An Eight-Year Study of the Viral Agents of Acute Gastroenteritis in Humans: Ultrastructural Observations and Seasonal Distribution With a Major Emphasis on Coronavirus-Like Particles

Claire M. Payne, C. George Ray, Virginia Borduin, Linda L. Minnich, and Michael D. Lebowitz

During an 8-yr period, 862 stool specimens from patients with gastroenteritis were examined by electron microscopy after negative staining with 2% phosphotungstic acid (pH 6.5). Forty-one percent of the specimens submitted over an 8-yr period were determined to be positive for virus or viruslike particles belonging to one or more of seven morphologically distinct viral groups. Coronavirus-like particles (CVLPs) were present in 69.8% of the positive stool specimens. Membranous profiles containing “complement-type” holes (10 nm in diameter) were identified in some preparations containing CVLPs. The second most prevalent viral agent found in stool specimens was the rotavirus (17% of all positive stools). The incidence of other viruses identified in the survey were as follows: adenovirus 4.5%, picorna/parvovirus agents 2.9%, Norwalk-like agent 2.9%, astrovirus 1.9%, and calicivirus 0.5%. Unclassified small round viruses (~25–30 nm in diameter) represented 0.5%. It was also determined that there was a seasonal distribution in excretion of all viruses except for CVLPs. A greater number of viruses were identified in the cooler, drier months of the year.

INTRODUCTION

Infectious diarrhea is a major cause of infant mortality in underdeveloped areas of the world and results in 5–18 million deaths annually (Elliott, 1976; Kapikian et al., 1980; Wolf and Schreiber, 1982). Bacterial (Sack, 1975) and parasitic (Knight, 1978) pathogens together account for less than 50% of all cases of pediatric diarrhea (Pickering et al., 1978). McLean (1931) first suspected a viral etiology of nonbacterial gastroenteritis because of the seasonal incidence of gastrointestinal symptoms. In 1947, Gordon et al. successfully transmitted epidemic gastroenteritis to human volunteers by oral administration of fecal filtrates. Although viruses were implicated by these studies, numerous attempts in later years to grow the presumed viral pathogens using conventional cell and organ culture techniques were largely unsuccessful (Wyatt and James, 1982). In 1972, Kapikian et al. then visualized, using immune electron microscopy (EM), 27-nm viral particles that were responsible for an outbreak of acute
nonbacterial gastroenteritis that occurred among school children in Norwalk, Ohio. Electron microscopy remains the single most effective laboratory technique available to detect the diverse viral pathogens that can cause gastroenteritis in humans (Yong and Peter, 1984). Viral diarrhea is now well recognized, and numerous reviews and overviews of ultrastructural findings have been published on the subject (Kjeldsberg, 1980; Tyrrell, 1982; Wolf and Schreiber, 1982; Brandt et al., 1984; Cukor and Blacklow, 1984; Yong and Peter, 1984). At least seven morphologically distinct viral or viral-like agents have been identified and include rotavirus, adenovirus, astrovirus, calicivirus, Norwalk-like agent, picorna-parvovirus, and coronavirus.

The present study encompasses our observations from December 1976 to December 1984 on stool specimens submitted to our laboratory from patients with a diagnosis of acute nonbacterial gastroenteritis. The frequency of viral agents, their morphologic pattern and size range, and their seasonal distribution are emphasized.

MATERIALS AND METHODS

Collection and Preparation of Specimens for Electron Microscopic Examination

The study population represented primarily hospitalized patients in southern Arizona with a clinical diagnosis of acute, nonbacterial gastroenteritis, in whom the attending physicians had requested EM of the stools. Aliquots (2–5 ml) of diarrheal stool were collected, transported on wet ice, and held at 4°C until processed. In instances where stool samples were not readily collected, rectal swabs were thoroughly soaked with the sample and immersed in 2 ml of distilled water for transport. The stools were prepared directly or diluted with 1–2 ml of distilled water depending upon their consistency. All specimens were vortexed and then centrifuged at 2,000 g for 30 min in a swinging-bucket clinical centrifuge (model No. J6B; Beckman Instruments, Palo Alto, CA) to remove bacteria and debris. A portion of the supernatant was removed with a Pasteur pipette and one drop was placed on the surface of each of two Formvar and carbon-coated, 300-mesh copper grids (Ernest Fullam, Schenectady, NY) (Vaucher et al., 1982) that were placed in microtiter wells containing 1% agar (Difco Laboratories) (Anderson and Doane, 1972). Grid No. 1 was immediately removed from the well with a pair of forceps and allowed to air-dry without blotting. Grid No. 2 was left in the well, and the drop was allowed to dry completely (~1 hr) onto the grid surface. Grid No. 1 was returned to the well, and both grids were negatively stained by adding several drops of 2% phosphotungstic acid (adjusted to pH 6.5 with 1 N KOH) directly to the well. The grids were removed after 3 min, blotted on filter paper, and examined under an electron microscope (model No. HU-12; Hitachi Scientific Instruments, Mountain View, CA). Positive stool specimens were photographed at a magnification of ×60,000 for measurement purposes and at lower magnifications to illustrate specific points. Calibration of the electron microscope was accomplished using a carbon grating at low magnifications and a catalase crystal at high magnifications.

