# Coronaviruslike Particles in Human Gastrointestinal Disease

## Epidemiologic, Clinical, and Laboratory Observations

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 Coronaviruslike particles (CVLPs) were visualized by direct electron microscopy (EM) of diarrheal stools in 49 of 126 infants and children between 1 month and 12 years of age during a threeyear observation period. The clinical and epidemiologic features of these patients were analyzed and compared with patients with diarrhea who were shedding rotaviruses, or whose stools were negative for enteric viruses by EM. Seasonal and age distributions for CVLP shedding were similar to those for rotaviruses (in most cases less than 1 year of age; peak months were September through January), as were the symptoms and median durations of illness. Prospective studies of three subsequent patients suggest that the duration of shedding in acute illness is five to at least 25 days. Multiple attempts to cultivate the CVLPs were unsuccessful. In addition, partial purification of CVLPs from stool specimens was performed, and immunologic analysis by immunoelectron microscopy and radial immunodiffusion showed no antigenic relatedness to prototype human (OC43 and 229E) or animal (bovine and canine) coronaviruses. These findings suggest that CVLPs may be an important cause of acute gastrointestinal illness in infancy, and may represent a virus antigenically unrelated to known human and animal coronaviruses.

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Coronaviruses are medium-sized **RNA-containing viruses that have** characteristic club-shaped projections or peplomers on their surface.<sup>1,2</sup> Some serotypes have been implicated as causative agents of enteric disease in animals and man.<sup>3-5</sup> Bovine coronaviruses are a cause of enteritis in neonatal calves and have been associated with necrotizing enterocolitis in these animals.<sup>3</sup> Coronaviruslike particles (CVLPs) in fecal samples have been inconsistently associated with diarrhea and other gastrointestinal symptoms in humans. Mathan et al<sup>6</sup> were first to report CVLPs seen in stool samples from children and adults with tropical sprue. In the same year, CVLPs were associated with an outbreak of explosive diarrhea in young adults.7 Subsequent reports have implicated CVLPs in outbreaks of diarrhea or necrotizing enterocolitis in neonates.<sup>8-10</sup> The relationship between CVLPs in fecal samples and illness, however, remains controversial, since some workers have found CVLPs in stools of controls as well as patients with gastrointestinal disease. Most of these latter reports were of children or adults in underdeveloped countries.<sup>11,12</sup>

Uncertainty about the association between CVLPs and human gastrointestinal disease may be due in part to the lack of detailed epidemiologic information, such as symptoms in relation to duration of particle shedding in feces and characterization of at-risk populations. Such data are difficult to gather because electron microscopy (EM) is the only available means for identifying CVLPs in fecal samples. Furthermore, identification of CVLPs requires a vigilant and experienced electron microscopist to distinguish the particles from other membrane profiles in the preparation.<sup>13</sup> With few exceptions,<sup>14,15</sup> attempts to cultivate CVLPs in organ or tissue culture, or in mycoplasma media, have been unsuccessful, thereby hampering efforts to develop an immunologic or other rapid diagnostic assay.<sup>9,12,16</sup>

At our institution, an outbreak of gastrointestinal illness among patients in the intensive care nursery<sup>9</sup> and sporadic cases of diarrhea associated with CVLPs among older infants and children have suggested that CVLPs may be an enteric pathogen. In this report, we describe clinical and epidemiologic characteristics of symptomatic infants and children over 1 month of age with CVLPs, the duration of CVLP shedding, attempts to cultivate and purify CVLPs, and tests of antigenic relatedness between CVLPs and prototype human and animal coronaviruses.

#### PATIENTS AND METHODS Epidemiologic and Clinical Observations

From May 1979 through April 1982, stool samples from 126 ill infants and children between 1 month and 12 years of age, of whom all but one were hospitalized, were studied by EM. Forty-nine of these samples were found to contain CVLPs. Of the remainder, 12 had rotavirus, three had adenovirus, six had Norwalk-like virus, one had astrovirus, and 55 were negative by EM. We analyzed the characteristics of the group with CVLPs and compared them

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with the rotavirus group and EM-negative group. The charts of these 126 patients were reviewed for pertinent clinical and laboratory data. Epidemiologic data included age, sex, seasonal distribution, and duration of symptoms. Clinical symptoms and signs included diarrhea; vomiting; mucousy, green, or bloody stools; fever; and nongastrointestinal symptoms. Laboratory data included: peripheral white blood cell counts, hematocrit, differential, platelet counts, stool culture, stool examination for ova and parasites by light microscopy, viruses by EM, abdominal roentgenograms, and miscellaneous studies associated with any nongastrointestinal diagnoses. Statistical comparisons were made with Student's t test.

