The Seoul Journal of Medicine
Vol. 36, No. 1: 29-34, March 1995

Cryptosporidiosis in a Leukemia Child with Severe Diarrhea

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= Abstract = **Protozoa of the genus Cryptoporidium are smallc occidian parasites that infect the mucosal epithelium of a variety of animals and humans, causing protracted diarrhea in immunodeficient or malnourished patients as well as self-limited illness in previously healthy individuals.**

Cryptosporidiosis in a resected appendix from a child with acute lymphoblastic leukemia is reported. He had had severe protracted watery diarrhea with abdominal pain for one month. The standard hematoxylin and eosin stain revealed many spherical, basophilic organisms on the apical surface of the mucosal epithelial cells. The various stages of the *Cryptosporidium parvum* were identified by electron microscopy.

It is important to recognize this organism because it is a widespread pathogen of diarrheal illness and may cause life-threatening disease in immunocompromised patients especially in acquired immune deficiency syndrome patients.

Key words : *Cryptosporidium, Protozoa, Appendix, Leukemia, Electron microscopy*

INTRODUCTION

Organisms of the genus Cryptosporidium are small coccidian parasites that infect the epithelial cells lining the digestive and respiratory tracts of vertebrates (Angus, 1983; Tzipori, 1983; Fayer and Ungar, 1986). They were first described by Tyzzer in 1907 from the gastric glands of laboratory mice, and since then more than 20 species have been named. Once thought to be rare and non-pathogenic, they are now considered to be one of the important and widespread causes of diarrheal illness in men and some domesticated animals (Tzipori, 1983; Fayer *et al.*, 1986).

In immunocompetent persons, *Cryptosporidium parvum*, the species that infects human beings, may cause a short-term(3 to 20 days) diarrheal illness that resolves spontaneously. However, in immunocompromised patients, it often causes a life-threatening, prolonged, cholera-like disease (Current *et al.*, 1983; Navin and Juranek, 1984; Koch

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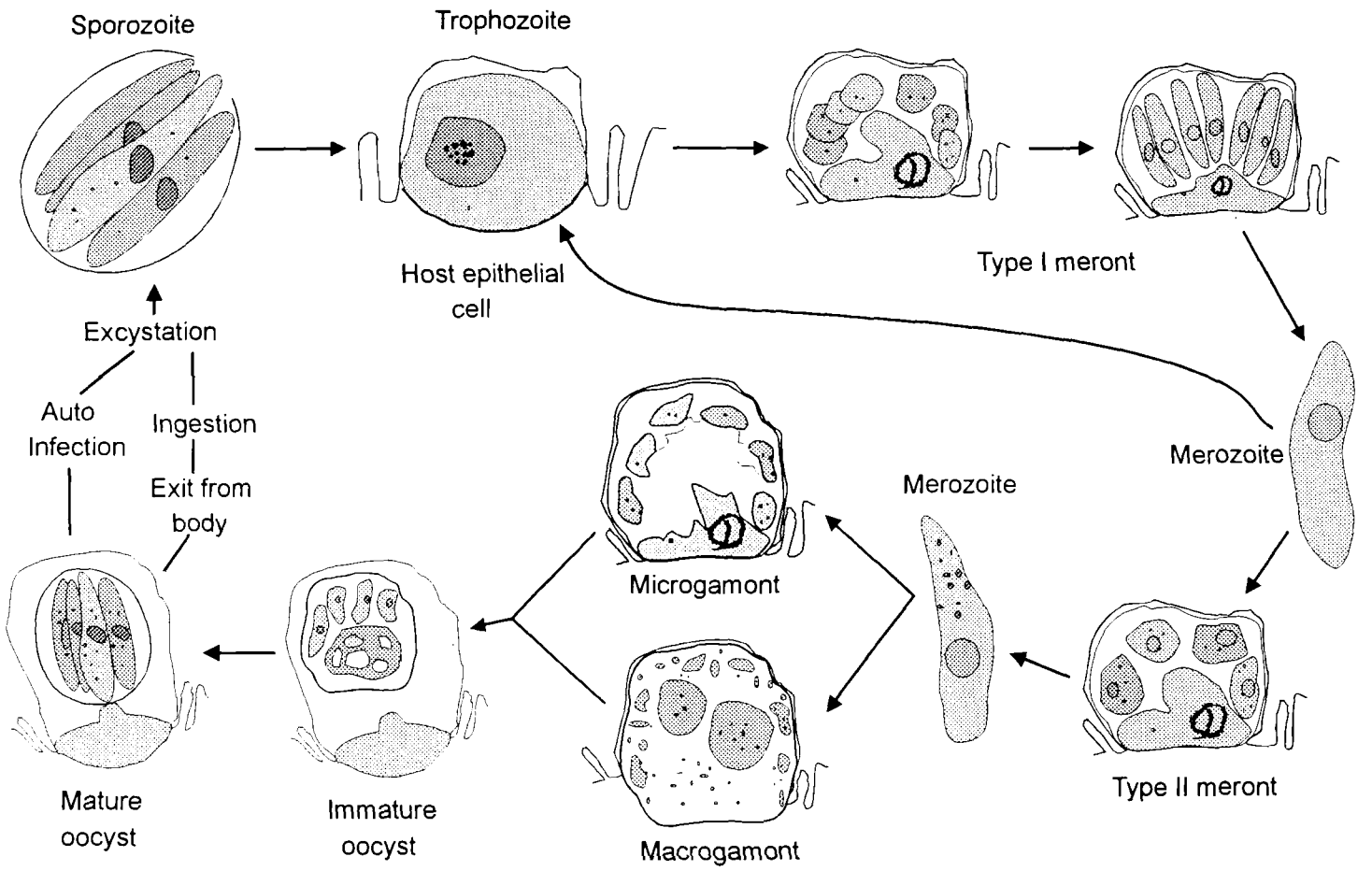

