Case report

Surface ultrastructure of giardial infestation

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Although the pathogenetic significance of giardial infestation in man has been questioned,[1] several studies have demonstrated that a malabsorption syndrome in children identical to celiac disease is associated with *Giardia lamblia* infestation.[2–4] Furthermore, successful treatment of the parasitic infestation in adults has been described. Until recently mucosal invasion by the parasite had not been demonstrated;[4–10] Brandborg et al[11] and others[12, 13] have recently documented mucosal invasion by the organism.

This report describes the light microscopy and surface ultrastructure of duodenal mucosa in a patient with giardial infestation and malabsorption.

Case report

A 58-year-old man was examined in the Department of Gastroenterology on October 16, 1975, for persistent diarrhea, left upper quadrant pain, belching, and abdominal distention. The symptoms had begun 2 years earlier; three to ten loose watery brown stools were passed daily. The patient had a 10-kg weight loss during the previous 3 years. An upper gastrointestinal series 8 months previously demonstrated edematous mucosal folds (*Fig. 1*); a barium enema at that time disclosed mild diverticulosis. The patient appeared thin. No other abnormalities were noted on the physical examination.

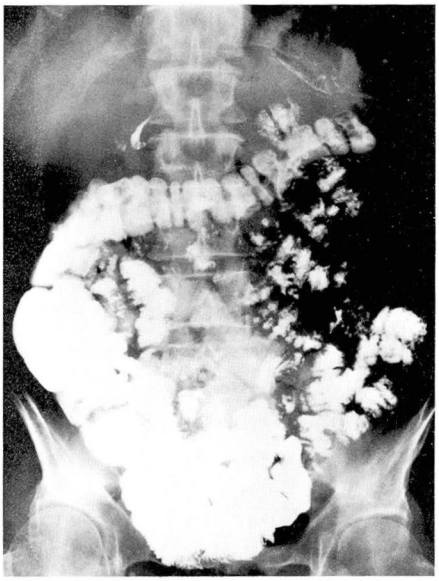

Fig. 1. Small bowel series. Note edematous mucosal folds.

Complete blood count, urinalysis, and chest x-ray were normal. The D-Xylose value was normal. Serum carotene was 40 mg/dl. A few *Giardia lamblia* cysts were present in the stool; a specimen obtained by duodenal aspiration contained many *Giardia* trophozoites. The patient was treated with metronidazole (Flagyl), 250 mg three times daily orally for 10 days, with prompt resolution of symptoms and weight gain of 3 kg. On April 13, 1975, a small bowel series and peroral biopsy were normal; no parasites were observed in a duodenal aspirate, and the serum carotene was 120 μg/dl. The patient was last examined April 21, 1976, was asymptomatic, and had noted a net weight gain of 9 kg.

Methods

Tissue was obtained from the duodenum with the Crosby-Kugler's capsule utilizing the method described by Crosby and Kugler.[14] The specimen, consisting of light tan tissue measuring 0.3 x 0.3 x 0.1 cm, was divided into two segments. One segment was fixed in modified Zenker's solution for light microscopy; the other was fixed overnight in gluteraldehyde, critical point dried in the Omar STC-900/Ex drier, coated with gold palladium, and examined in the AMR-1200 scanning electron microscope.

Sections submitted for light microscopy were stained with hematoxylin and eosin, periodic acid Schiff pre- and post-diastase digestion, and Gridley's method for *Endamoeba histolytica*.[15] The latter consistently stained the trophozoite nucleus red, and inconsistently stained the organism a light reddish-orange sufficient to differentiate the parasite from adjacent tissue. Consistent red staining of the organism was obtained using the technique of Brandborg et al;[11] a reduction of exposure time to scarlet red from 5 to 2½ minutes resulted in better differentiation from the surrounding tissues.

Histopathology

The biopsy specimen consisted of a small bowel mucosal and submucosal segment. No villus atrophy, mucosal edema, or abnormal epithelial mitotic rate were identified (*Fig. 2*). Mild chronic lymphoplasmacytic inflammation of the lamina propria was noted. Many *Giardia* trophozoites were identified within the lumen and in the crypts (*Fig. 3*); no parasites were observed in the mucosa or submucosa. The repeat small bowel biopsy obtained after treatment and resolution of symptoms was normal.