Methods Used in Scanning for Viruses Under the Electron Microscope

Each specimen was read out in ~10–15 min. Both grids prepared from each specimen were examined. One routinely began with grid No. 1, which had a thinner film of stool than grid No. 2. Coronaviruses and coronavirus-like particles (CVLPs) were most easily seen with this preparation. The thicker stool film of grid No. 2 tended to obscure the delicate filaments that make up the fringe of the CVLPs. Each grid was viewed initially through the attached ×10 ocular system at a scope magnification
of \times 10,000. The CVLPs were identified as fringed membranous profiles that are flexible and tend to collapse onto the grid surface, exclude the negative stain, and contain a somewhat electron-dense "nucleoid" in the interior of the particle (see Results section for details of morphology). The viruses that have distinctive geometric shapes were recognized initially at a magnification of \times 10,000 by the presence of a ring of electron-dense stain that surrounds the individual particles making them stand out against a background of debris. Suspicious particles were then examined at \times 60,000 and the size was estimated from a calibrated millimeter scale that is drawn on the fluorescent screen of the microscope. The small (\sim 20 nm) featureless particles were only scored as present if aggregates of the same-sized particles were present or if defective particles could be identified. Defective particles have no nucleic acid and become penetrated by stain revealing the geometric shape of the capsid. Photographs were taken at \times 60,000 for exact measurements of viral size and for comparison purposes.

Measurement of Viral Particles

The diameter of individual viral particles was obtained by measuring the distance between the furthest projections on either side of the particle using a calibrated loop. If the particle was not round, the diameter was obtained by averaging the lengths of the long and short axes. The mean diameters of viral populations were statistically compared using the Student's t-test. The area of pleomorphic viral particles was obtained using a MOP-3 computerized planimeter (Carl Zeiss, Inc., Thornwood, NY).

Statistical Methods Used in the Determination of Seasonal Distribution

The proportionate occurrence of each of the four morphologically distinct groups (rotavirus, adenovirus, small round virus, and CVLPs) was compared with the expected occurrence if month was an independent factor using the \chi^2 test. The correlation of viral occurrence with each of the climatologic conditions using monthly data was analyzed using the Spearman rank order correlation coefficient.

RESULTS

Viral Agents Identified in Stool Specimens by Direct Electron Microscopy

Morphology. Seven morphologically distinct viral or viral-like agents were identified in this survey. The six viral agents that displayed cubic symmetry (Figures 1A–1F) were easily identified under the electron microscope because of their characteristic geometric shapes, capsomere pattern, and size. The classic rotavirus is 65–70 nm in diameter and has a characteristic wheel-like appearance with surface "holes" (Figure 1A). The adenovirus (65–70 nm in diameter) displays closely spaced capsomeres on 20 triangular facets that are arranged to form an icosahedron (Figure 1B). The Norwalk-like agent has no distinctive capsomere pattern; the spikelike projections on the surface are, however, most characteristic (Figure 1C). Although all particles that displayed spike-like projections with no discernible surface capsomere pattern were classified as Norwalk-like for survey purposes, there was a considerable range in size (27–39 nm in diameter). The astrovirus (25–30 nm in diameter) has a characteristic surface pattern in the form of a five- or six-pointed star (Figure 1D). Caliciviruses (25–35 nm in diameter) have a variable surface pattern that sometimes appears as cup-like depressions (Figure 1E) or has a "star of David" configuration (Figure 1E, insert). Picorna/parvoviruses are 25–30 nm in diameter and
are ultrastructurally featureless in that no distinct surface capsomere pattern can be discerned and the contour of the particle appears smooth (Figure 1F).