Fig. 1. Diagrammatic representation of the life cycle of Cryptosporidium. Sporozoites enter the microvillus of an epithelial cell, where they differentiate into trophozoites. Trophozoites undergo nuclear proliferation to form type I meronts. A type I merozoite leaves the meront to form either type I or II meront. Type II merozoites form microgametes or a macrogamont. After fertilization the macrogamont develops into an oocyst. Oocysts sporulate and either release sporozoites for autoinfection or pass from the body in the feces(Fayer and Ungar, 1986).

et al., 1985).

After ingestion by a susceptible host, this parasite infects the intestinal epithelial cells, where it remains extracytoplasmic but in an intracellular location and undergoes development through asexual multiplication, gametogony and oocyst formation. Autoinfection by completion of the life cycle markedly increases the number of the parasite (Current, 1985; Fayer and Ungar, 1986). The complex life cycle of Cryptosporidium resembles that of other coccidia (Fig. 1).

We report a case with cryptosporidiosis diagnosed histologically and electron microscopically in the resected appendix.

Case History

This 9-year-old boy was admitted to Seoul Paik Hospital because of several episodes of watery diarrhea, nausea, vomiting, abdominal pain and mild fever. He had been diagnosed as acute lymphoblastic leukemia (ALL, L2) one year previously and has been treated with maintenance chemotherapy agents (vincristin, prednisolone, 6-mercaptopurine, methotrexate). After admission re-induction chemotherapy was scheduled and started with vincristin, prednisolone, adriamycin and L-Asp paraginase because a bone marrow examination revealed leukemic relapse. Diarrhea and abdominal pain started 3 weeks after the chemotherapy and the diarrhea rapidly aggravated to a frequency of up to 40/day. At that time, laboratory findings were as follows : hemoglobin 9.1g/dl, hematocrit 28%, WBC 200/cmm, platelet 38K/cmm, ESR 5 mm/hr, Na 128 mmol/l, K 2.6