#### Duration of CVLP Shedding

Subsequent to the retrospective review, during June 1982 through March 1983, any hospitalized nonneonate with CVLPs identified by EM in a fecal sample was eligible. Bacterial pathogens were not identified in fecal samples of these subjects. Twice or three times weekly, stool samples were collected and prepared for EM (described below). A subject was considered to have ceased shedding CVLPs when particles were not observed in two consecutive samples.

#### Laboratory Investigations

Virus Detection.—Electron microscopy of stool specimens was done by a previously described method.9 Stool diluted with distilled water was vortexed and centrifuged at 3,500 rpm for ten minutes, then the supernatant was removed and centrifuged again at 3,000 rpm for ten minutes in a clinical centrifuge to remove debris. Supernatant, placed on 300-mesh copper grids previously covered with a carbon-coated polyvinyl formal (Formvar) layer. was airdried, negatively stained with 2% phosphotungstic acid (pH, 6.5), and then directly examined under an EM. The pleomorphic appearance of characteristic CVLPs is shown in Fig 1. These particles are morphologically distinct from respiratory coronavirus in that CVLPs have a flexible-appearing fringe compared with the more rigid-appearing fringe that characterizes coronaviruses. Coronaviruslike particles also have more closely spaced peplomers compared with typical coronaviruses.

Cultivation Attempts.—Five to 20 stool samples that were positive for CVLPs by EM and five stools negative by EM were used in each of various culture attempts. These were processed according to standard methods<sup>17</sup> from either fresh sam-



Fig 1.—Representative coronaviruslike particles in negatively stained stool specimen from patient with gastroenteritis. Eight distinct pleomorphic, fringed, viral-like particles are shown. Fringe surrounding individual viral particles varies in length and appears "flexible." Although ends of some filaments appear "bulbous," others may simply represent end-on views of the flexible filaments. Among viral-like particles are two nonspecific membranelike profiles with thickened electron-lucent areas (phosphotungstic acid,  $\times 154,000$ ).

ples or ones that had been kept frozen at -60 °C. Aliquots (0.1 mL) were inoculated undiluted and diluted 1:10 onto the following cell cultures: a rhabdomyosarcoma line (RD), human embryonic intestine (intestine 407), a human rectal tumor line (HRT 18), a heteroploid lung line, human fetal lung (Flow 2,000), primary cynomolgus monkey kidney, and four strains of human embryonic tonsil fibroblasts developed in our laboratory. In addition, six continuous cell lines obtained from the American Type Culture Collection (Vero, BHK21, LLC-MK2, Madin-Darby canine kidney, Buffalo green monkey, and SIRC) were used. All specimens were observed for cytopathic effect and passaged on days 5 to 7 for five to 15 times.

Processed specimens were also diluted with equal volumes of trypsin at concentrations of 250, 25, 2.5, and 0.25  $\mu$ g/mL, incubated at 4 °C, 25 °C, and 35 °C for 30 minutes, and inoculated onto intestine 407 and two lines of human embryonic tonsil fibroblasts. These were passaged five times on days 6 and 7 with and without trypsin treatment.

Cultures were also tested for the presence of hemagglutinin at 4  $^{\circ}$ C, 25  $^{\circ}$ C, and 35  $^{\circ}$ C with human O, mouse, and guinea pig erythrocytes.

Mycoplasma culture attempts included inoculation of SP4 broth and agar plates with 10% horse serum. The plates were incubated in 5% carbon dioxide at 35  $^{\circ}$ C, and observed for colonies for ten days. Broth cultures were observed for 30 days for any changes in pH.

