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INFECTIOUS GASTROENTEROCOLITIDES IN CHILDREN

An Update on Emerging Pathogens

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Infectious agents have long been known to cause acute enterocolitis,[38] particularly in infants and young children[14, 96, 97]; however, a proportion of both children[30, 53] and adults[65] have clinical symptoms typical of acute enterocolitis in the absence of an identifiable microbial pathogen. Failure to isolate pathogenic organisms from the infected stools of such patients does not conclusively demonstrate that the illness was not infectious. For instance, these individuals may have been infected but the organism cleared from the gut at the time the samples were obtained for testing.[107] For cost-containment purposes, some diagnostic laboratories do not test for all known enteropathogens but focus their isolation procedures on those organisms that historically have been identified most commonly in the local community. For example, despite increasingly widespread acceptance of enterohemorrhagic *Escherichia coli* as a recognized cause of disease, not all medical centers or commercial laboratories routinely test for these organisms in standard stool cultures.[3]

The presence of unrecognized enteropathogens may provide an-

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other explanation for the failure to identify an infectious agent in the stool. For example, *Campylobacter jejuni* and *Campylobacter coli* infections were not recognized as a major health concern until the organisms could be cultured routinely on selective medium under microaerophilic culture conditions.[79] The selective media that are used contain antibiotics to suppress the growth of the fecal microflora while permitting the replication of resistant campylobacters. The limitation of the technique is that intestinal campylobacters that are sensitive to the antibiotics in the selective culture medium are not identified. For example, *Campylobacter upsaliensis* likely is an important cause of disease that is underdiagnosed because standard methods for culturing intestinal campylobacters use antibiotics to which this organism is sensitive.[40]

The lack of suitable culture techniques to successfully grow some microbes is another possible reason for the failure to identify enteropathogens. The polymerase chain reaction (PCR) has been used to identify nonculturable microbial pathogens, including, for example, the Whipple's bacillus in intestinal biopsy specimens.[84, 85] This technology also has demonstrated that microbial pathogens can cause gastric disease in the absence of enterocolitic symptoms.

This review highlights selected, recently recognized and emerging bacterial and viral pathogens that infect the gastrointestinal tract. Protozoal, helminthic, and fungal infections are not considered in this review. These are, nevertheless, important causes of disease involving the gastrointestinal tract, especially in the immunocompromised host. Several of these agents, including cryptosporidia, microsporidia, cyclospora, and possibly *Dientamoeba fragilis* and *Blastocystis hominis* have increasingly been recognized as human enteropathogens. The interested reader is referred to an accompanying article by Winter and Chang in this issue and to several recent relevant reviews.[18, 42, 113, 114]

Some newly recognized agents may ultimately prove not to be true causes of disease. Nevertheless, several considered in this review likely are to be proven as important gastroenteric pathogens in children. They also are considered to emphasize that much remains to be learned about the interactions of the gastrointestinal tract with the multitude of microbes that enter into it on a daily basis.

A recent review provides a summary of the therapeutic approach to acute infectious diarrhea, including, most importantly, oral rehydration therapy.[99] The future promises the development, field testing, and widespread delivery of mucosal vaccines for use in preventing gastroenteric infections in humans.[20, 88]

THE STOMACH

Bacteria

Helicobacter pylori

Helicobacter pylori, a gastric pathogen, was first successfully cultured from mucosal biopsies obtained from the antrum of patients in the

early 1980s by Marshall and Warren in Perth, Western Australia.[67] The presence of this urease-producing, gram-negative, microaerobic organism was correlated with histopathologic evidence of chronic-active (type B) gastritis[25, 67] (Table 1). Specificity of the infection for certain subtypes of gastritis,[25, 77] resolution of gastritis with antibacterial therapy,[24] and the induction of type B gastritis in both human volunteers[68, 71] and in the appropriate experimental animals[37] fulfill each of Koch's postulates and thereby establishes *H pylori* as a human pathogen. Infection of the stomach also is strongly correlated with recurring peptic ulcer disease and with gastric cancers.[73, 95] Readers are referred to Chapter 21 in this volume for a more detailed review of *H pylori* infection.

Helicobacter heilmannii

Helicobacter heilmannii, a urease-producing, spiral-shaped organism, also is associated with gastritis in both the antrum of infected humans and in domesticated animals, including cats.[104] As shown in Figure 1, the organism can be distinguished from *H pylori* both with brightfield microscopy and by using transmission electron microscopy because *H heilmannii* is longer and more tightly coiled, presenting with a corkscrew-like appearance.[112] Formerly referred to as *Gastrospirillum hominis*,[104] *H heilmannii* is identified in inflamed antral tissues less commonly than is *H pylori*. The incidence of infection by this organism is likely underestimated because it cannot be cultured yet.[100, 101] The organism has been identified by staining with silver of mucosal biopsies in the stomachs of children and adolescents.[76, 104] A study is needed to determine the prevalence of *H heilmannii* infection in children with and without type B gastritis.

Viruses

Cytomegalovirus

In adults, Ménétrier's disease (hypertrophic gastritis) is a chronic remitting and relapsing disease[91, 92] that seems to be related to an excessive production of transforming growth factor-alpha in the gastric mu-

Table 1. RESULTS OF SILVER STAINING, CULTURE, AND UREASE TESTING FOR *HELICOBACTER PYLORI* IN ANTRAL BIOPSY SPECIMENS FROM 67 CHILDREN

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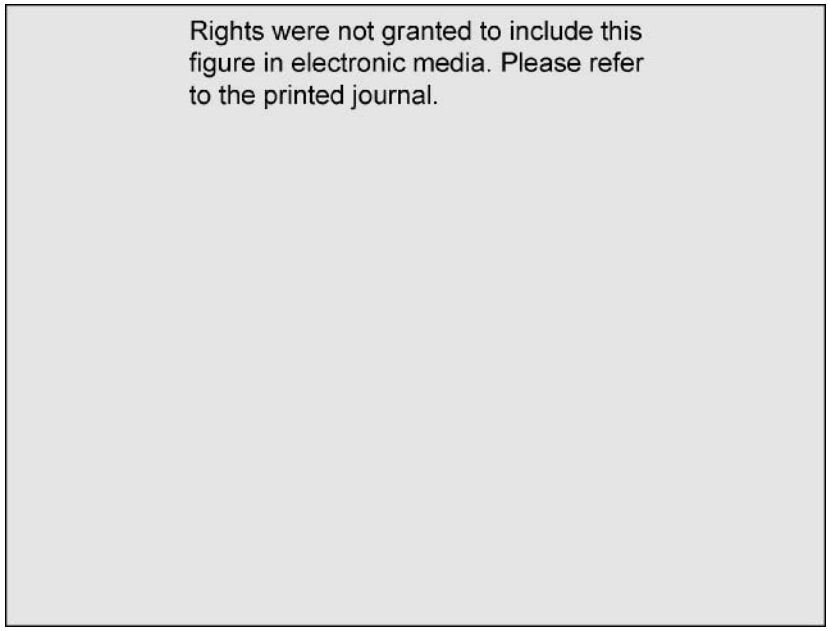

