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DIETARY FIBER AND GIARDIASIS

**DIETARY FIBER REDUCES INTESTINAL INFECTION BY
GIARDIA LAMBLIA IN THE GERBIL**

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SUMMARY

Gerbils were maintained on a low fiber (5%) or a high fiber (20%) diet in which the major fiber source was cellulose. Low fiber diet group animals were significantly more likely to become infected when inoculated with 100 Giardia lamblia cysts than were animals from the high fiber diet group. No differences were detected between the high and the low fiber diet animals at the time of cysts inoculation in gastrointestinal transit, gastric and small intestinal luminal pH, or in duodenal mucus blanket acidic glycoprotein. Further, the fiber content of the diet after, rather than before, cyst inoculation determined the Giardia infection rate. These data suggest that the dietary fiber effect occurred at the time of Giardia trophozoite colonization of the small intestine rather than at the time of excystation.

When infected low fiber diet animals were placed on the high fiber diet for 24 hours, trophozoite clearing occurred in the lower small intestine. In the jejunum, the number of trophozoites attached to the mucosal surface decreased while the number associated with luminal mucus increased. It is concluded that the fiber-induced mucus secretion and the bulk movement of the insoluble fiber reduced the attachment of Giardia trophozoites to the intestinal mucosa, decreasing the probability of establishing a sustained colonization of the mucosa by trophozoites.

Giardia lamblia is a common protozoan parasite of man. The signs and symptoms of giardiasis are very variable and extend from an almost asymptomatic disease to a prolonged, life threatening malabsorption in individuals with a compromised immune system. (1, 2). A Giardia infection occurs when an individual ingests viable cysts which apparently are stimulated to excyst by passage through the acidic environment of the stomach (3). The motile trophozoites then emerge from the cysts to establish an infection in the upper small intestine (4). Parasite adherence lectins (5) and components in the duodenal and jejunal mucus (6) may contribute to the fact that the parasite load is greatest in the jejunum.

Giardiasis is known to have numerous effects on the host's nutritional status (7, 8), but relatively little is known about the effects of host nutritional status and diet on the pathogenesis of the disease. Protein energy malnutrition appears to exacerbate the infection, at least in terms of the severity of mucosal invasion (9), and clinically undernutrition is believed to exacerbate the signs and symptoms of the disease (10).

Several animal models have been developed to study giardiasis. Recently the Mongolian gerbil, Meriones unguiculatus, has been successfully infected with Giardia lamblia cysts (11, 12). The present study employed this animal model to determine the effects of host diet, specifically insoluble dietary fiber, on the pathogenesis of giardiasis.

METHODS:

ANIMAL MODEL:

Young adult male gerbils were obtained from Tumblebrook Farms and gang housed in wire floored cages until inoculated with Giardia cysts, after which they were individually housed in metabolism cages. The two diets employed in this study were a pelleted complete gerbil diet and a high fiber version of this diet manufactured by ICN Biochemicals, Cleveland, Ohio. The complete or low fiber diet contained 5% Alphacel, while the high fiber diet contained 20% Alphacel. Alphacel is approximately 90% cellulose and 10% hemicellulose. All feeding was ad lib.

The general protocol that was followed involved maintaining the animal on one of the diets for a specific period of time, starving for 24 hours, and then inoculating the animal per os with a known number of Giardia lamblia cysts (strain CDC:0284:1) in 0.1 ml water (12). The cysts used were isolated from stool samples of infected gerbils and were maintained in water at 4° C for no more than 4 days prior to inoculation.

CYST AND TROPHOZOITE COUNTS:

Damp pads were placed under the floors of each cage and 24 hour stool samples were collected on days 2, 4, 7, 9, 11 and 14. The stool samples were macerated in saline, filtered through cheese cloth, and the Giardia cysts were collected by flotation in 0.75M sucrose, washed twice in water and counted using a hemocytometer (12). The animals were sacrificed on day 14 by the intraperitoneal injection of pentobarbital and the caecal contents were removed and prepared for cyst isolation and counting. The proximal 6 cm of small intestine was also removed from each animal at this time, opened at the antimesenteric border, and the mucosa was harvested by lightly scraping with a glass slide. The mucosal scraping and any luminal content were then pooled and fixed in 1%

formalin-saline overnight. Thorough, gentle agitation was then used to separate the trophozoites from mucosal tissue and mucus, and the trophozoites were then counted using a hemocytometer.