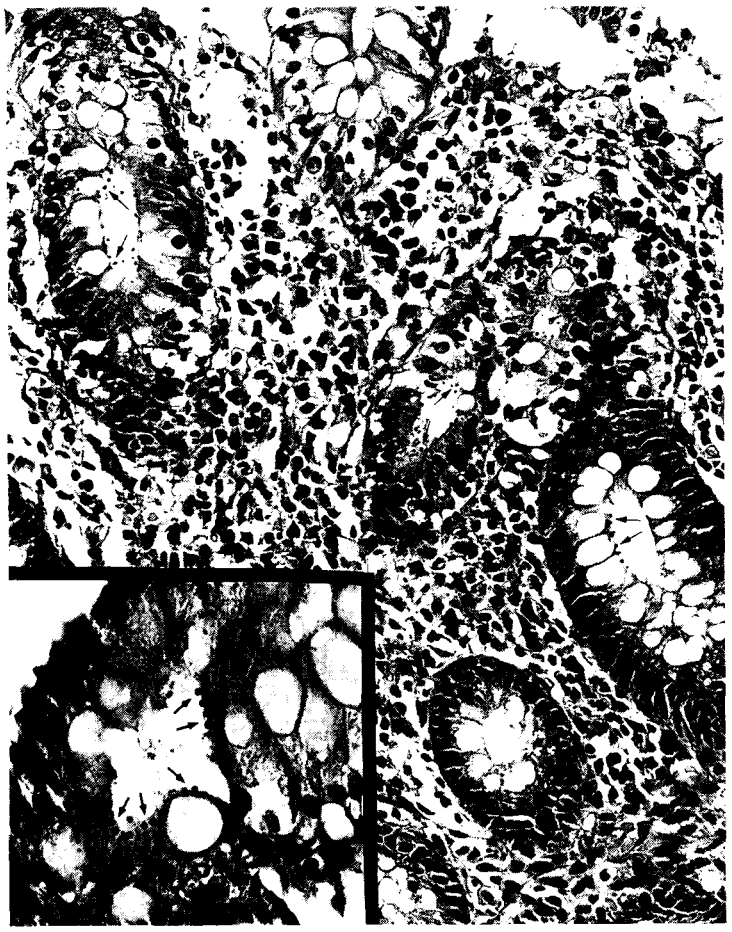

Fig. 2. Many tiny spherical organisms of the Cryptosporidium species (arrows) are noted along the apical surface of the luminal border of the crypt epithelial cells. The organisms show marked basophilia by the standard hematoxylin and eosin staining.

mmol/l, Cl 87 mmol/l, BUN 16 mg/dl, creatinine 1.4 mg/dl and lactic dehydrogenase 498 IU/l. Simple abdomen radiography revealed no bowel gas shadow.

With the clinical impression of either infection or mucositis of drug toxicity, all chemotherapeutic agents were discontinued followed by supportive intravenous hydration and granulocyte-colony stimulating factor therapy. Stool examination was done five times but neither pathologic bacteria nor protozoa was found.

Two weeks after the discontinuation of chemotherapy, his WBC count recovered to 5,700/cmm and the diarrhea which had lasted for one month began to subside. However, sudden onset of acute abdominal pain and rebound tenderness of the right lower quadrant of the abdomen was found. With the clinical impression of acute appendicitis, an emergency appendectomy was done.

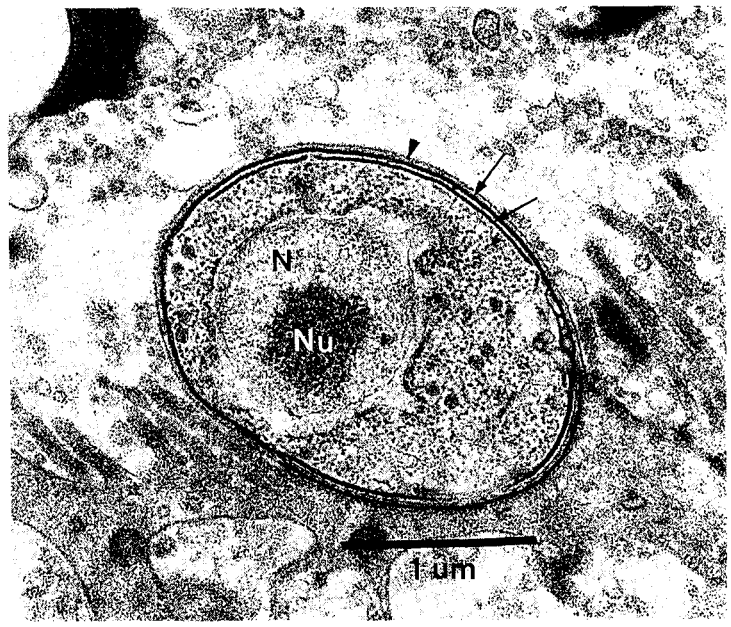

Fig. 3. Transmission electron microscopy reveals a trophozoite found within the parasitophorous vacuole surrounded by host cell membrane (arrowhead). It has two electron-dense membranes (arrows), a nucleus (N) and a prominent nucleolus (Nu) (× 40, 000).