Attempt to Artifactually Produce CVLP.-Mucosa was teased from a portion of human jejunum obtained at autopsy from a patient without gastrointestinal symptoms. The jejunal contents were examined by EM to determine the absence of CVLPs, thereby excluding the possibility of subclinical infection. A 4-mL aliquot of homogenized jejunum was incubated at 31 °C with either 1 mL of 0.25% trypsin or physiologic saline solution (control) for ten or 120 minutes. At the end of incubation, 3 mL of 5% bovine serum albumin was added. Specimens were immediately prepared for EM. In addition a sample of untreated homogenized and jejunal mucosa was examined by EM.

Antigen Purification.—To obtain a sufficient quantity for purification, approximately 25 CVLP-positive fecal specimens were pooled. Ultrapure ammonium chloride was added to the pooled specimen to produce a 60% saturated solution. The mixture was stirred at 4 °C for three hours and then centrifuged at 10,000 rpm (7,800 g) for 20 minutes. The supernatant was dialyzed overnight at 4 °C against phosphate-buffered saline (pH, 7.4). In the final stage the dialysate was centrifuged at 90,000 g for two hours, resulting in supernatant and pellet. Prior to purification and following each step in the procedure, an aliquot of the

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Fig 2.—Age distribution of patients. Solid bars indicate coronaviruslike particles; shaded bars, rotavirus; and open bars, electron microscopy-negative.

pooled specimen was examined by EM to verify the presence of CVLPs.

Mouse Inoculation.—Suckling white Swiss mice (24 hours old) received 0.1 mL of pellet antigen by the intraperitoneal (n = 4) or intragastric (n = 3) route. During the next seven days, the animals were observed for signs of illness (diarrhea, ascites) or death. On day 7, one mouse (intragastric injection) was killed and the gastric contents and large intestine were homogenized and centrifuged at 3,500 rpm (1,564 g). The pellet was resuspended in approximately 0.5 mL of sterile, distilled water and prepared for EM.

Antisera.-Porcine anti-bovine enteric coronavirus (anti-BEC) and guinea pig anti-canine coronavirus (anti-CCV) sera were contributed privately and Guinea pig anti-229E serum and murine anti-OC43 ascitic fluid were obtained from the Centers for Disease Control, Atlanta. Human anti-CVLP serum was obtained from a laboratory technologist who had the onset of CVLP-associated acute diarrhea within 24 hours after the accidental aerosolization of **CVLP-positive fecal samples.** Pre-illness serum was that obtained approximately two years before the episode and convalescent serum was obtained two weeks after the onset of illness. Paired serum samples were also obtained from one infant studied for duration of CVLP fecal excretion; however, the earliest available serum was obtained approximately ten days after the onset of diarrhea ("acute"), and a later postillness serum specimen was collected 30 days after onset.

Immunodiffusion Studies.—Periph-

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Fig 3.—Monthly distribution of electron microscopic stool examinations over three-year period. Solid bars indicate coronaviruslike particles; shaded bars, rotavirus; and open bars, electron microscopy-negative.

eral wells of four radial immunodiffusion plates were each filled with 0.1 mL of the following: normal goat serum (two control wells), CVLP-positive fecal samples (three wells), and rotavirus-positive fecal sample (one well). The central well of each plate was filled with one of the following sera: anti-BEC, anti-CCV, anti-OC43, or anti-229E. After incubation at 4 °C the plates were examined at 24 and 48 hours. The study was performed on two occasions, using the same fecal samples and goat serum.

Immuno-EM.-A fecal sample contain-

ing numerous intact, nonaggregated CVLPs was selected. In addition to antisera against the four prototype coronaviruses, rabbit anti-herpes simplex (antiserum control) and normal goat sera (serum control) were used. Bovine serum albumin (0.22%) was used to make serial twofold dilutions of antisera and goat serum, from 1:20 through 1:320.