MEASUREMENT OF MUCUS BLANKET ACIDIC GLYCOPROTEIN CONTENT, GASTROINTESTINAL TRANSIT AND ESTIMATION OF LUMINAL pH:

The small intestine was removed from sacrificed animals, rinses with room temperature saline and opened at the antimesenteric border. The method of Corne et al (13) was used to assess the acidic glycoprotein content of small intestinal segments. This method involved staining tissue samples with alcian blue dye in a buffered sucrose solution, pH 5.8, for 2 hours, rinsing the tissue of any uncomplexed dye using three rinses of 0.5M sucrose over a 45 minute period, and recovering complexed dye by soaking the tissue in 0.5M MgCl₂ for 2 hours. Results are expressed as the number of μ g dye recovered per gm wet weight of tissue.

Gastrointestinal transit was measured in animals that had been denied food for 24 hours. Animals were administered 0.5 ml 2% phenol red and 0.25% methylcellulose per os (14). Ten minutes later, the animals were sacrificed and the stomachs and small intestines removed. The small intestines were divided into three equal segments and the stomachs and intestinal segments were flushed with saline to recover phenol red. The phenol red content of these samples were measured by the addition of NaOH to each sample to increase the pH to approximately 10, and the sample absorbences were measured at 600 nm. Results are given as the phenol red content of a sample expressed as a percentage of the total phenol red recovered from that animal.

The gastric and intestinal luminal pH was assessed in animals denied food for 24 hours by measuring the pH of saline used to flush the stomach, upper and middle thirds of the small intestine. A constant volume (3 ml) of saline was used to irrigate each sample.

HISTOLOGICAL AND ULTRASTRUCTURAL OBSERVATIONS OF JEJUNAL TISSUE:

At the time an animal was sacrificed, the distal 2 cm of the upper third of the small intestine was sampled for histological and ultrastructural study. A 1 cm segment was fixed in neutral formalin, embedded in paraffin, and 5 μ m sections were stained using hematoxylin and eosin, periodic acid-Schiff or alcian blue dye at pH 2.0. Sections were examined to assess the degree of trophozoite attachment to the mucosa, and the amount of neutral or acidic glycoprotein remaining in crypt and villus goblet cells.

In ultrastructural studies, segments of jejunum were opened at the antimesenteric border, pinned out, mucosa facing upwards, and fixed overnight with 0.1 M cacodylate buffered, 2.5% glutaraldehyde, pH 7.4, at 4° C. TEM specimens were postfixed for 1 hour in cacodylate buffered 1% osmium tetroxide. Both TEM and SEM samples were then dehydrated in a graded series of ethanol solutions. SEM samples were dried in a critical point drier and sputter coated with gold-palladium prior to being mounted and examined with a JOEL JSH 820 scanning electron microscope. TEM specimens were embedded in Epon 812, and thin sections were picked up on copper-rhodium grids, stained with 4% uranyl acetate and Reynold's lead citrate, and examined with a JOEL 1200 EX transmission electron microscope.

RESULTS:

Gerbils were gang housed on wire floored cages and fed the high or the low fiber diet for 2 weeks. They were then denied food for 24 hours and inoculated with 100 G. lamblia cysts prior to being individually housed and returned to their original diet. Figure 1 illustrates the daily cyst excretion of these animals during the next 2 weeks and the trophozoite count obtained from the upper 6 cm of small intestine at the time of sacrifice (14th day postinoculation). The infection rate was significantly lower for animals on the

high fiber diet as judged by a Chi squared test ($p \leq 0.05$). At the time of sacrifice, all infected animals appeared to have an accumulation of fluid in the small intestine (enteropooling) which was reflected in an increased small intestinal weight. This small intestinal weight is expressed as a percentage of the body weight in Table 1. There was a statistically significant increase in the small intestinal weight ($p \leq 0.01$) as judged by Student t tests comparing the mean values of infected animals to uninfected animals on the same diet.

A variety of parameters were tested in low and high fiber diet fed animals that had been denied food for 24 hours in an attempt to determine which of these parameters could have accounted for the observed difference seen in infection rates in animals on these two diets. Table 2 summarizes these data. Using Student t tests, no significant differences were found between mean values for luminal pH, gastrointestinal transit or mucus blanket acidic glycoprotein content between low fiber and high fiber diet groups. There was less complete small intestinal emptying in the high fiber diet group and this was reflected in a significantly higher small intestinal weight as determined by a Student t test. There was also significant caecal enlargement in the high fiber diet group, reflected in a significantly larger mean caecal weight.