Coronavirus-like particles are pleomorphic, enveloped viral-like particles that possess a surface fringe consisting of closely spaced filaments with a bulbous tip (Figures 2A–2D). The width of the fringe varies from particle to particle and ranges from 10 nm to 31 nm at its greatest dimension. The individual filaments that make up the fringe have a diameter of 3–5 nm. Some CVLP profiles were rather large and covered as much as 0.21 μm² area of the grid surface (Figure 2A). Most of the CVLPs

FIGURE 2. Electron micrographs showing the morphologic variation of CVLPs. (Phosphotungstic acid, × 138,400.) A. A large, irregularly shaped CVLP with a flexible appearing fringe. A small CVLP appears to be budding off (arrow) from the larger particle. B. A large CVLP with a rigid appearing fringe. The bulbous ends of the individual filaments that make up the fringe appear prominent. C. A cluster of three CVLPs with a flexible appearing fringe. A distinct nucleoid (arrow) can be seen in the interior of one of the particles. D. A smaller CVLP with a rigid appearing fringe.
FIGURE 3. Low power electron micrographs comparing a stool specimen containing a large number of CVLPs with nonspecific membranes. (Phosphotungstic acid, × 47,800.) A. CVLPs present in a stool specimen from a patient with gastroenteritis. The particles appear to collapse onto the surface of the grid, and a distinct nucleoid can be seen in most of the particles. Note the absence of flagella and other debris commonly observed in stool specimens. B. Nonspecific membrane profiles present in a stool specimen submitted for diagnosis. The membranes are elevated in part from the grid surface giving the profiles a refractile appearance (arrow) under the electron microscope.
identified in stool specimens had a "flexible" appearing fringe (Figures 2A and 2C) whose bulbous tips measured ~5 nm in diameter. Occasionally, other particles were present that possessed a "rigid" appearing fringe (Figures 2B and 2D) whose bulbous tip measured up to 7 nm in diameter. The CVLPs with the "flexible" appearing fringe appeared to exclude the negative stain from the interior of the particle to a greater extent than the CVLPs with the "rigid" appearing fringe. The former also displayed an electron-dense "nucleoid" that was obvious in most of the particles examined (Figures 2A, 2C, and 3A). In some stool specimens the small particles were present in such large numbers (to the exclusion of other debris normally found in stools) (Figure 3A) that they appeared similar to viral-enriched fractions experimentally prepared in vitro. It was noted in some stool specimens that the small CVLPs possessing a flexible appearing fringe appeared to "bud off" from larger particles by forming a narrow stalk (Figure 2A). A similar process was not observed with the CVLPs possessing a rigid appearing fringe. The large CVLPs were not observed in neonates but were seen in stools of infants (as young as 3 months of age), children, and adults. Features of CVLPs that serve to distinguish them from nonspecific membranes are the width of the surface fringe, the nonrefractile nature of the envelope (see Figure 3B for comparison), the exclusion of negative stain from the interior of the particle, the presence of a distinct nucleoid, and the presence of "budding" forms.

Frequency. Forty-one percent of the 862 stool specimens submitted to our laboratory from December 10, 1976 to December 9, 1984 (Table 1) were determined to be positive for one or more of the seven morphologically distinct viral agents shown in Figures 1, 2, and 3A. Coronavirus-like particles were the most frequently identified viral-like particle and were present in 244 patients (70% of all positive stool specimens). Rotavirus was second in frequency and was excreted as either mixtures of double-shelled ("smooth") and single-shelled ("rough") particles, or as homogeneous populations of rough particles. Fifty-nine percent of all rotavirus-positive stools consisted of only rough particles that had varied in size from 30–61 nm in diameter. Twenty-four specimens had two different viral agents identified and one specimen had three (CVLPs, adenovirus, and picorna/parvovirus agent). Coronavirus-like particles were also found in association with rough rotavirus (seven specimens), smooth rotavirus (two specimens), adenovirus (five specimens), and picorna/parvovirus (three specimens).

The relatively low prevalence of rotavirus in the study population raised the

<table>
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<th>TABLE 1. Frequency of Enteric Viruses Identified by Electron Microscopy</th>
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<td><strong>Viral agent identified</strong></td>
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<tr>
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<tr>
<td>Coronavirus-like</td>
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<tr>
<td>Rotavirus and minirotavirus</td>
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<td>Adenovirus</td>
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<td>Picorna/parvovirus agents</td>
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<td>Norwalk-like agent</td>
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<td>Astrovirus</td>
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<td>Calicivirus</td>
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<td>Unidentified small round viruses ( ~ 25–30 nm in diameter)</td>
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*Total no. of stool specimens examined = 862.
Total no. of positive stools = 350.
Total no. of viral agents identified = 377.
FIGURE 4. Electron micrographs of CVLPs showing evidence of immune system activation. A. Low power electron micrograph of a stool specimen from a patient with gastroenteritis. Distinct clusters of CVLPs representing possible natural immune aggregates are present. (Phosphotungstic acid, ×47,800.) B. High magnification electron micrograph of a CVLP showing complement-like lesions. Many ring-shaped structures containing an electron-dense center or hole surrounded by an electron-lucent rim are present. (Phosphotungstic acid ×204,300.)
question of sensitivity of our EM methods for the detection of these viruses. We investigated this possibility by applying an enzyme-linked immunosorbent assay (ELISA) (Rotazyme, Abbott Laboratories, Inc.) with confirmation by blocking antibody testing. Of 466 specimens negative for rotavirus by EM, including 80 in which only CVLPs were observed, none were positive by ELISA. On the other hand, 30 known rotavirus-positive specimens were all ELISA-positive. Therefore, we conclude that the EM method we employed was adequately sensitive for the detection of rotavirus.