Pathologic findings

Grossly, the resected appendix was not remarkable. Microscopically there was no evidence of acute appendicitis. On high power field of standard hematoxylin and eosin stained sections, many tiny spherical organisms were noted along the apical surface of the luminal border of the crypt epithelial cells. They were measured 2 to 5μ. The organisms showed remarkable basophilia and could be distinguished from the other cellular or mucinous materials (Fig. 2). Protozoal infection of Cryptosporidium species was suspected, and electron microscopy from the mucosal portion was performed. Transmission electron microscopy showed various stages of Cryptosporidium including trophozoites, meronts, merozoites and oocysts. The trophozoites (Fig. 3) were round to oval, 2μ in diameter and found within the parasitophorous vacuoles surrounded by host cell membranes, with an electron-dense attachment zone at the host cell interface. They had two electron-dense membranes, a large nucleus, a prominent nucleolus, scattered rough endoplasmic reticulums and ribosomes. At the parasitic attachment por-

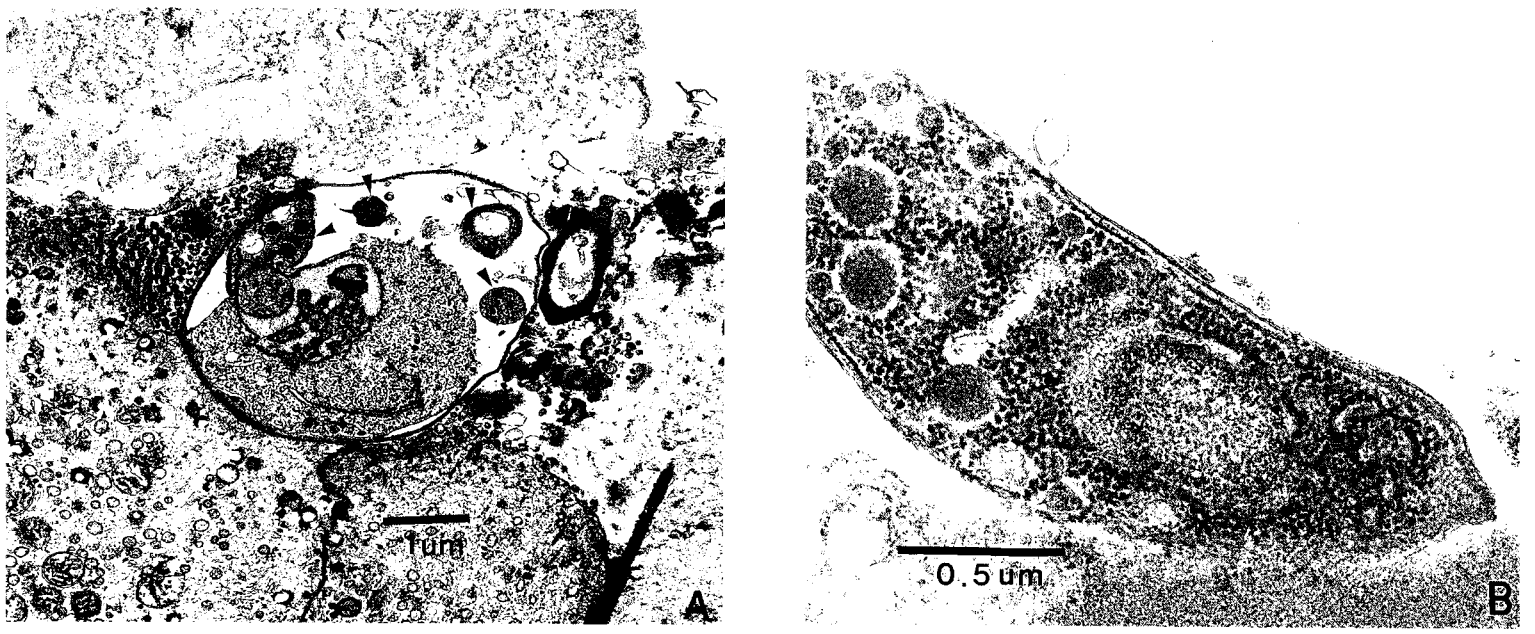

Fig. 4. A: A meront containing at least 4 merozoites (arrowheads) is noted. The microvillous projections of the epithelial cells are lost at the dense parasite attachment zone (× 17,500). B: A crescent shape merozoite measuring 3×1μ is noted within the crypt lumen (× 74,000).