Surface ultrastructure

The surface ultrastructure of *Giardia* infested duodenal mucosa is illustrated in *Figures 4 through 7*. Mild to absent shortening of microvilli was

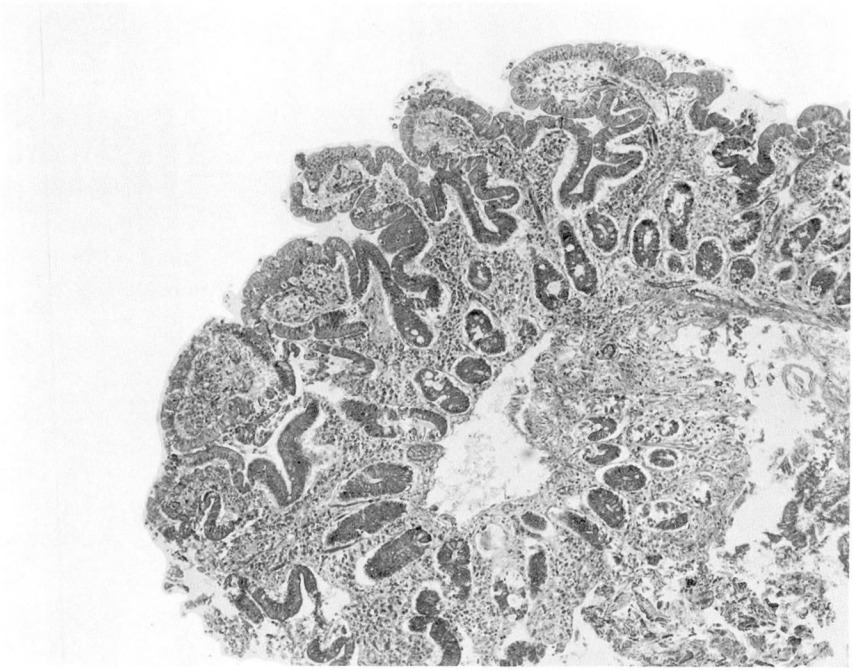

Fig. 2. Duodenal peroral biopsy. No villus atrophy is apparent. Mild chronic inflammation is apparent in the lamina propria and submucosa (hematoxylin and eosin stain, ×64).

noted, an observation which Takano and Yardley[9] made utilizing transmission electron microscopy. Trophozoites were observed lying free on the luminal surface (*Fig. 6*). Most of the parasites were attached to the mucosal surface, so that only the pear-shaped dorsal aspect was observed (*Figs. 5 and 7*). This configuration probably results from a mode of attachment described by Morecki and Parker,[13] who have demonstrated by transmission electron microscopy that the parasite frequently extends over the mucosal surface apparently attached to the mucosa by its ventral suction disk.

Comment

Although most investigators have been unable to identify mucosal invasion in man,[4–9] experimental studies[12] of murine small intestine infested with *Giardia lamblia* documented the presence of the parasite in the intestinal wall. Brandborg et al[11] and Morecki and Parker[13] have demonstrated mucosal invasion, attributing the failure of other investigators to demonstrate tissue invasion to (1) inadequate differential stains, (2) absence of inflammatory response to invasion, (3) the subtle nature of mucosal damage apparent on light microscopy, (4) the random sampling nature of the small bowel biopsy, and (5) the relative paucity of invading organisms with respect to the luminal population.

The surface ultrastructure of the duodenal mucosa reveals that both attached and nonattached trophozoites are present with absent or mild shortening of microvilli. It is possible that scanning electron microscopy of the duodenum in symptomatic pa-