The lack of any significant dietary fiber-induced differences in upper gastrointestinal luminal pH, transit and duodenal mucus blanket acidic glycoprotein suggested the possibility that diet controlled conditions in the intestine at the time of cyst inoculation were not responsible for the observed dietary effect on infection rates. To clarify whether the dietary fiber effect took place prior to, or following, Giardia cysts inoculation, animals were placed on the high fiber diet for 2 weeks prior to cyst inoculation and switched to the low fiber following cyst inoculation. The infection rate resembled that seen in animals maintained on the low fiber diet for the entire experiment. Similarly, when animals were fed the low fiber diet prior to cyst inoculation and the high fiber diet

following cyst inoculation, the infection rate resembled that seen in animals fed the high fiber diet throughout the experiment. Figure 2 illustrates the results of these experiments. When Chi squared tests were used to determine the significance of differences between mean infection rate values the difference between the two switched diets groups was significant at $p \leq 0.01$. The difference in infection rates between the two groups fed the same diet for the entire experiment was significant at $p \leq 0.05$.

To determine if the protective effect of the high fiber diet involved bile salt binding, animals were maintained on the low fiber diet, inoculated with 100 *G. lamblia* cysts, and returned to the low fiber diet. Six animals were given 0.5 ml water per os twice a day for 14 days, while six animals were given 0.5 ml water containing 5 mg cholestyramine twice daily. Five of the six animals given water became infected, while six of the six cholestyramine-treated animals became infected.

Morphological studies were performed using a group of animals maintained on the low fiber diet, all of which were infected by the administration of 1,000 cysts. On day 5 following cyst inoculation, half of these animals were placed on the high fiber diet, and on day 6, all animals were sacrificed. Trophozoite counts in these animals suggested that even 24 hours on the high fiber diet reduced the magnitude of the infection in the lower two thirds of the small intestine (Table 3). The trophozoite counts were not heterogenous for the lower small intestine, necessitating the use of a non-parametric test. A Wilcoxon two-sample test indicated a significant difference ($p \leq 0.05$) between the high and low fiber diet groups in the lower small intestine.

Scanning electron micrographs of the jejunum of these animals revealed a great deal of variability between and within animals in both the distribution of trophozoites on the surface of villi and in the intestinal lumen. Figure 3 summarizes the major SEM observations. In general, trophozoites were more widely distributed on the surfaces of villi in low fiber diet animals (Figure 3a), while animals given the high fiber diet for 24 hours

tended to have fewer trophozoites actually on or near the mucosa, and more in the mucus between the villi and in the lumen at the tips of the villi (Figure 3b and c). In addition, the ventral disc impressions observed in the brush borders of the mucosal epithelial cells were fainter and fewer in the animals exposed to the high fiber diet (Figure 3d) than in the animals on the low fiber diet (Figure 3e).

Transmission electron microscopy of jejunal tissue from the same animals indicated that Giardia lamblia trophozoites attached to gerbil jejunum in the same manner as has been described for the human intestinal mucosa. Attached trophozoites flattened against the mucosal surface, with the edges of the ventral disc extended between the microvilli (Figure 4a). Trophozoites were also observed attached to the mucosal surface by the dorsal surface (Figure 4b). In keeping with the SEM data, trophozoites were observed attached to the brush border by their ventral discs more frequently in sections from low fiber diet animals. Dorsal, flange or flagellar attachment, both immediately adjacent to, and in the mucus more distant from, the mucosa was seen more frequently in the high fiber diet animals.

Histological studies in these animals confirmed the conclusion that 24 hours exposure to the high fiber diet resulted in a redistribution in the trophozoites from the surfaces of the villi to the mucus of the intestinal lumen. Alcian blue and periodic acid-Schiff stained sections failed to reveal any differences between these two groups of animals in the acidic and neutral glycoprotein contents of jejunal villus goblet cells.

DISCUSSION:

In the present study Mongolian gerbils were fed complete diets that were high or low in insoluble fiber. The average daily wet stool weight of animals on the high fiber diet was 3.13 ± 0.36 gm, which was essentially the same as that of animals on a commercial lab chow, while the average stool weight of animals on the low fiber diet was 1.07 ± 0.18 . The infection rates of animals inoculated with G. lamblia cysts were the same for animals on the complete high fiber diet and on a commercial lab chow. However, following inoculation with 100 cysts, the infection rate was significantly higher in animals on the low fiber diet when compared with animals on the high fiber diet.