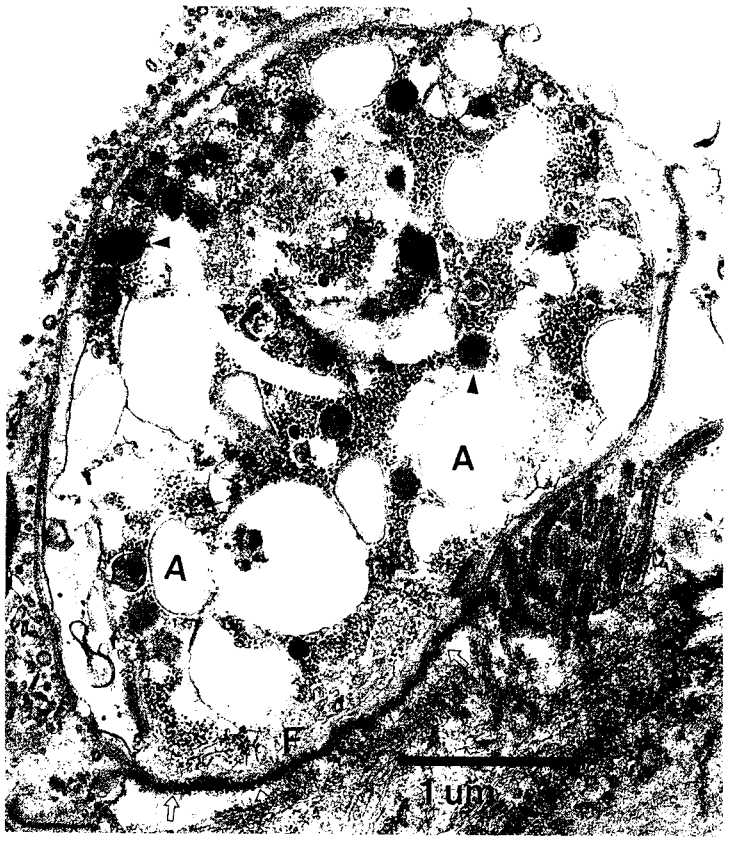

Fig. 5. A 5μ sized young oocyst containing many amylopectin granules (A) and residual bodies (arrowheads) are noted. Also revealed are an electron-dense attachment zone at the parasite-host cell interface (arrows) and thread-like feeding organelle (F) (× 31,000).

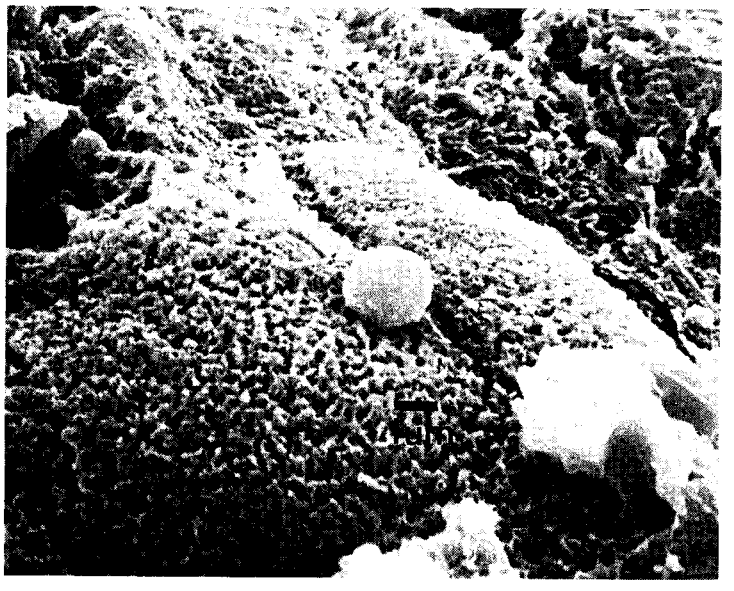

Fig. 6. Scanning electron microscopy shows a few round to ovoid trophozoite forms of the Cryptosporidium species (× 15,000).

tion, the microvillous projections of the epithelial cells were lost. Several meronts (Fig. 4A) were noted. They were 4μ in diameter and contained several merozoites. The merozoites (Fig. 4B) were noted within the crypt lumina, apart from the epithelial cells. They were 3 x 1μ sized and showed

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many amylopectin granules and residual bodies were also encountered. Scanning electron microscopy also showed a few trophozoite forms of the organism (Fig. 6).