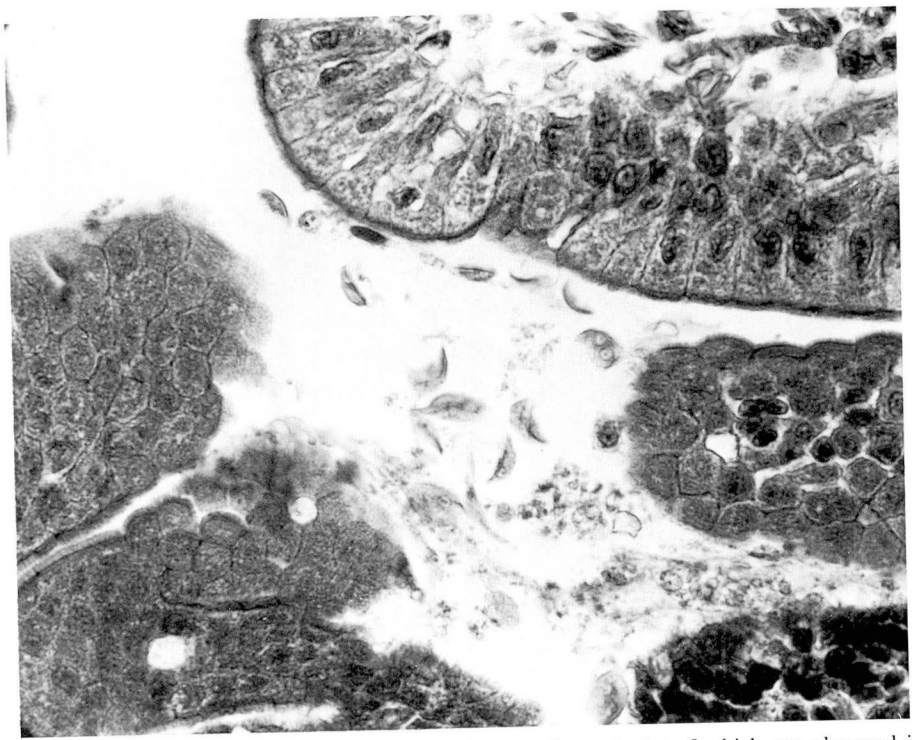

Fig. 3. Abundant *Giardia* within lumen of duodenum, the majority of which are observed in a transverse plane (Gridley *E. histolytica* stain, ×640).

Fig. 4. Note slight shortening of microvilli and pear-shaped parasite lying upon the duodenal mucosa (×2000).

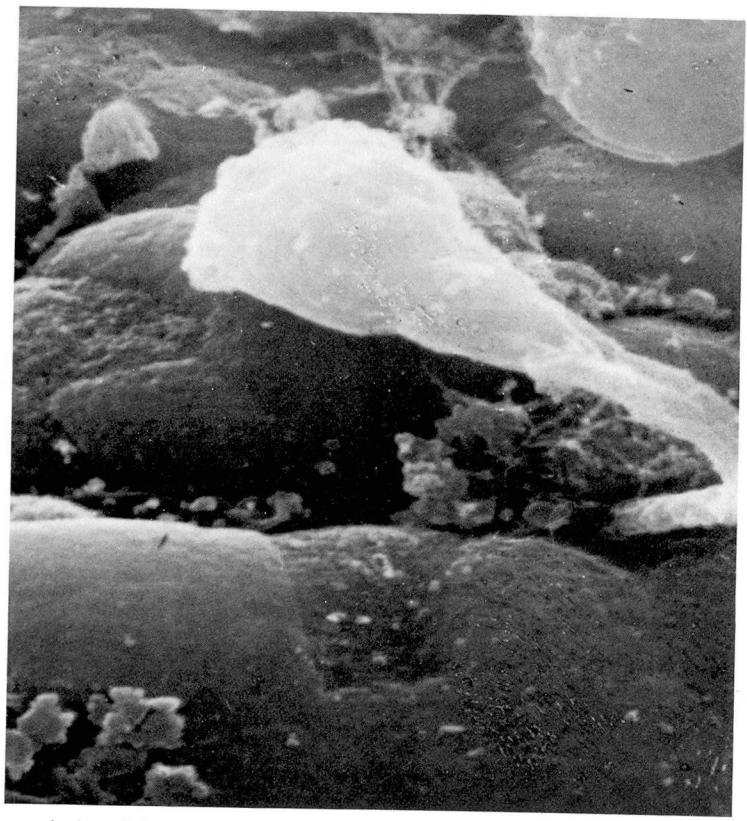

Fig. 5. The majority of the parasites were attached to the mucosa so that only the dorsal aspect of the organism was apparent (×2000).