Figure 1. *A*, Gastric biopsy demonstrating several long corkscrew-like organisms typical for "Gastrospirillum hominis" overlying the mucosa (hematoxylin and eosin, original magnification, × 1250). *B*, Transmission electron photomicrograph showing tightly coiled *H heilmannii* with corkscrew appearance. Dark stained gastric mucin vacuoles are shown in the mucosa on the top left (original magnification, × 20,000). (*Reproduced with permission of* Van Zanten SJO, Malatjalian DA, Desmormeau LM, et al: Gastritis induced by the helicobacter 'Gastrospirillum hominis.' Can J Gastroenterol 8:257, 1994.)

cosa (Fig. 2).[21, 106] In contrast, childhood Ménétrier's disease is a short-lived, acute event[12] recently suggested to be of an infectious etiology. Several investigators have identified the cytomegalovirus (CMV) virions and genomic DNA in the antral mucosa of children with a protein-losing gastropathy and the hypertrophic gastritis that is characteristic of Ménétrier's disease.[33, 50, 74, 75, 81] A cluster of cases of CMV-associated Ménétrier's disease was reported by Kovacs and colleagues[54] in five children under 4 years of age; however, restriction enzyme DNA fingerprinting did not support the hypothesis that the gastric infections were due to a single virulent strain of the virus. In fact, at least four different CMV strains were identified among the five infants.

SMALL INTESTINE

Bacteria

Vibrio cholerae O139

The past 2 years have seen the emergence of a new *Vibrio cholerae* serogroup, O139, that has caused epidemic diarrheal disease primarily

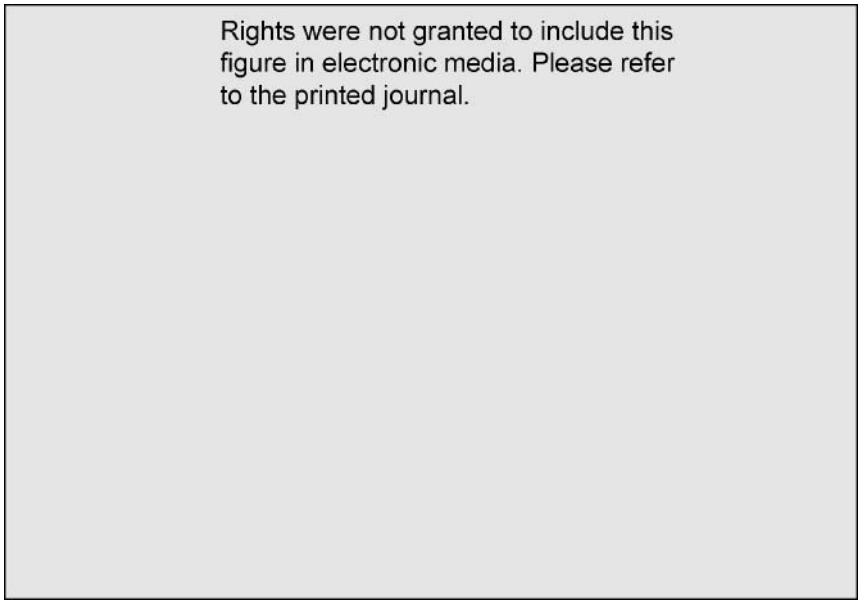

Figure 2. Histology of gastric mucosa in a 3-year-old with Ménétrier's disease reveals glandular epithelial cell *(closed arrow)* with cytomegalovirus inclusion and positive immune reactivity for early antigen by immunoperoxidase using monoclonal antibody against cytomegalovirus early antigen and counterstained with hematoxylin-eosin (original magnification, × 300). These consist of large cells with a large prominent eosinophilic intranuclear inclusion surrounded by a thin clear halo. A dilated gland *(open arrow)*, regenerative epithelium *(arrowhead)*, and mixed acute and chronic inflammatory cell infiltrate are present in the lamina propria. (*From* Eisenstat DDR, Griffiths AM, Cutz E, et al: Acute cytomegalovirus infection in a child with Ménétrier's disease. Gastroenterology, 109:592, 1995.)

in the Indian subcontinent.[56, 59] Although the lipopolysaccharide and, hence, the serogroup of this strain is distinct from classic O1 epidemic strains, *V cholerae* O139 contains the same virulence operon (i.e., cholera toxin, toxin-coregulated pilus) as the O1 organisms traditionally causing worldwide cholera pandemics.[56]

The severe dehydration of cholera is related to the Gs-protein–mediated activation of cyclic adenosine monophosphate by cholera toxin that causes reduced sodium-chloride–coupled absorption in villus enterocytes and increased chloride secretion in crypt cells.[102] Recent studies show that these effects are mediated through the enteric nervous system and immunocytes.[41] Increasingly, a complex interaction of nerves, muscle, mast cells, T cells, and B cells with the host epithelium is recognized to ultimately modulate the host response to the infecting organisms and their toxins.[10, 48] Indeed, the concept has sponsored the formation of a new multidisciplinary field of investigation referred to as *neuroimmunophysiology*.[80, 94, 103]

Deletion of the genes encoding for the A (active adenosine diphos-

phate ribosylating) and B (binding) subunits of the cholera toxin attenuates but does not completely block the diarrheal response in infected human volunteers[105] or in experimental studies using animal tissues. This has led to the recognition that *V cholerae* harbor additional virulence factors in the bacterial genome that may contribute to diarrheal disease and serve as factors requiring consideration in the design of an optional, nontoxic vaccine. Two newly recognized toxins produced by *V cholerae* are the zona occludens toxin (ZOT)[35] and the accessory cholera enterotoxin (ACE).[110]