Dietary fiber has been shown to cause many gastrointestinal effects including changes in small intestinal morphology (15), mucus secretion (16), and transit time (17), and bile salt binding (18), depending upon the nature of the fiber being consumed. There are at least two points in the establishment of an intestinal infection with Giardia lamblia where a dietary fiber effect could be manifested, at the level of excystation, and at the level of intestinal colonization by trophozoites. In order to determine whether the observed dietary fiber effect was the result of a fiber induced change in the upper gastrointestinal tract affecting excystation, luminal pH, transit time and duodenal mucus blanket acidic glycoprotein content were assessed in animals under the conditions existing at the time of cyst inoculation. No differences were observed between animals on the low fiber diet and the high fiber diet in any of the upper gastrointestinal tract parameters measured. Incomplete emptying of the lower small intestine and caecal enlargement accounted for significantly larger small intestine and caecum: body weight ratios in the high fiber diet group. This apparent lack of an effect of dietary fiber on the gastric and upper small

intestinal environment at the time of cyst inoculation suggested that the fiber effect on experimental giardiasis was at the level of trophozoite colonization rather than at the level of excystation. This suggestion was supported by the observation that when animals were changed from the low fiber diet to the high fiber diet at the time of cyst inoculation, the infection rate resembled that of animals continuously maintained on the high fiber diet. Similarly, when animals were switched from the high fiber diet to the low fiber diet at the time of cyst inoculation, the infection rate resembled that of animals maintained on the low fiber diet. Thus the fiber effect was manifested at the time when trophozoite colonization and growth were taking place, and not at the time of excystation.

The protection that the insoluble dietary fiber afforded the gerbils against experimental giardiasis appeared to be limited to reducing the incidence of infection when the animals were infected with a small number of cysts. When animals were inoculated with a large number of cysts ($\geq 1,000$), all became infected whether they were fed the high or the low fiber diet. In addition, the enteropooling observed in infected animals was of the same magnitude in animals on either diet, indicating that this aspect of the consequence of the experimental giardiasis was not influenced by dietary fiber once an infection had been established.

Giardia lamblia trophozoites grown in axenic culture require a bile supplement (19). It seems unlikely, however, that the high fiber diet interfered with the intestinal colonization by Giardia trophozoites by denying the parasite necessary growth promoting factors from bile and bile salts (20) as cholestyramine had no effect on the infection rate in animals inoculated with 100 cysts while fed the low fiber diet, and cellulose would not be expected to effectively bind bile salts (21).

The existence of ventral disc impressions in the mucosal brush border in giardiasis (22) points to the trophozoites passing through cycles of attachment to the brush borders, followed by detachment and subsequent reattachment elsewhere. Carbohydrate binding

lectins on the surfaces of the trophozoites (5) probably account for the fact that many parasites appear to also randomly attach to the intestinal mucosa by their dorsal surface or to the luminal mucus. The probability of the latter occurring would be expected to be increased by the hypersecretion of mucus seen in giardiasis (23).

In the present study, we infected animals maintained on the low fiber diet by the inoculation with 1,000 Giardia cysts per os. This was followed by changing one half of the animals to the high fiber diet for 24 hours on day 5 of the experiment. The morphological data suggested that exposure to the high fiber diet for 24 hours caused a redistribution of the trophozoites in the upper small intestine. The parasites were seen to attach more to the luminal mucus and less to the mucosal surfaces of the villi (Figure 3a, b and c). The high fiber diet appeared to reduce the number and size of the ventral disc impressions left by the attachment of Giardia trophozoites to jejunal enterocytes (Figure 3d and e). The significance of the magnitude of the ventral disc impression is not known at this time. It may correlate with the duration of time a trophozoite remained attached or be inversely related to the time elapsed since the trophozoite detached. Transmission electron microscopy and histology also confirmed that 24 hours on the high fiber diet increased the probability that trophozoites could attach to the mucosal surface by the dorsal surface or remain attached to the luminal mucus. The fiber-induced redistribution of trophozoites in the small intestine, coupled with the reduction in trophozoite counts, particularly in the lower small intestine, suggests that the high fiber diet increased mucus secretion and that this, together with the increased bulk of the intestinal chyme, caused a clearing of trophozoites from target sites in the upper small intestine, accounting for the observed protective effect of the high fiber diet on animals inoculated with a small number of G. lamblia cysts.

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Table 1.