A 0.05-mL aliquot of antiserum (or serum) was pipetted into the U-shaped well of a microtiter plate. Next, 0.05 mL of stool extract was added to each well and the plate was gently agitated. Following a 45-minute

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Clinical and Laboratory Characteristics of the Three Study Groups*			
	Group		
	CVLP	Rotavirus	EM-Negative
Symptoms, No. positive/No. reported (%) Diarrhea	46/49 (94)	12/12 (100)	50/55 (89)
Fever (>38 °C)	31/49 (63)	7/12 (58)	35/55 (63)
Vomiting	25/49 (51)	8/12 (67)	36/55 (64)
Stool characteristics, No. positive/No. reported (%) Occult blood	7/38 (18)	0/12 (0)	12/30 (40)
Watery	25/38 (66)	11/12 (92)	43/55 (77)
Mucoid	12/38 (32)	1/12 (8)	13/43 (24)
Green	10/38 (26)	5/12 (42)	18/55 (32)
Duration of symptoms, days Mean	18	12	12
Median	7	5	9
Range	2-60	2-90	1-60
Peripheral blood counts, mean WBCs/cu mm	14,200	10,600	12,600
PMNs/cu mm	5,274	4,322	4,361
Hematocrit	35.8	32.7	34.7
Platelets/cu mm	449,000	412,000	466,000

\*CVLP indicates coronaviruslike particle; EM, electron microscopy; WBCs, white blood cells; and PMNs, polymorphonucleocytes.

incubation at 37  $^{\circ}$ C, an EM grid was dipped into each well, air-dried, and processed as previously described.

The electron microscopist examining and scoring immune aggregates on the grids was blinded to the specific antiserum used, and to the sequences of antisera and dilutions. For each microtiter well, ten fields per grid were examined at  $\times 4,800$  magnification and scored for clumping of enveloped particles (0 for no immune aggregates to 4 for extensive aggregation). The mean of the ten fields was used for comparison among the different antisera.

#### RESULTS Epidemiologic and Clinical Observations

**Epidemiology.**—Of the 49 patients with CVLPs in their stools, 59% were male, 88% were 2 years old or less, and 71% were 1 year old or less. The median age was 7 months (Fig 2). Although sporadic cases occurred throughout the year, the majority were detected in the fall and early winter (Fig 3). Thirty-eight (78%) of the cases occurred between September and the end of January.

Nine patients (18%) with CVLP also had at least one other identifiable stool pathogen. These included *Salmonella* (four patients), *Shigella* (three patients), *Campylobacter* (one patient) and an enterovirus (one patient).

Nine of the 12 patients with rotavirus were male. Ages ranged from 2 months to 12 months, with a median of 7 months. Eleven of these patients were ill in the fall and early winter. Three patients with rotavirus infection also had other pathogens concurrently identified in their stools: two patients had Norwalk-like particles and one had *Shigella*.

Sixty-four percent of the patients with EM-negative stools were male, with ages ranging from five weeks to 11 years. The median age was 7 months. The seasonal distribution for this group is shown in Fig 2. No other pathogens were detected in these patients.

Clinical Findings.—The clinical characteristics of patients in each of the three groups (CVLP-positive, rotavirus-positive, and EM-negative) are summarized in the Table.

The median duration of symptoms in all patients with CVLP was seven days, compared with five and nine days in the rotavirus- and EM-negative groups, respectively. These differences were not statistically significant.

The most common gastrointestinal symptoms described in the CVLP

group included diarrhea (94%), fever (63%), and vomiting (51%). The presence of these symptoms was not significantly influenced by the presence of another pathogen in addition to CVLPs. The occurrence of these symptoms in the other two groups was similar to the CVLP group. There were no statistically significant differences. Stools were commonly described as watery (66%), green (26%), and mucoid (32%) in the CVLP group, and occult blood was detected in the stools of seven patients (18%). No grossly bloody stools were reported. Of these stool characteristics, only occult blood in the stool was more frequent in the EM-negative group than the CVLP group (P < .05). None of the rotavirus group had occult blood. However, only slightly more than one half of the patients with stools negative by EM had stools tested for blood: stool guaiac tests were not done on one fourth of the CVLP group. Green stools were more common in the rotavirus group and mucus was more common in the CVLP group, but these differences were not statistically significant. Comparisons of peripheral blood cell counts (Table) showed no significant distinguishing characteristics among the three groups. Statistical evaluation was also done that excluded all patients with more than one intestinal pathogen. This did not change the analysis.