Enteroaggregative Escherichia coli

Enteroaggregative *E coli* (EAggEC) are characterized by their pattern of bacterial adhesion to infected tissue culture cells.[89] These organisms produce enterotoxins that are heat-labile[4] and heat-stable.[90] Epidemiologic studies provide supportive evidence for the pathogenic role of EAggEC in disease, especially as a cause of persistent diarrhea. In some studies, the organism is identified in the stool samples of children with diarrhea more frequently than in age-matched controls.[11, 78, 89] For example, Cravioto and colleagues[16] identified EAggEC in 51% of Mexican children under 2 years of age with protracted diarrhea but in only 5% of asymptomatic age-matched infants. The relative importance of EAggEC as a cause of disease in the United States remains to be established. The biologic relevance of the in vitro adhesion properties and toxins produced by these bacteria also need to be addressed in further experimental evaluation.

Diffusely Adherent Escherichia coli

Diffuse adherence *E coli* (DAEC) are characterized by binding of organisms evenly over the surface of tissue culture cells in in vitro adhesion assays.[89] DAEC have been associated with acute and chronic diarrhea in some case-controlled epidemiologic studies.[5, 44, 60] The reproducibility and generalizability of these findings now need to be defined. Genetic probes to defined bacterial adhesins, such as F1845[8] and AIDA-I[7] should help in achieving these goals.

Enterotoxigenic Bacteroides fragilis

Although generally considered a constituent of the normal colonic microflora, some investigators report that a toxin-producing variant of *Bacteroides fragilis* is enteropathogenic.[70] Additional studies are required to fulfill each of Koch's postulates for this organism. The recognition that a normal constituent of the fecal flora can cause disease is not, however, without precedent. For example, *Clostridium difficile* can be a part of the normal colonic microflora. Toxin-producing *C difficile* colonize in normal infants without apparent ill effect,[109] perhaps because the receptor(s) required for binding of the toxins that are elaborated by the

organism are not present until later in life.[32b] Alteration of the normal flora by the use of antibiotics can create a microenvironment in which the organism proliferates and, in the susceptible (i.e., age-appropriate) host, ultimately results in disease. The concept that the commensal microflora influences health also has been used to develop medical biotherapy in which nonpathogenic organisms, such as *Saccharomyces boulardii*,[69] *Bifidobacterium bifidum*,[87] and *Lactobacillus* species,[83] are used to treat and prevent infectious enterocolitides.

Viruses

Astrovirus

In addition to rotaviruses, adenoviruses of serotype 40 and 41, Norwalk virus, and related 27 nm enteritis-associated viruses, recent epidemiologic studies have demonstrated that the astrovirus is an etiologic agent of childhood enteritis.[15] In controlled studies in Thailand using a monoclonal antibody-based immunoassay, astrovirus was detected in 8.6% of symptomatic children compared with 2.0% of asymptomatic, age-matched controls ($P < 0.001$,[43] Table 2). Comparable findings have been reported in day-care centers in North America.[61] The recently derived sequence of the astrovirus RNA genome reveals that this agent is sufficiently unique to be classified in its own family as Astroviridae.[46]

Calicivirus

The RNA genomes of the Norwalk virus, several Norwalk-like viruses (also termed *small round structured viruses* [SRSV]), and the calicivirus have been sequenced.[47, 55, 62] The RNA sequences of these viruses are related, and these agents are now considered members of the family Caliciviridae. Using reverse transcription-PCR, the SRSV can be subclassified into four distinct genotypes that can also be identified by

Table 2. FREQUENCY OF ASTROVIRUSES, GROUP A ROTAVIRUSES, ENTERIC ADENOVIRUSES, AND NONENTERIC ADENOVIRUSES AS ETIOLOGIC AGENTS OF ENTERITIS IN THAI CHILDREN

immunoelectron microscopy.[2] This has greatly simplified our understanding of the classification of these agents.

Torovirus

Fringed, pleomorphic particles resembling toroviruses were first reported in stool specimens of children with diarrhea by Beards and colleagues.[6] Because of their pleomorphic structures, electron microscopy alone was not sufficiently convincing for these agents to gain immediate acceptance as viral enteropathogens. The more recent finding that torovirus-like particles are reactive in an immunoassay using antiserum to a well-established, morphologically similar Breda virus of calves has provided supporting evidence that these particles are indeed toroviruses.[52] Preliminary evidence shows that torovirus-like particles (Fig. 3), which also may be reported as coronavirus-like agents, are commonly found in stool samples of children with diarrhea.[45, 72] During a 9-month period beginning in October, 1993, torovirus-like particles accounted for more than half of all stool specimens that were positive for viruses when examined by electron microscopy.[45] By contrast, torovirus was identified in only 4 of 49 stool specimens obtained in age-matched children without intestinal symptoms. Additional investigations at multiple centers are now required to confirm the enteropathogenicity of toroviruses and to clarify the epidemiology of infection.

COLON

Bacteria

Brachyspira aalborgi

Intestinal spirochetosis refers to colonization of the large bowel mucosa by *Brachyspira aalborgi* or related spirochetes.[57] The organism is identified in biopsies of colonic mucosa obtained at colonoscopy (Fig. 4). Although some investigators report that the spirochetes are a cause of bloody diarrhea,[19, 64] appropriately controlled studies have not been performed to ascertain the prevalence of *B. aalborgi* adherent to colonic mucosa in asymptomatic children. In fact, some studies in adults indicate that asymptomatic colonization of the large bowel does occur.[82] Additional studies to characterize the presence of potential virulence properties, such as toxin production, invasion potential, and the ability to induce a signal transduction response following adhesion to colonocytes,[9, 36] are clearly warranted.

