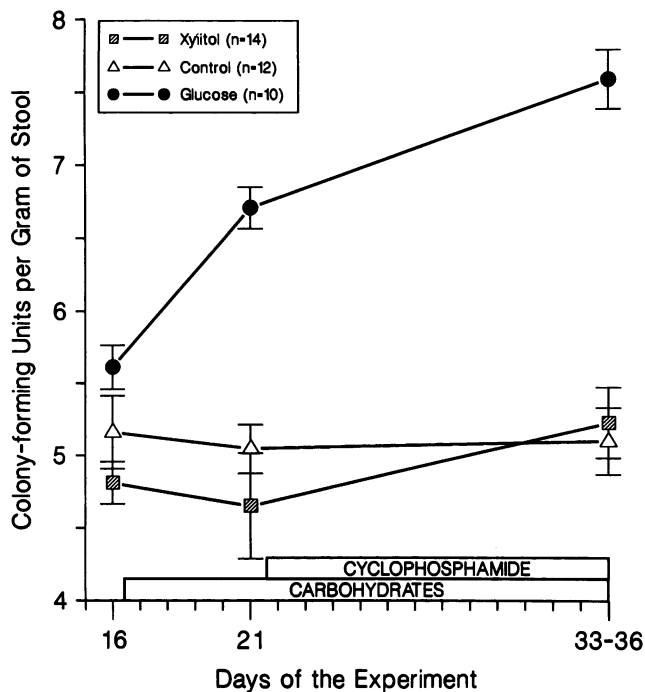


FIG. 3. Changes in the fecal *C. albicans* concentration after carbohydrate supplementation. Values are means \pm standard errors for \log_{10} -transformed data. There was a significant difference between the glucose group and both the xylitol and control groups on day 21 and days 33 through 36 ($P \leq 0.05$ by Fisher's protected least significant difference test). None of the differences between the xylitol and control groups were statistically significant.

puscular particles, such as yeast cells, through the epithelial layer of the GI mucosa (41). Use of this mechanism depends largely on the number of particles present in the gastrointestinal lumen; thus, high concentrations of *C. albicans* overlying the mucosa, as seen in mice fed supplemental glucose, would be expected to trigger presorption. Nevertheless, we could not identify a definitive threshold surface concentration of *C. albicans* leading to gastric wall invasion. In general, $\geq 10^6$ CFU/g correlated with invasive growth (19, 37–39); however, several mice with lower counts had mucosal infiltration, and several with counts of $\geq 10^6$ CFU/g lacked infiltration altogether. At concentrations of $\leq 10^5$ CFU/g, only three mice showed mucosal invasion, suggesting that certain low levels of *C. albicans* in the GI tract can be tolerated without undue risk of dissemination. This idea is supported by studies in which small inocula of *C. albicans* failed to induce colonization or dissemination (37, 38).

A second mechanism of GI wall invasion is penetrative growth by mycelial-phase organisms, which demonstrate increased binding to host epithelial cells (14). This pathway appears most relevant to hosts ingesting proportionally larger-than-average amounts of glucose, as compared with that ingested by our control group of mice (total of 1.05 g [equivalent to 127 g, which is low for an average 6-year-old child] [6]). Glucose stimulates transformation of *C. albicans* to the mycelial phase (26), and organisms in this stage of development are more resistant to phagocytosis and killing by granulocytes and macrophages (14, 26). Whether xylitol also stimulates penetrative growth by *C. albicans* is not clear from the available information; however, this compound has been reported to increase candida adherence to human

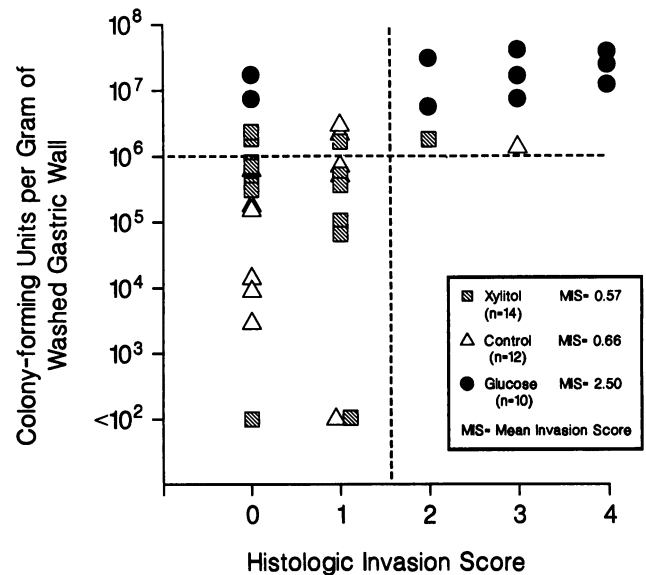


FIG. 4. Relation between the histologic invasion score and the concentration of *C. albicans* on the GI surface. The association toward higher invasion scores with increasing colonization is highly significant ($P = 0.00008$ by Fisher's exact test).

epithelial (HeLa) cells in vitro more than glucose does (36). We were unable to document any impressive stimulatory effect of the xylitol on candida growth in the GI tract, regardless of the mechanism of mucosal invasion.

There was significant variability of the cecum percentage of body weight among the three experimental groups, suggesting that xylitol and glucose have different effects on the GI microflora (19). The higher cecum percentages observed in the xylitol group indicate the presence of a simpler flora (19, 45). A shift to an increased fecal proportion of gram-positive versus gram-negative organisms has been described by others for animals receiving xylitol (22, 33, 35). In fact, we have seen the disappearance of nonlactose fermentors from the stools of mice receiving xylitol in previous experiments (39a). There is also evidence that important transformations of the GI flora can be induced by the addition of other slowly absorbed carbohydrates, such as lactose, to the diets of different species (17) or even by administering a diet restricted to milk (8).

We conclude that dietary glucose supplementation leads to higher rates of candida growth and invasion. Thus, it may be possible to control this organism in patients by restricting the availability of this carbohydrate. Two strategies, each based on the different efficiencies with which candida cells utilize carbohydrates, might be considered. One would be to develop drugs capable of blocking the pathway for glucose utilization by candida organisms. The other would be to limit the patient's intake of dietary glucose, for example, by substituting carbohydrates such as xylitol. Xylitol is naturally present in many vegetables, and its palatability, safety, and oral use are well documented (23, 43). It may therefore constitute a suitable carbohydrate for use in immunocompromised patients during periods of high risk for mucositis or candidemia.

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