Small intestine weight as a percentage of body weight of gerbils maintained on the low fiber or the high fiber diet for 4 weeks. After 2 weeks, experimental animals were inoculated with 100 G. lamblia cysts per os.

	Low fiber	High fiber
Control	2.17 ± 0.07 (N = 5)	2.59 ± 0.06 (N = 5)
Inoculated, not infected	2.23 ± 0.13 (N = 4)	2.59 ± 0.05 (N = 10)
Inoculated, infected	4.01 ± 0.22 (N = 16)	4.27 ± 0.35 (N = 6)

Means ± SEM

Table 2.

Gastrointestinal parameters of gerbils maintained on the low fiber or the high fiber diet for 2 weeks and starved for 24 hours.

	Low fiber	High fiber	Difference
pH:			
Stomach	2.74 ± 0.11	3.42 ± 0.53	NS
Upper small intestine	7.55 ± 0.06	7.64 ± 0.09	NS
Middle small intestine	8.18 ± 0.21	8.15 ± 0.13	NS
Gastrointestinal transit, % dye recovered from			
Stomach	44.7 ± 9.9	58.2 ± 7.4	NS
Upper small intestine	21.0 ± 3.3	19.0 ± 3.8	NS
Middle small intestine	29.7 ± 8.5	22.4 ± 5.1	NS
Lower small intestine	4.7 ± 2.6	0.4 ± 0.3	NS
Duodenal mucus blanket acidic glycoprotein*	160 ± 18	164 ± 15	NS
Small intestine weight: body weight ratio, %	1.69 ± 0.08	2.55 ± 0.17	p ≤ 0.01
Caecum weight:body weight ratio, %	1.26 ± 0.12	1.76 ± 0.20	p ≤ 0.01

Means ± SEM

*μg alcian blue dye recovered per gm tissue

Table 3.

G. lamblia trophozoites recovered from the lumen and mucosa of gerbils maintained on the low fiber diet after being inoculated with 1,000 Giardia cysts per os. On day 5, half of the animals were placed on the high fiber diet for 24 hours and all animals were then sacrificed.

	Trophozoite Count x 10 ³		
	Low Fiber	High Fiber	Difference
Upper Small Intestine	31,125 ±9,613	34,250 ±2,420	NS
Middle Small Intestine	17,114 ±5,722	7,107 ±1,641	NS
Lower Small Intestine	3,576 ±1,542	421 ±134	p ≤ 0.05

Means ± SEM

Figure 1.