Enterohemorrhagic Escherichia coli

Enterohemorrhagic *E coli* (EHEC) refers to *E coli* of a limited number of serotypes, including O157:H7 and O26:H11.[58] These bacteria cause

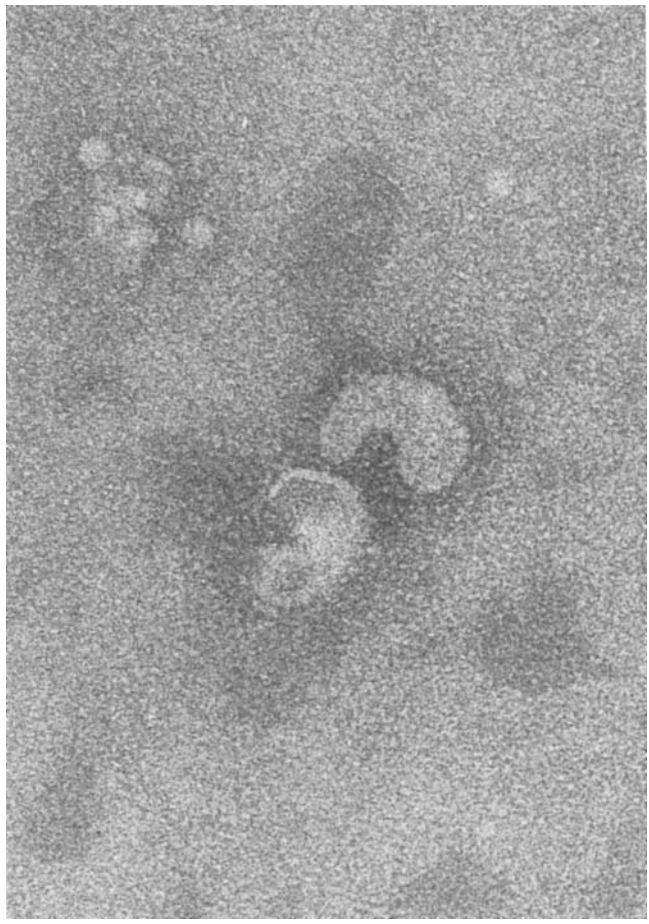

Figure 3. Transmission electron micrograph of torovirus-like particles in the stool specimen of a child with diarrhea (original magnification, × 20,000).

hemorrhagic colitis and systemic complications, including the hemolytic uremic syndrome (i.e., microangiopathic hemolytic anemia, thrombocytopenia, and renal failure) and thrombotic thrombocytopenic purpura (i.e., microangiopathy with neurologic involvement). The morbidity, mortality, and immense financial implications of this food-borne infection are only now being fully appreciated.[66] Hemorrhagic colitis and the systemic complications of the disease also can be caused by infection with other *E coli* serotypes, albeit less frequently than by O157:H7.[14, 49] All strains are characterized by the production of one or more phage-encoded cytotoxins variously referred to as *Shiga-like toxin* or *Verocytotoxin*.[1, 20] These toxins function as an N-glycosidase that depurinates a

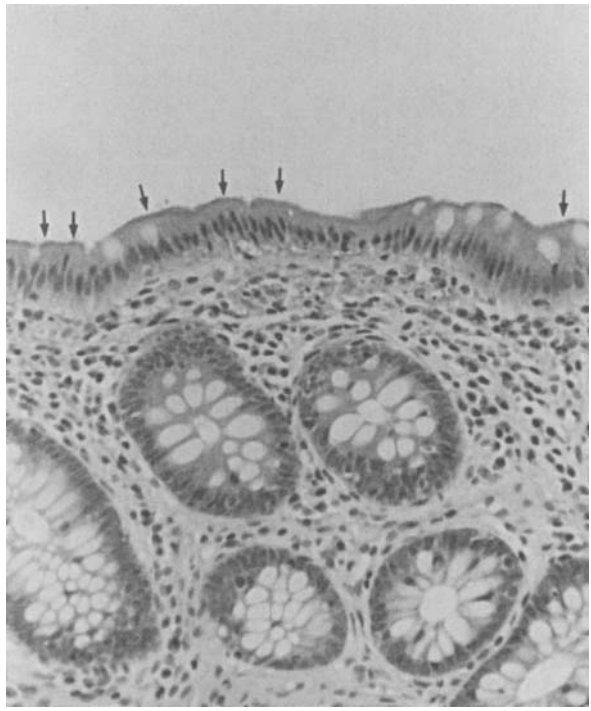

Figure 4. Brightfield microscopy photomicrograph showing spirochetes adherent to surface epithelial cells *(arrows)* in the transverse colon of a 16-year-old male adolescent with hematochezia. The infection may have been incidental, however, because a juvenile polyp was observed in the rectum and removed by snare polypectomy (hematoxylin and eosin, original magnification, × 150).

specific residue (adenine 4324) on 28S ribosomal RNA and thereby block eukaryotic cell protein synthesis, ultimately resulting in cell death.[14, 49]

The precise role of the cytotoxin in EHEC-induced disease is currently under active investigation. Studies using animal models have reported evidence both for[86, 89] and against[63, 111] the role of Verocytotoxins in experimentally induced enterocolitis. If these toxins are important in beginning the cascade that results in a systemic disease, preventing toxin binding to enterocyte receptors may interrupt the pathophysiology of this disease. The hypothesis that receptor analog therapy is an effective and safe form of treatment to prevent the systemic complications of EHEC infection is now being tested.[93] This or other novel treatment approaches are required because antimicrobial agents are of unproven benefit. Theoretically, antibiotics could cause harm because the cytotoxins are released from the periplasm of the organism following exposure to agents that disrupt the prokaryotic outer membrane. An increased

prevalence of antibiotic resistance among EHEC isolates also has been recently described.[51]

EHEC attach to infected epithelial cells in vitro and in experimental animals in a morphologically distinct pattern referred to as *attaching and effacing adhesion* (Fig. 5).[29, 108] The attaching and effacing lesion is dependent on a chromosomally encoded bacterial outer membrane protein.[22] The role of the outer membrane protein in mediating EHEC-induced diarrheal disease is under active investigation because the adhesin is a potential vaccine candidate. Whether all cytotoxin-producing strains induce the attaching and effacing lesion is doubtful. The authors have reported, for example, that Verocytotoxin-producing *E coli* of the serotype O113:H21 does not contain a homologous gene for the outer membrane adhesin and does not cause attaching and effacing adhesion either in vitro or in vivo.[28]

As is typical of infectious colitis, the lamina propria of large intestine is infiltrated by polymorphonuclear leukocytes following EHEC infection. Using a lapine model, Elliot and colleagues[34] showed that a mono-