Discussion

Recently, the Cryptosporidium species was recognized as a widespread pathogen having many hosts. More than 20 have been were named according to the host in which the parasite was found, and only two species, *C. parvum* and *C. muris* were known to infect mammals. On the basis of oocyst morphology almost all of the previously well-documented cases of cryptosporidiosis in mammals are *C. parvum* which has smaller oocysts (Upton and Current, 1985). In the early 1980s the association of the Cryptosporidium species with the diarrheal illness of patients with acquired immune deficiency syndrome (AIDS) was reported and since then human infection with Cryptosporidium species has been described on six continents, in developed and less developed countries, and in urban and rural areas (Fayer and Ungar, 1986). The prevalence began at less than 1% and reached 10 to 20%, and children may be more susceptible to infection (Alpert *et al.*, 1984; Hart *et al.*, 1984).

Diarrhea is the sine qua non of symptomatic infection which is profuse, watery and appears to be more copious in immunocompromised persons. Crampy abdominal pain, nausea, vomiting and low grade (<39℃) fever are less frequent symptoms. Symptoms may wax and wane and may correlate with the intensity of oocyst shedding (Hart *et al.*, 1984; Holley and Dover, 1986). Duration of symptoms and outcome typically vary according to the immunologic status of the host. Immunologically healthy persons usually have shorter duration of symptoms (<20 days) and have a spontaneous complete recovery (Holley and Dover, 1986). In AIDS patients, infections of long duration followed by death are most frequent, although spontaneous clinical recovery has been reported. Patients with reversible immune deficiencies as in our patient usually recover when the cause of immunosuppression is removed (Berkowitz and Seidel, 1985; Lewis *et al.*, 1985). Oocysts may be found more than a week after the cessation of diarrhea and this may be important in the transmission of the organisms (Fayer and Ungar, 1986).

Pathologically the organisms are usually found in the intestinal tract, particularly attached to the surface epithelial cells of the villi and crypts of the small intestine. They have also been found in the other gastrointestinal tract including the stomach, appendix, rectum, gallbladder and pancreatic duct (Fayer and Ungar, 1986). Special staining procedures have not provided any marked improvement over the use of hematoxylin and eosin for histologic diagnosis. Transmission electron microscopy can be used to confirm the diagnosis and reveals distinct life cycle forms, each within a parasitophorous vacuole confined to the microvillous or crypt region of the host cells. The location of these parasites has been described as intracellular-extracytoplasmic : intracellular because they are contained in a parasitophorous vacuole, and extracytoplasmic because they are confined to the microvillous region of the host cell (Fayer and Ungar, 1986). Other histologic changes include blunting and loss of villi, lengthening of crypts, infiltration of the lamina propria with lymphocytes, polymorphonuclear leukocytes and plasma cells.

There have been some doubts as to whether the Cryptosporidium species is an agent of clinical disease or simply a commensual which occasionally causes illness. However, clustering of cases in families, in day-care centers points to person-to-person or animal-to-person transmission of a single agent (Holly and Dover, 1986).

The mechanism by which Cryptosporidium causes disease in humans is unknown. Recently, Adams *et al.* (1994) studied an in vitro model of *C. parvum* infection of intestinal epithelium and suggested an alteration in barrier function, with a marked change in permeability to macromolecules. The important host immune response required for the clearance of parasites are probably local antibodies (secretory IgA) coupled with cell mediate immune mechanisms (Current, 1989).

Discontinuation of immune suppressive agents is an important treatment in patients with reversible immune deficiencies. Supportive care with intravenous hydration is the primary therapeutic intervention. There is no specific antimicrobial agent

available. Spiromycin is the only one reported to have some efficacy (Portnoy *et al.*, 1984). Hyperimmune bovine skim colostrum have therapeutic and preventive effect reported in recent studies (Tzipori *et al.*, 1986).