**Duration of Shedding.**—During the fall of 1982, three hospitalized infants with CVLPs identified in fecal specimens were serially studied.

A 1-month-old boy was found to have CVLPs after 13 days of vomiting and diarrhea. An extensive workup ruled out bacterial pathogens (Salmonella, Shigella, Yersinia enterocolitica, Campylobacter jejuni, and toxin-producing Clostridium difficile), intestinal parasites, immunoglobulin deficiency, and metabolic disease. The CVLPs were detected in fecal samples for an additional five days, and the disappearence of the particles coincided with the resolution of diarrhea.

A 2-month-old boy with chronic lung disease had fever with a temperature of 39 °C and loose stools, but no other symptoms of illness. Bacterial cultures of blood, urine, and cerebrospinal fluid



Fig 4.—Composite electron micrographs comparing typical coronaviruslike particles with jejunal homogenates (phosphotungstic acid, ×154,000). Top left, Typical coronaviruslike particle from patient with gastrointestinal illness. Note particle's irregular shape, thick fringe, and lack of stain penetration into particle interior. Top right, Membrane profile from jejunal homogenate incubated at 37 °C (ten minutes) without trypsin. Note distinct surface fringe and penetration of negative stain (electron-dense areas) into particle. Bottom left, Membrane profile from jejunal homogenate incubated at 37 °C (ten minutes) with at 37 °C (ten minutes) with trypsin. Note distinct surface fringe irregular, jagged contour and penetration of negative stain (large electron-dense areas) into particle interior. Bottom right, Smooth-surfaced membrane profile from jejunal homogenate incubated at 37 °C (ten minutes) with trypsin.

were sterile, as were viral cultures of cerebrospinal fluid and nasopharyngeal and rectal swabs. There were CVLPs in fecal samples collected over the next 25 days. The patient was intermittently febrile for seven days. Although his stools were noted to be somewhat loose and occasionally watery, he continued to tolerate gavage feedings of breast milk without difficulty. A 3-month-old girl had watery diarrhea and required intravenous hydration and parenteral alimentation. This secretory diarrhea subsided only after all oral feedings were discontinued at 14 days after onset. No enteric pathogens were isolated from the feces, but CVLPs were seen in a sample submitted 19 days after the onset of diarrhea and CVLPs were shed in the feces for an additional four days.

#### Laboratory Investigations

Cultivation Attempts.—All attempts to cultivate the CVLPs failed. Aside from some irregularly observed, nonspecific cytopathic effects that could not be serially passaged or confirmed by EM of the culture supernatant or a cell pellet, the only isolates were two polioviruses of presumed vaccine origin and one adenovirus. No detectable hemagglutinin was found, and all mycoplasma cultures were negative.

Mouse Inoculation.—None of the suckling mice became ill or died during the seven days of observation. No CVLPs were noted in the intestinal homogenate of a mouse killed on the seventh day after intragastric inoculation.

Attempts to Produce CVLPs Artifactually.—The jejunal contents were negative for CVLPs by EM. A characteristic CVLP (Fig 4, top left) with a surface fringe approximately 20 nm thick was used to compare with the homogenized jejunum (Fig 4, top right, bottom left and right). A nontrypsinized jejunal homogenate incubated at 37 °C for ten minutes disclosed the presence of membrane profiles, some of which displayed a surface fringe approximately 10 nm thick (Fig 4, top right). The individual filaments that made up the fringe were of a width (1.9 to 3.2 nm) similar to that associated with characteristic CVLPs (Figs 1 and 4, top left). The CVLPs had, in addition, bulbous tips, some of which measured up to 5 nm in diameter. Jejunal homogenates trypsinized for ten minutes showed either membrane profiles with a jagged irregular surface fringe (Fig 4, bottom left) or smooth-surfaced membrane profiles with no fringe (Fig 4, bottom right). Homogenates exposed to trypsin for two hours contained a sparse number of smooth-surfaced membrane profiles. In addition to the filament characteristics, the negative-contrast staining features of typical CVLPs differed from the membrane profiles found in the homogenate. The CVLPs appear to exclude the negative stain from the interior of the membrane profiles (Figs 1 and 4, top left), whereas the fringed membrane profiles from the homogenized jejunum appear to allow the stain to penetrate (Fig 4, top right and bottom left). Despite an exhaustive search, no typical CVLPs were identified in any of the homogenate preparations.