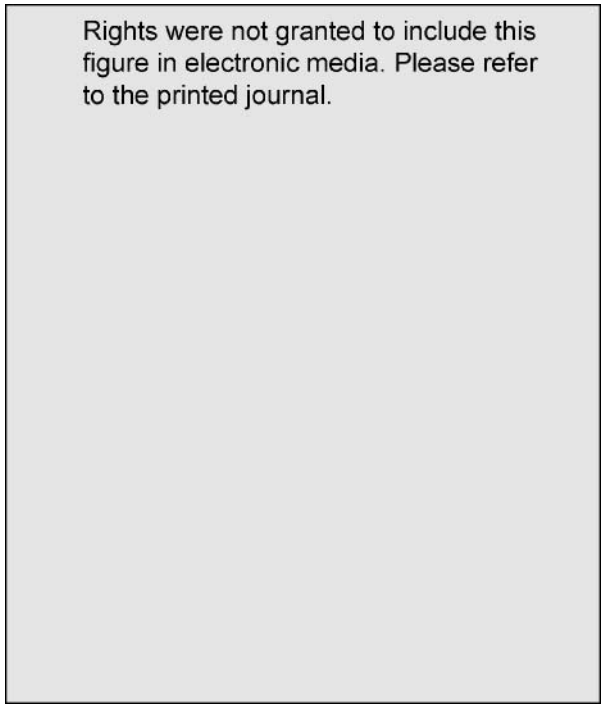

Figure 5. Transmission electron photomicrograph of Verocytotoxin-producing *E coli*, serotype O121:H$^-$, adherent to the eukaryotic cell plasma membrane in an attaching and effacing phenotype with loss of the microvillus membrane and collection of electron-dense material (F-actin, alpha-actinin) below the adherent organisms *(arrows)*. (*From* Sherman P, Soni R, Karmali M: Attaching and effacing adherence of Verocytotoxin-producing *Escherichia coli* to rabbit intestinal epithelium in vivo. Infect Immun 56:756, 1988; with permission.)

clonal antibody against C18 vascular adhesion epitopes on leukocytes reduces the secretory response of the colon to *E coli* O157:H7 infection. Thus, anti-inflammatory molecules might serve to provide novel therapy regardless of the role of cytotoxin and outer membrane protein in the etiopathogenesis of EHEC infection.

SUMMARY

The recognition that bacterial infections induce signal transduction responses in infected epithelial cells also provides new avenues to consider as novel forms of therapy. For example, the chemokine interleukin-8, which attracts neutrophils to sites of mucosal infection, is produced by epithelial cells of gastric[17] and intestinal[31, 32] origin in response to bacterial infection. Inhibitors of chemokine production or inhibition of the biologic effects of neutrophil chemoattractants have the potential to reduce both mucosal inflammatory responses and the attendant clinical sequelae. Eukaryotic cells also respond to infection with elevations in cytosolic second messengers, including inositol triphosphate (IP_3) and calcium ($[Ca^{2+}]_i$).[27] In intestinal epithelium, these second messengers can mediate the diarrheal response to infection.[23]

Calcium/calmodulin inhibitors may have a beneficial effect in treating those gastrointestinal infections mediated through changes in the level of cytosolic free calcium. DuPont and colleagues[26] showed, for example, that oral therapy with zaldaride maleate relieves symptoms of disease and shortens the duration of diarrhea in travelers with ETEC-induced diarrhea. Evaluation of additional signal transduction responses to microbial infections should provide both new insights into the pathogenesis of gastrointestinal infectious diseases and novel approaches to consider for the prevention and therapy for these human illnesses.

References

1. Acheson DWK, Keusch GT: The shigella paradigm and colitis due to enterohaemorrhagic *Escherichia coli*. Gut 35:872, 1994
2. Ando, T, Monroe SS, Gensch J, et al: Detection and differentiation of antigenically distinct small round-structured viruses (Norwalk-like viruses) by reverse transcription-PCR and Southern hybridization. J Clin Microbiol 33:6, 1995
3. Anonymous: Panel calls *E coli* screening inadequate. J Am Med Assoc 272:501, 1994
4. Baldwin TJ, Knutton S, Sellers L, et al: Enteroaggregative *Escherichia coli* strains secrete a heat-labile toxin antigenically related to *E. coli* hemolysin. Infect Immun 60:2092, 1992
5. Baqui AH, Sack RB, Black RE, et al: Enteropathogens associated with acute and persistent diarrhea in Bangladeshi children <5 years of age. J Infect Dis 166:792, 1992
6. Beards GM, Green J, Hall C, et al: An enveloped virus in stools of children and adults with gastroenteritis that resembles the Breda virus of calves. Lancet 1:1050, 1984
7. Benz I, Schmidt MA: AIDA-I, the adhesin involved in diffuse adherence of the diarrhoeagenic *Escherichia coli* strain 2787 (O126:H27) is synthesized via a precursor molecule. Mol Microbiol 6:1539, 1992