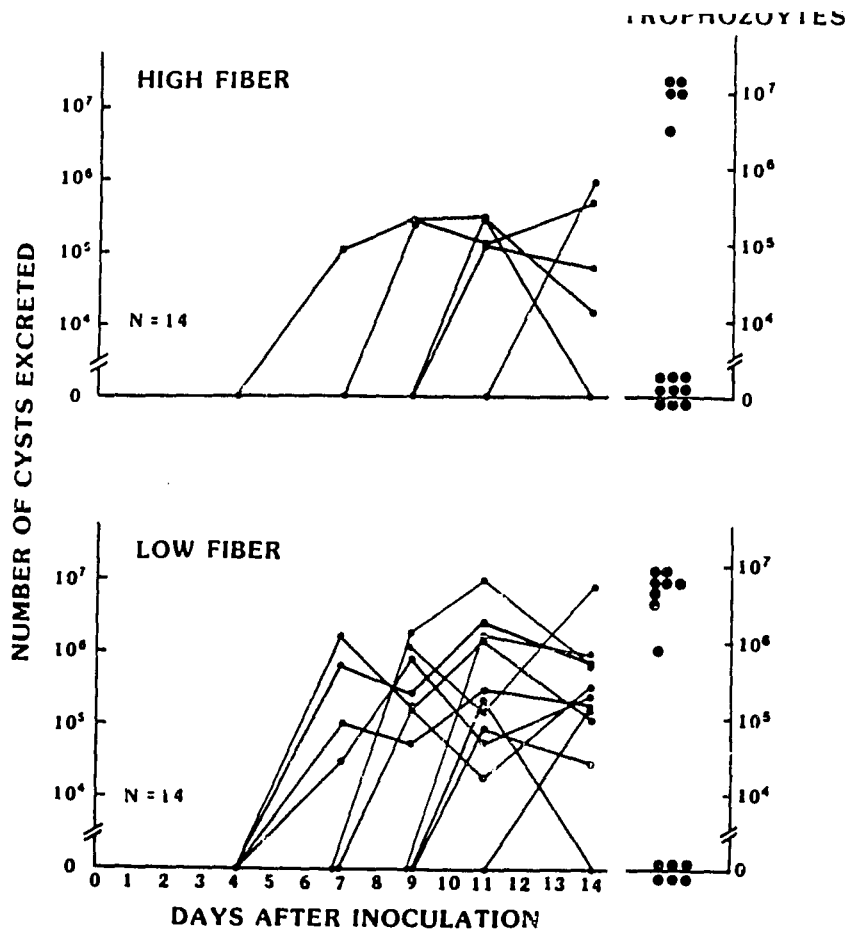
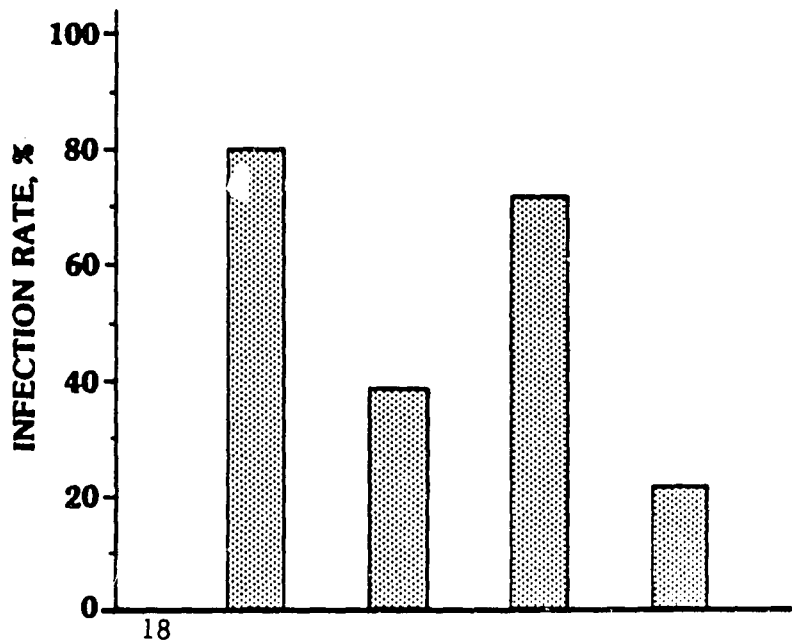


Figure 2.

DIET

2 WK PREINOCULATION	LF	HF	HF	LF
2 WK POSTINOCULATION	LF	HF	LF	HF



24

FIGURE LEGENDS

FIGURE 1. Daily G. lamblia cyst excretion by gerbils maintained on the high or the low fiber diet for 2 weeks prior to the 2 weeks following the per os administration of 100 cysts. Trophozoite counts represent the number of trophozoites recovered from the proximal 6 cm of small intestine on the 14th day postinoculation.

FIGURE 2. Infection rates of gerbils inoculated per os with 100 G. lamblia cysts. Animals were maintained on the low fiber diet (LF) or the high fiber diet (HF) for 2 weeks prior to and 2 weeks following cyst inoculation.

FIGURE 3. Gerbils were maintained on low fiber diet for 2 weeks and inoculated with 1,000 G. lamblia cysts per os. On the 5th day postinoculation, half of the animals were changed to high fiber diet, while the remainder remained on the low fiber diet. All animals were sacrificed on the 6th day. (a) Small intestinal villi of low fiber diet animal. Giardia trophozoites are seen to cover much of the surface of the villi, although many are attached by their dorsal rather than by their ventral surface. (b) Small intestinal villi of high fiber diet animal. Relatively few trophozoites are seen on the villus surface. Most appear to be attached to the mucus in the luminal space between the villi. (c) Trophozoites are seen to adhere to the mucus that is associated with fiber fragments in the lumen of a high fiber diet animal. The Giardia ventral disc impressions (arrows) seen in the brush border of high fiber diet animals (d) were fewer and shallower than those seen in low fiber diet animals (e) Bars represent 50 μm in (a) and (b), 10 μm in (c), and 1 μm in (d) and (e).

FIGURE 4. (a). Giardia trophozoite attached by the ventral disc to the jejunal brush border of the low fiber diet animal shown in Figure 3a. (b). Giardia trophozoite attached to the jejunal brush border of the high fiber diet animal shown in Figure 3b. In this case, the parasite attached to the epithelial cell glycocalyx by the dorsal surface.

Fig 3



27

a