Antigen Purification.—Absence of rotavirus antigen in the pooled stool specimen was confirmed using a commercial enzyme-linked immunosorbent assav (ELISA) kit. The partially purified supernatant fraction contained greater than ten CVLPs per 300-mesh grid square. Some of the particles had characteristic fringes. No flagella and few nonspecific membrane profiles were seen. The pellet fraction contained greater than 100 CVLPs per 300-mesh grid square. No contaminants were seen and the particles did not appear to be clumped. The fractions were used without success in attempts (not detailed herein) to develop specific antisera in rabbits to apply ELISA methods for CVLP antigen detection and characterization.

Immunodiffusion.—There was no evidence of reaction between the prototype antisera (BEC, CCV, 229E, and OC43) or rotavirus antiserum and CVLP antigens in the stool samples. Similarly, the goat serum control did not react with any of the stool antigens.

Immuno-EM.—For all anticoronavirus sera tested, aggregates of particle envelopes appeared at random serum dilutions. These aggregates consisted of both fringed and nonfringed membrane profiles, as previously described and illustrated.<sup>9</sup> No consistent pattern of enhanced aggregation with changing serum dilution was discernible for any of these antisera. These aggregates were as likely to be seen in control wells (anti-herpes simplex virus serum) as in a well containing prototype antiserum of the same dilution.

### COMMENT

A variety of coronaviruses or "coronaviruslike" agents have been detected in mammalian and avian species. Langpap et al<sup>3</sup> demonstrated the presence of coronaviruses in the stools of calves by negative-staining EM and by fluorescent antibody examination of intestine and colon tissue sections. The calves ranged in age from 1 day to 3 months. Green, mucoid, fecal casts were seen in the colon at necropsy and the histologic characteristics were compatible with necrotizing enterocolitis.<sup>3</sup> This bovine enteric coronavirus has been shown to possess membrane antigens that cross-react with human respiratory coronavirus OC43.<sup>18</sup>

The association of CVLPs with gastrointestinal symptoms in humans has been reported recently, and their role in such disease is less clear. Identicalappearing particles have also been demonstrated in the stools of asymptomatic persons, making it more difficult to prove a causal relationship.<sup>5,1,12</sup>

Caul and Clarke<sup>15</sup> have reported successful in vitro propagation of CVLPs from one patient. More recently, CVLPs were associated with gastroenteritis in children and adults residing in England and France, two of whom also had a hemolytic-uremic syndrome<sup>19</sup>; Gerna et al<sup>20</sup> found CVLPs by EM in 34 (16.3%) of 208 Italian infants and children with acute gastroenteritis and only three (1.6%) of 182 age-matched control subjects.<sup>20</sup>

Evidence has been presented to support an association between neonatal disease and the presence of CVLPs in stool. We have previously described an outbreak of gastrointestinal disease in a neonatal intensive care unit, and a subsequent 40-week survey demonstrated a statistically significant association between the presence of CVLP and gastrointestinal symptoms, including blood in stools, watery stools, gastric retention, bilious gastric aspirates, and abdominal distension.<sup>9</sup> Most CVLPs were identified during the fall and early winter.

Chany et al<sup>10</sup> have reported a cluster of cases of neonatal necrotizing enterocolitis associated with CVLPs in the stool. The association occurred in both premature and full-term infants. A high incidence of CVLPs was also found in the staff members caring for these infants.

We have characterized the gastroenteritis associated with CVLPs among hospitalized patients beyond the neonatal age group. Over a three-year period, the incidence of CVLPs in feces was greatest in fall and early winter. Diarrhea was the most common symptom (94% of patients), but vomiting and fever were frequently present (63% and 51%, respectively). Stools were often characterized as being watery, green, and mucus-containing.