8. Bilge SS, Clausen CR, Law W, et al: Molecular characterization of a fimbrial adhesin, F1845, mediating diffuse adherence of diarrhea-associated *Escherichia coli* to HEp-2 cells. J Bacteriol 171:4281, 1989
9. Bliska JB, Galan JE, Falkow S: Signal transduction in the mammalian cell during bacterial attachment and entry. Cell 73:903, 1993
10. Castagliuolo I, LaMont JT, Letourneau R, et al: Neuronal involvement in the intestinal effects of *Clostridium difficile* toxin A and *Vibrio cholerae* enterotoxin in rat ileum. Gastroenterology 107:657, 1994
11. Chan KN, Phillips AD, Knutton S, et al: Enteroaggregative *Escherichia coli*: Another cause of acute and chronic diarrhoea in England? J Pediatr Gastroenterol Nutr 18:87, 1994
12. Chouraqui JP, Roy CC, Brochu P, et al: Menetrier's disease in children: Report of a patient and review of sixteen other cases. Gastroenterology 80:1042, 1981
13. Cohen MB: Etiology and mechanisms of acute infectious diarrhea in infants in the United States. J Pediatr 118(suppl):34, 1991
14. Cohen MB, Giannella RA: Hemorrhagic colitis associated with *Escherichia coli* O157:H7. Adv Intern Med 37:173, 1991
15. Cook N, Myint S: Astroviruses. J Med Microbiol 42:1, 1995
16. Cravioto A, Tello A, Navarro A, et al: Association of *Escherichia coli* HEp-2 cell adherence patterns with type and duration of diarrhoea. Lancet 337:262, 1991
17. Crowe SE, Alvarez L, Dytoc M, et al: Expression of interleukin 8 and CD54 by human gastric epithelium after *Helicobacter pylori* infection. Gastroenterology 108:65, 1995
18. Current WL, Garcia LS: Cryptosporidiosis. Clin Microbiol Rev 4:325, 1991
19. daCunha Ferreira RMC, Phillips AD, Stevens CR, et al: Intestinal spirochaetosis in children. J Pediatr Gastroenterol Nutr 17:333, 1993
20. Dellert SF, Cohen MB: Diarrheal disease. Established pathogens, new pathogens, and progress in vaccine development. Gastroenterol Clin North Am 23:637, 1994
21. Dempsey PJ, Goldenring JR, Soroka CJ, et al: Possible role of transforming growth factor alpha in the pathogenesis of Menetrier's disease: Supporting evidence from humans and transgenic mice. Gastroenterology 103:1950, 1992
22. Donnenberg MS, Tzipori S, McKee ML, et al: The role of the *eae* gene of enterohemorrhagic *Escherichia coli* in intimate attachment in vitro and in a porcine model. J Clin Invest 92:1418, 1993
23. Donowitz M, Cohen ME, Gould M, et al: Elevated intracellular Ca^{2+} acts through protein kinase C to regulate rabbit ileal NaCl absorption. J Clin Invest 83:1953, 1989
24. Drumm B, Sherman P, Chiasson D, et al: Treatment of *Campylobacter pylori* associated gastritis in children with bismuth subsalicylate and ampicillin. J Pediatr 113:908, 1988
25. Drumm B, Sherman P, Cutz E, et al: Association of *Campylobacter pylori* on the gastric mucosa with antral gastritis in children. N Engl J Med 316:1557, 1987
26. DuPont HL, Ericsson CD, Mathewson JJ, et al: Zaldaride maleate, an intestinal calmodulin inhibitor, in the therapy of traveler's diarrhea. Gastroenterology 104:709, 1993
27. Dytoc MT, Fedorko L, Sherman PM: Signal transduction in human epithelial cells infected with attaching and effacing *Escherichia coli* in vitro. Gastroenterology 106:1150, 1994
28. Dytoc MT, Ismaili A, Philpott DJ, et al: Distinct binding properties of *eae*A negative Verocytotoxin-producing *Escherichia coli* of serotype O113:H21. Infect Immun 62:3494, 1994
29. Dytoc MT, Soni R, Cockerill III F, et al: Multiple determinants of Verotoxin-producing *Escherichia coli* O157:H7 attachment-effacement. Infect Immun 61:3382, 1993
30. Echeverria P, Hoge CW, Bodhidatta L, et al: Etiology of diarrhea in a rural community in Western Thailand: Importance of enteric viruses and enterovirulent *Escherichia coli*. J Infect Dis 169:916, 1994
31. Eckmann L, Kagnoff MF, Fierer J: Epithelial cells secrete the chemokine interleukin-8 in response to bacterial entry. Infect Immun 61:4569, 1993
32. Eckmann L, Jung HC, Schurer-Maly C, et al: Differential cytokine expression by human intestinal epithelial cell lines: Regulated expression of interleukin 8. Gastroenterology 105:1689, 1993

32a. Eglow R, Pothoulakis C, Itkowitz S, et al: Diminished *Clostridium difficile* toxin A sensitivity in newborn rabbit ileum is associated with decreased toxin A receptor. J Clin Invest 90:822, 1992
33. Eisenstat DDR, Griffiths AM, Cutz E, et al: Acute cytomegalovirus infection in a child with Menetrier's disease. Gastroenterology, 109:592, 1995
34. Elliott E, Li Z, Bell C, et al: Modulation of host response to *Escherichia coli* O157:H7 infection by anti-CD18 antibody in rabbits. Gastroenterology 106:1554, 1994
35. Fasano A, Baudry B, Pumplin DW, et al: *Vibrio cholerae* produces a second enterotoxin, which affects intestinal tight junctions. Proc Natl Acad Sci U S A 88:242, 1991
36. Finlay BB, Falkow S: Common themes in microbial pathogenicity. Microbiol Rev 53:210, 1989
37. Fox JG: *In vivo* models of gastric Helicobacter infections. *In* Hunt RJ, Tytgat GNJ (eds): Helicobacter Pylori: Basic Mechanisms to Clinical Cure. Lancaster, UK, Kluwer Academic Publishers, 1994, p 3
38. Giannella RA: Enteric infections: 50 years of progress. Gastroenterology 104:1589, 1993
39. Giron JA, Jones T, Millan-Velasco F, et al: Diffuse adhering *Escherichia coli* (DAEC) as a putative cause of diarrhea in Mayan children in Mexico. J Infect Dis 163:507, 1991
40. Goossens H, Vlaes L, DeBoeck M, et al: Is "*Campylobacter upsaliensis*" an unrecognised cause of human diarrhoea? Lancet 335:584, 1990
41. Guerrant RL: Lessons from diarrheal diseases: Demography to molecular pharmacology. J Infect Dis 169:1206, 1994
42. Gupta TP, Ehrinpreis MN: Candida-associated diarrhea in hospitalized patients. Gastroenterology 98:780, 1990
43. Herrmann JE, Taylor DN, Echeverria P, et al: Astroviruses as a cause of gastroenteritis in children. N Engl J Med 324:1757, 1991
44. Jallat C, Livrelli V, Darfeuille-Michaud A, et al: *Escherichia coli* strains involved in diarrhea in France: High prevalence and heterogeneity of diffusely adhering strains. J Clin Microbiol 31:2031, 1993
45. Jamieson F, Kellner J, Bain C, et al: Torovirus-like particles (TVLPs) in pediatric gastroenteritis. Annual Meeting of the Canadian Association of Medical Microbiologists, Montreal, Quebec, November 1994. Abstract N-1
46. Jiang B, Monroe SS, Koonin EV, et al: RNA sequence of astrovirus: Distinctive genetic organization and a putative retrovirus-like ribosomal frameshifting signal that directs the viral replicase synthesis. Proc Natl Acad Sci U S A 90:10539, 1993
47. Jiang X, Wang M, Wang K, et al: Sequence and genome organization of Norwalk virus. Virology 195:51, 1993
48. Jodal M, Holmgren S, Lundgren O, et al: Involvement of the mesenteric plexus in the cholera toxin-induced fluid secretion in the rat small intestine. Gastroenterology 105:1286, 1993
49. Karmali MA, Petric M, Lim C, et al: The association between idiopathic hemolytic uremic syndrome and infection by Verotoxin-producing *Escherichia coli*. J Infect Dis 151:775, 1985
50. Khoshoo V, Alonzo E, Correa H, et al: Menetrier's disease with cytomegalovirus gastritis. Arch Pediatr Adolesc Med 148:611, 1994
51. Kim HH, Samadpour M, Grimm L, et al: Characteristics of antibiotic-resistant *Escherichia coli* O157:H7 in Washington state, 1984–1991. J Infect Dis 170:1606, 1994
52. Koopmans M, Petric M, Glass R, et al: Enzyme-linked immunosorbent assay reactivity of torovirus-like particles in fecal specimens from humans with diarrhea. J Clin Microbiol 31:2738, 1993
53. Kotloff KL, Wasserman SS, Steciak JY, et al: Acute diarrhea in Baltimore children attending an outpatient clinic. Pediatr Infect Dis J 7:753, 1988
54. Kovacs AA, Churchill MA, Wood D, et al: Molecular and epidemiologic evaluations of a cluster of cases of Menetrier's disease associated with cytomegalovirus. Pediatr Infect Dis J 12:1011, 1993
55. Lambden PR, Caul EO, Ashley CR, et al: Sequence and genome organization of a human small round-structured (Norwalk-like) virus. Science 259:516, 1993
56. Lang DR, Guerrant RL: Summary of the 29th United States-Japan joint conference on cholera and related diarrheal diseases. J Infect Dis 171:8, 1995