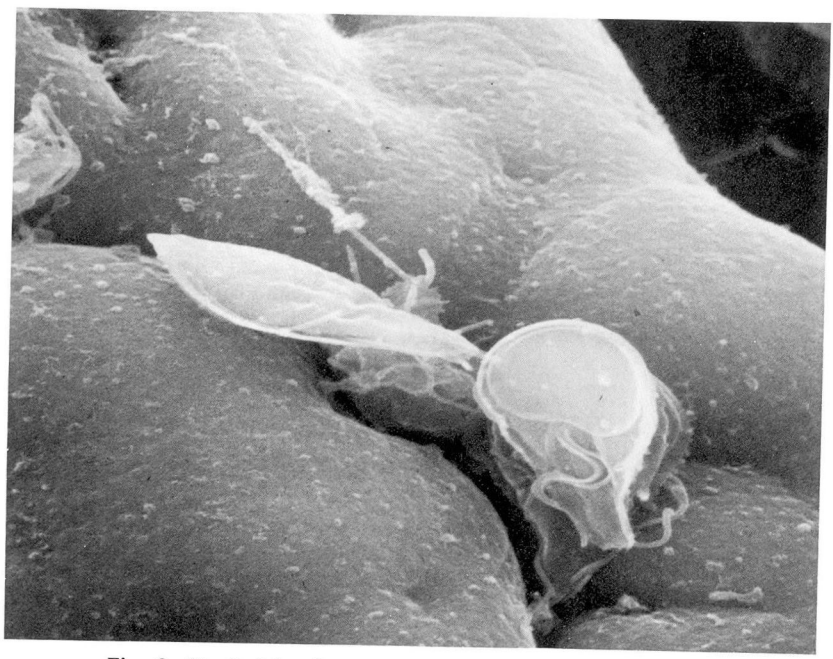

Fig. 6. *Giardia* lying free upon the duodenal mucosa (×2000).

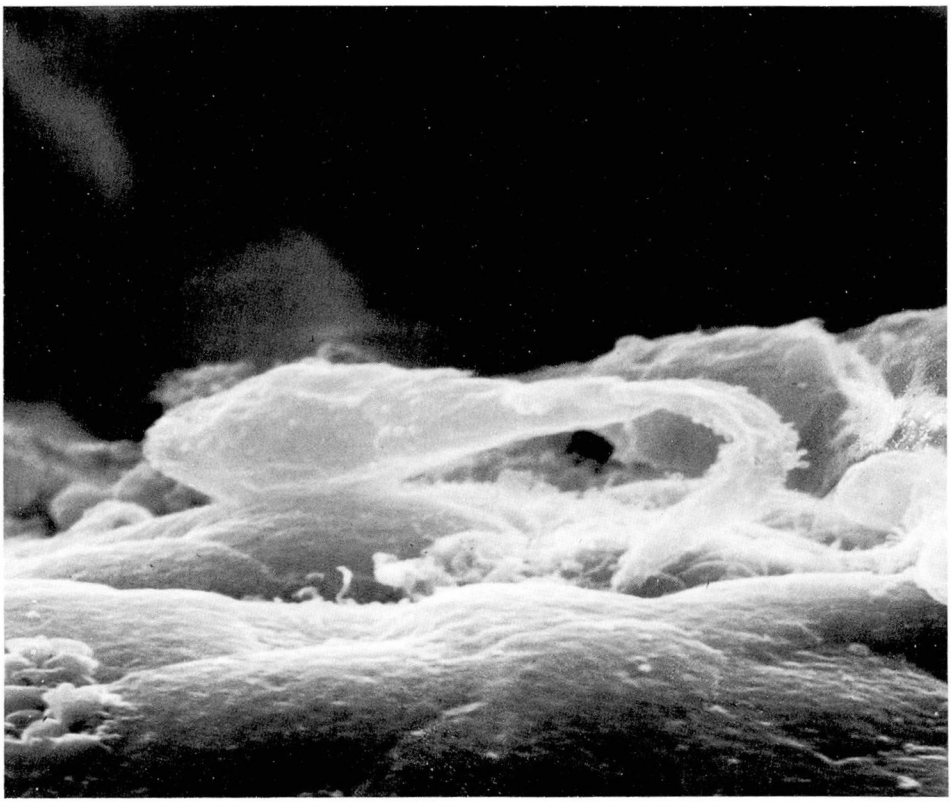

Fig. 7. Scanning electron micrograph (×1000); the organism is attached to the small bowel mucosa by a sucking disk, the flagella lying free on the surface.