Seasonal distribution was similar for rotavirus and CVLP-associated gastroenteritis, with the peak incidence for both occurring in fall and early winter. The age distribution for both agents was similar, with the majority of patients being less than 3 years old. The clinical manifestations and duration of illness associated with both agents were not significantly different: however, occult blood was more frequent in the stools of CVLP-infected than rotavirus-infected patients. Smears and Gram stains of acute-phase fecal specimens were not regularly recorded, so data were not available to determine if the hemepositive stools reflect a greater enteroinvasiveness of CVLPs than rotavirus.

In samples of nonhuman primate feces. CVLPs were rarely found in nonweaned compared with weaned simians (1/35 vs 23/34, respectively).<sup>21</sup> The protective effect of nursing may extend to humans; premature infants receiving intensive care are often weaned, and seem to be at risk for severe CVLP-associated disease.<sup>9,10</sup> The relationship between gastrointestinal illness and weaning status of infants in our retrospective study was not examined because of incomplete information about whether infants were formula- or breast-fed, or if recent changes in feeding practice had been made prior to the illness.

The three patients prospectively studied were not sufficient to determine specific correlation between fecal CVLP excretion and duration or severity of illness; it is apparent that a larger number of patients will need to be similarly studied to determine these relationships.

Because some investigators have questioned the accuracy of CVLP diagnosis by EM,<sup>13,22</sup> we attempted to propagate the agent in cell culture and suckling mice, and to develop an ELISA for detecting fecal CVLPs. As in other studies, we were unable to cultivate the agent in a variety of cell lines or by using mycoplasma media. We were unsuccessful in producing gastrointestinal symptoms by inoculating suckling mice with the purified CVLP antigen. Because of the small amount of antigen available, we did not orally infect the animals. In the future, this and perhaps other routes should be considered.

To determine if CVLPs might be an artifact of enzyme action on the mucosal lining of the intestine, we treated a jejunal homogenate with trypsin. Our inability to produce any particles resembling CVLPs suggests that the agent is not artifactual. The fact that CVLPs can also be observed in the stools of symptomatic adults precludes the possibility that CVLPs are artifacts produced only in the intestinal contents of children infected with an as yet undiagnosable virus. The definitive proof of their viral nature may come from the demonstration of nucleic acid in these particles and their

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We were unable to purify fecal CVLPs sufficiently to determine whether the particles are antigenically unrelated to prototype coronaviruses (OC43, 229E, CCV, or BEC). However, when antisera to prototype coronaviruses were used for immune EM, only spontaneous, random aggregation was seen. Similarly, immunodiffusion results indicated a lack of antigenic sharing between CVLPs and the prototype coronaviruses. Gerna et al<sup>20</sup> suggested antigenic relatedness between their patients' CVLPs and OC43 coronavirus, but others have suggested that CVLPs are antigenically unique. Beards et al<sup>19</sup> showed antigenic unrelatedness of their human subjects' CVLPs to prototype coronaviruses, and suggested that these may represent a new viral genus, related to the Breda and Berne viruses of calves and horses, respectively.<sup>19</sup>

The lack of consistent results using

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mature nursery during an outbreak of necrotizing enterocolitis (NEC) and diarrhea (D). *Pediatr Res* 1983;17(suppl):181A.

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15. Caul EO, Clarke SKR: Coronavirus propagated from patient with non-bacterial gastroenteritis. *Lancet* 1975;2:953-954. convalescent sera in immune EM experiments may reflect the presence of a dominant local immune response in some or most patients. Clarification of this will require further investigation of humoral and mucosal immune mechanisms, perhaps utilizing methods other than immuno-EM.

In conclusion, we have described the epidemiology and clinical features of CVLP-associated gastroenteritis in hospitalized nonneonates. Using several techniques, we were unable either to cultivate or to purify adequately the agent. We believe that these particles are of potential importance in human disease, and do not merely represent fecal or mucosal artifacts. However, better definition of their role in disease may require other methods of in vitro cultivation and antigen purification.

Brian A. David, PhD, provided the HRT 18, Alan Liss, PhD, Binghamton, NY, provided the SP4 broth and agar plates, and E. A. Carbrey, VMD, MS, Ames, Iowa, provided the anti-CCV and anti-BEC sera.

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Librarian's Note.—See also: Rettig PJ, Altshuler GP: Fatal gastroenteritis associated with coronaviruslike particles (AJDC 1985;139:245-248).