57. Lee JI, Hampson DJ: Genetic characterisation of intestinal spirochaetes and their association with disease. J Med Microbiol 40:365, 1994
58. Levine MM: *Escherichia coli* that cause diarrhea: Enterotoxigenic, enteropathogenic, enterinvasive, enterohemorrhagic, and enteradherent. J Infect Dis 155:377, 1987
59. Levine MM, Levine OS: Changes in human ecology and behavior in relation to the emergence of diarrheal diseases, including cholera. Proc Natl Acad Sci U S A 91:2390, 1994
60. Levine MM, Ferreccio C, Prado V, et al: Epidemiologic studies of *Escherichia coli* diarrheal infections in a low socioeconomic level peri-urban community in Santiago, Chile. Am J Epidemiol 138:849, 1993
61. Lew JF, Moe CL, Monroe SS, et al: Astrovirus and adenovirus associated with diarrhea in children in day care settings. J Infect Dis 164:673, 1991
62. Lew JF, Petric M, Kapikian AZ, et al: Identification of minireovirus as a Norwalk-like virus in pediatric patients with gastroenteritis. J Virol 68:3391, 1994
63. Li Z, Bell C, Buret A, et al: The effect of enterohemorrhagic *Escherichia coli* O157:H7 on intestinal structure and solute transport in rabbits. Gastroenterology 104:467, 1993
64. Lindboe CF, Tostrup NE, Nersund R, et al: Human intestinal spirochaetosis in mid-Norway. APMIS 101:858, 1993
65. Loosli J, Gyr K, Stalder H, et al: Etiology of acute infectious diarrhea in a highly industrialized area of Switzerland. Gastroenterology 88:75, 1985
66. Marks S, Roberts T: *E. coli* O157:H7 ranks as the fourth most costly foodborne disease. Food Review 16:51, 1993
67. Marshall BJ, Warren JR: Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1:1311, 1984
68. Marshall BJ, Armstrong JA, McGechie DB, et al: Attempt to fulfil Koch's postulates for pyloric campylobacter. Med J Aust 142:436, 1985
69. McFarland LV, Bernasconi P: *Saccharomyces boulardii*: A review of an innovative biotherapeutic agent. Microb Ecol Health Dis 6:157, 1993
70. Moncrief JS, Obiso Jr R, Barroso LA, et al: The enterotoxin of *Bacteroides fragilis* is a metalloprotease. Infect Immun 63:175, 1995
71. Morris AJ, Ali MR, Nicholson GI, et al: Long-term follow-up of voluntary ingestion of *Helicobacter pylori*. Ann Intern Med 114:662, 1991
72. Mortensen ML, Ray CG, Payne C, et al: Coronavirus-like particles in human gastrointestinal disease. Am J Dis Child 139:928, 1985
73. NIH Consensus Development Panel: *Helicobacter pylori* in peptic ulcer disease. J Am Med Assoc 272:65, 1994
74. Occena RO, Taylor SF, Robinson CC, et al: Association of cytomegalovirus with Menetrier's disease in childhood: Report of 2 new cases and a review of the literature. J Pediatr Gastroenterol Nutr 17:217, 1993
75. Oderda G, Cinti S, Cangiotti AM, et al: Increased tight junction width in two children with Menetrier's disease. J Pediatr Gastroenterol Nutr 11:123, 1990
76. Oliva MM, Lazenby AJ, Perman JA: Gastritis associated with "Gastrospirillum hominis" in children. Mod Pathol 6:513, 1993
77. Ormand JE, Talley NJ, Shorter RG, et al: Prevalence of *Helicobacter pylori* in specific forms of gastritis. Further evidence supporting a pathogenic role for *H pylori* in chronic nonspecific gastritis. Dig Dis Sci 36:142, 1991
78. Paul M, Tsukamoto T, Ghosh AR, et al: The significance of enteroaggregative *Escherichia coli* in the etiology of hospitalized diarrhoea in Calcutta, India and the demonstration of a new honey-combed pattern of aggregative adherence. FEMS Microbiol Lett 117:319, 1994
79. Penner JL: The genus *Campylobacter*: A decade of progress. Clin Microbiol Rev 1:157, 1988
80. Powell DW: New paradigms for the pathophysiology of infectious diarrhea. Gastroenterology 106:1705, 1994
81. Qualman SJ, Hamoudi AB: Pediatric hypertrophic gastropathy (Menetrier's disease). Pediatr Pathol 12:263, 1992
82. Quinn TC, Stamm WE, Goodell SE, et al: Intestinal spirochetosis in homosexual men. N Engl J Med 310:392, 1984