In Korea, several histologic studies on animals have been done (Rhee *et al.*, 1991). However, few human cases have been reported. This is the first human case which was confirmed by electron microscopy. According to the smaller size of 2-5μ, this species proved to be a *C. parvum*. We think that this parasite will be increasingly important since it is a widespread pathogen of diarrheal illness and may cause life-threatening diseases in immunocompromised patients, especially in AIDS patients.

Acknowledgment

The authors would like to thank Chul Jong Yoon, M. P. H. of the electron microscope laboratory of Seoul National University Children's Hospital for processing tissues, cutting ultrathin sections and preparing prints.

REFERENCES

Adams RB, Guerrant RL, Zu S, Fang G, Roche JK. *Cryptosporidium parvum* infection of intestinal epithelium : morphologic and functional studies in an in vitro model. J Infect Dis 1994; 169 : 170-7

Alpert G, Bell LM, Kirkpatrick CE, Budnick LD, Campos JM, Friedman HM, Poltkin SA. Cryptosporidiosis in a day care center. N Engl J Med 1984; 311 : 860-1

Angus KW. Cryptosporidiosis in man, domestic animals and birds : a review. J R Soc Med 1983 ; 76 : 62-70

Berkowitz CD, Seidel JS. Spontaneous resolution of cryptosporidiosis in a child with acquired immunodeficiency syndrome. Am J Dis Child 1985 ; 139 : 967

Current WL, Reese NC, Ernst JV, Bailey WS, Heyman MB, Weinstein MD. Human cryptosporidiosis in immunocompetent and immunodeficient persons. Studies of an outbreak and experimental transmission. N Engl J Med 1983; 308 : 1252-7

Current WL. Cryptosporidiosis. J Am Vet Med Assoc 1985; 187 : 1334-8Current WL. Cryptosporidium Spp. In Walzer PD, Genta RM (Ed) Parasitic infections in the compromised host. Marcel Dekker, New York, 1989 : pp. 281-342

Fayer R, Ungar BLP. Cryptosporidium spp. and cryptosporidiosis. Microbiol Rev 1986; 50 : 458-83

Hart CA, Baxby D, Blundell N. Gastroenteritis due to Cryptosporidium : a prospective survey in a hildren's hospital. J Infect 1984; 9 : 264-70

Holley HP, Dover C. Cryptosporidium : a common cause of parasitic diarrhea in otherwise healthy individuals. J Infect Dis 1986; 153 : 365-8

Koch KL, Phillips DJ, Aber RC, Current WL. Cryptosporidiosis in hospital personnel : evidence for person-to-person transmission. Ann Intern Med 1985; 102 : 593-6

Lewis IS, Hart CA, Baxby D. Diarrhea due to Cryptosporidium in acute lymphoblastic leukemia. Arch Dis Child 1985; 60 : 60-2

Navin TR, Juranek DD. Cryptosporidiosis : clinical, epidemiologic, and parasitologic review. Rev Infect Dis 1984; 6 : 313-27

Portnoy D, Whiteside ME, Buckley E, MacLeod CL. Treatment of intestinal cryptosporidisis with spiromycin. Ann Intern Med 1984; 101 : 202-4

Rhee JK, Seu YS, Park BK Isolation and identification of Cryptosporidium from various animals in Korea. II. Identification of *Cryptosporidium muris* from mice Kor J Parasit 1991 ; 29 : 149-59

Rhee JK, Seu YS, Park BK. Isolation and identification of Cryptosporidium from various animals in Korea. I. Prevalence of Cryptosporidium in various animals. Kor J Parasit 1991 ; 29 : 139-48

Tzipori S, Robertson D, Chapman C. Remission of diarrhea due to cryptosporidiosis in an immunodeficient child treated with hyperimmune bovine colostrum. Br Med J 1986; 293 : 1276-7

Tzipori S. Cryptosporidiosis in animals and humans. Microbiol Rev 1983; 47 : 84-96Upton SJ, Current WL. The species of Cryptosporidium (Apicomplexa : Cryptosporiidae) infecting mammals. J Parasitol 1985; 71 : 625-9