tients without trophozoites in the small bowel aspirate or stool may demonstrate *Giardia lamblia* attached to the mucosa. Studies testing this hypothesis are under investigation.

Summary

Although the pathogenetic significance of infestation with *Giardia lamblia* has been debated, most investigators agree that the organism is capable of producing a sprue-like syndrome in both adults and children. Successful treatment of these patients with antimicrobial agents and the demonstration of tissue invasion by the organism have provided convincing evidence for the pathogenicity of the organism in humans.

A case of giardial infestation and malabsorption syndrome with resolution following metronidazole therapy is described. Light microscopy demonstrated the presence of organisms within the lumen of the small bowel and especially in the mucosal crypts. Surface ultrastructural studies confirmed the findings and demonstrated attachment of the organism to the mucosal surface and mild atrophy of villi.

References

1. Monat HA, McKinney WL Jr: Giardiasis; question of pathogenicity. U. S. Nav Med Bull **46:** 1204–1206, 1946.
2. Cortner JA: Giardiasis, a cause of celiac syndrome. Am J Dis Child **98:** 311–316, 1959.

3. Court JM, Anderson CM: The pathogenesis of *Giardia lamblia* in children. Med J Aust **46:** 436–438, 1959.
4. Veghelyi P: Celiac disease imitated by giardiasis. Am J Dis Child **57:** 894–899, 1939.
5. Zamcheck N, Hoskins LC, Winawer SJ, et al: Histology and ultrastructure of the parasite and the intestinal mucosa in human giardiasis; effects of antabrine therapy. (Abstr) Gastroenterology **44:** 860, 1963.
6. Vachon A, Paliard P, Moulinier B, et al: Diagnostic par biopsie jéjunal d'une diarrhée chronique à *lamblia* avec syndrome de dénutrition. Arch Mal Appar Dig **52:** 355–359, 1963.
7. da Silva JR, Coutinho SG, Dias LB, et al: Histopathologic findings in giardiasis; a biopsy study. Am J Dig Dis **9:** 355–365, 1964.
8. Yardley JH, Takano J, Hendrix TR: Epithelial and other mucosal lesions of the jejunum in giardiasis; jejunal biopsy studies. Bull Johns Hopkins Hosp **115:** 389–406, 1964.
9. Takano J, Yardley JH: Jejunal lesions in patients with giardiasis and malabsorption; an electron microscopic study. Bull Johns Hopkins Hosp **116:** 413–429, 1965.
10. Barbieri D, De Brito T, Hoshino S, et al: Giardiasis in childhood; absorption tests and biochemistry, histochemistry, light, and electron microscopy of jejunal mucosa. Arch Dis Childh **45:** 466–472, 1970.
11. Brandborg LL, Tankersley CB, Gottlieb S, et al: Histological demonstration of mucosal invasion by *Giardia lamblia* in man. Gastroenterology **52:** 143–150, 1967.
12. Dekkan-Khodzhaeva N: On the ability of *lamblia* to penetrate the tissues. Med Parazit (Moskva) **29:** 226–228, 1960.
13. Morecki R, Parker JG: Ultrastructural studies of the human *Giardia lamblia* and subjacent jejunal mucosa in a subject with steatorrhea. Gastroenterology **52:** 151–164, 1967.
14. Crosby WH, Kugler HW: Intraluminal biopsy of the small intestine; the intestinal biopsy capsule. Am J Dig Dis **2:** 236–241, 1957.
15. Armed Forces Institute of Pathology: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, ed 2. Luna LG, ed. New York, McGraw-Hill Book Co, 1968, pp 228–229.