83. Reid G, Bruce AW, McGroarty JA, et al: Is there a role for lactobacilli in prevention of urogenital and intestinal infections? Clin Microbiol Rev 3:335, 1990
84. Relman DA, Schmidt TM, MacDermott RP, et al: Identification of the uncultured bacillus of Whipple's disease. N Engl J Med 327:293, 1992
85. Relman DA: The identification of uncultured microbial pathogens. J Infect Dis 168:1, 1993
86. Richardson SE, Rotman TA, Jay V, et al: Experimental Verocytotoxemia in rabbits. Infect Immun 60:4154, 1992
87. Saavedra JM, Bauman NA, Oung I, et al: Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhea and shedding of rotavirus. Lancet 344:1046, 1994
88. Sack DA, Freij L, Holmgren J: Prospects for public health benefits in developing countries from new vaccines against enteric infections. J Infect Dis 163:503, 1991
89. Savarino SJ: Diarrhoeal disease: Current concepts and future challenges. Enteroadherent *Escherichia coli*: a heterogeneous group of *E. coli* implicated as diarrhoeal pathogens. Trans Roy Soc Trop Med Hyg 87(Suppl 3):49, 1993
90. Savarino SJ, Fasano A, Watson J, et al: Enteroaggregative *Escherichia coli* heat-stable enterotoxin represents another subfamily of *E. coli* heat-stable toxin. Proc Natl Acad Sci U S A 90:3093, 1993
91. Scharschmidt BF: The natural history of hypertrophic gastropathy (Menetrier's disease). Report of a case with 16 year follow-up and review of 120 cases from the literature. Am J Med 63:644, 1977
92. Searcy RM, Malagelada J-R: Menetrier's disease and idiopathic hypertrophic gastropathy. Ann Intern Med 100:565, 1984
93. Service RF: *E. coli* scare spawns therapy search. Science 265:475, 1994
94. Sheridan JF, Dobbs C, Brown D, et al: Psychoneuroimmunology: Stress effects on pathogenesis and immunity during infection. Clin Microbiol Rev 7:200, 1994
95. Sherman PM: Peptic ulcer disease in children. Diagnosis, treatment, and the implication of *Helicobacter pylori*. Gastroenterol Clin North Am 23:707, 1994
96. Sherman PM, Lichtman SN: Mucosal barrier function and colonization of the gut. *In* Walker WA, Durie PR, Hamilton JR (eds): Pediatric Gastrointestinal Disease, ed 2. Mosby Press, New York, 1996, p 103
97. Sherman PM, Lichtman SN: Pediatric considerations relevant to enteric infection. *In* Blaser MJ, Smith PD, Ravdin JI, et al (eds): Infections of the Gastrointestinal Tract. Raven Press, New York, 1995, p 143
98. Sjogren R, Neill R, Rachmilewitz D, et al: Role of Shiga-like toxin 1 in bacterial enteritis: Comparison between isogenic *Escherichia coli* strains induced in rabbits. Gastroenterology 106:306, 1994
99. Snyder JD: Evaluation and treatment of diarrhea. Semin Gastrointest Dis 5:47, 1994
100. Solnick JV, O'Rourke J, Lee A, et al: An uncultured gastric spiral organism is a newly identified *Helicobacter* in humans. J Infect Dis 168:379, 1993
101. Solnick JV, O'Rourke J, Lee A, et al: Molecular analysis of urease genes from a newly identified uncultured species of *Helicobacter*. Infect Immun 62:1631, 1994
102. Spangler BD: Structure and function of cholera toxin and the related *Escherichia coli* heat labile enterotoxin. Microbiol Rev 56:622, 1992
103. Stead RH, Perdue MH, Cooke H, et al: Neuro-immuno-physiology of the gastrointestinal mucosa. Implications for inflammatory diseases. Ann New York Acad Sci 664:1, 1992
104. Stolte M, Wellens E, Bethke B, et al: *Helicobacter heilmannii* (formerly *Gastrospirillum hominis*) gastritis: An infection transmitted by animals? Scand J Gastroenterol 29:1061, 1994
105. Tacket CO, Losonsky G, Nataro JP, et al: Safety and immunogenicity of live oral cholera vaccine candidate CVD110, a Δ*ctx*AΔ*zot*Δ*ace* derivative of El Tor Ogawa *Vibrio cholerae*. J Infect Dis 168:1536, 1993
106. Takagi H, Jhappan C, Sharp R, et al: Hypertrophic gastropathy resembling Menetrier's disease in transgenic mice overexpressing transforming growth factor α in the stomach. J Clin Invest 90:1161, 1992
107. Tarr PI, Neill MA, Clausen CR, et al: *Escherichia coli* O157:H7 and the hemolytic

uremic syndrome: Importance of early cultures in establishing the etiology. J Infect Dis 162:553, 1990
108. Tesh VL, O'Brien AD: Adherence and colonization mechanisms of enteropathogenic and enterohemorrhagic *Escherichia coli*. Microb Pathog 12:245, 1992
109. Triadafilopoulos G, LaMont JT: Pseudomembranous colitis. *In* Walker WA, Durie PR, Hamilton JR, et al (eds): Pediatric Gastrointestinal Disease: Pathophysiology, Diagnosis, Management. Philadelphia, BC Decker, 1:619, 1991
110. Trucksis M, Galen JE, Michalski J, et al: Accessory cholera enterotoxin (Ace), the third toxin of a *Vibrio cholera* virulence cassette. Proc Natl Acad Sci U S A 90:5267, 1993
111. Tzipori S, Karch H, Wachsmuth KI, et al: Role of a 60-megadalton plasmid and Shiga-like toxins in the pathogenesis of infection caused by enterohemorrhagic *Escherichia coli* O157:H7 in gnotobiotic piglets. Infect Immun 55:3117, 1987
112. Van Zanten SJO, Malatjalian DA, Desormeau LM, et al: Gastritis induced by the helicobacter 'Gastrospirillum hominis.' Can J Gastroenterol 8:257, 1994
113. Weber R, Bryan RT, Schwartz DA, et al: Human microsporidial infections. Clin Microbiol Rev 7:426, 1994
114. Wurtz R: Cyclospora: A newly identified intestinal pathogens of humans. Clin Infect Dis 18:620, 1994

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