**Ultrastructural Support Implicating Coronavirus-Like Particles as Possible Etiologic Agents of Gastroenteritis: Activation of Host Immune System**

Natural immune-like aggregates. In many of the CVLP-positive stool specimens, distinct aggregates of CVLPs could be seen (Figure 4A). In some aggregates, antibody-like bridges could be seen between some of the particles. Occasional aggregates were found to be coated with a fuzzy substance that completely obscured the surface fringe of the particles within the aggregate.

Complement-Like Lesions. In some of the CVLP-positive stool specimens, membrane profiles were found that contained distinct ring-shaped structures with an electron-dense center (Figure 4B). The electron-dense center or hole measured 10–12 nm across (Table 2) and was surrounded by a distinct electron-lucent rim 5 nm in thickness. These CVLP profiles containing complement-like lesions were seen in newborns, infants, and one adult (Table 2).

**Seasonal Distribution of Enteric Viruses.** Fifty percent of all virus-positive stool specimens were identified between the months of September and December and corresponds to the onset of cooler weather (Figure 5). The percentage of virus-positive stool decreased by 50%, however, between December and January. The difference in average temperature between December and January was negligible (1.7°F cooler in January) over the 8-yr survey, whereas January experienced twice as much rainfall (1.7 in.) as December (0.9 in.) and a 10% higher average humidity. The lowest percentage (4.0%) of virus-positive stools occurred in the month of July, the beginning

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<th>Patient</th>
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<tr>
<td>1</td>
<td>1 day</td>
<td>11.4 ± 2.1</td>
<td>8.4–17.1</td>
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<td></td>
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<td>11.4 ± 1.6</td>
<td>9.6–16.5</td>
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<td>2</td>
<td>4 days</td>
<td>11.3 ± 0.9</td>
<td>9.8–13.0</td>
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<td>3</td>
<td>6 months</td>
<td>10.2 ± 2.2</td>
<td>8.5–16.6</td>
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<td>4</td>
<td>6 months</td>
<td>11.6 ± 2.6</td>
<td>9.2–17.2</td>
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<td>5</td>
<td>11 months</td>
<td>10.9 ± 1.8</td>
<td>7.9–13.9</td>
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<td>6</td>
<td>69 yr</td>
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<td>9.4–11.4</td>
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<td>11.6 ± 0.7</td>
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aEach mean represents the average diameter of all electron-dense centers present on the representative CVLPs photographed from each case for measurement purposes.

bEach value represents a single CVLP.
An Eight-Year Study of Viral Gastroenteritis

FIGURE 5. Seasonal distribution of enteric viruses and viral-like particles in Arizona. Top graph represents the mean monthly climatologic data obtained from the Weather Service Office (Division of the United States Department of Commerce, National Oceanic and Atmospheric Administration) at Tucson International Airport. The mean monthly relative humidity represents an average of the monthly humidity recorded daily at 5 AM, 11 AM, 5 PM, and 11:00 PM. Bottom bar graph represents the monthly distribution of enteric viruses. The total number of specimens in each virus group was derived from Table 1. The Norwalk-like agent, astrovirus, calicivirus, and picorna/parvovirus agents are classified as small round viruses.

of our summer rainy season. The average monthly rainfall in July (2.2 in.) was 12 times higher than in June, and the relative humidity was twice as high. No adenoviruses or small round viruses and only 2% of the rotavirus-positive cases occurred at this time. The most dramatic seasonal variation occurred with rotavirus. Forty-one percent of all rotavirus-positive stools occurred in the month of December. The incidence of increased rotavirus excretion with decreased precipitation was determined to be statistically significant \( p < 0.05 \). The seasonality of each of the four virus-specific groups (adenoviruses, small round viruses, rotaviruses, and CVLPs) was evaluated statistically. The adenovirus, small round virus, and rotavirus groups all differed from chance occurrence \( p < 0.005 \), whereas the CVLP group did not \( p > 0.1 \).

**Age and Sex Distributions Among Coronavirus-Like Particles.** Of the CVLP-positive specimens, 73% were from patients <1 yr of age. The remainder were almost equally divided among other age groups (12–24 months of age, 8%; 25 months–5 yr, 3%; 6–15 yr, 6%; 16–30 yr, 4%; >30 yr, 6%). Four patients were 80–99 yr of age. Patient sex was known in all but four patients, with a calculated male to female ratio of 1.4 : 1.

**DISCUSSION**

Examination of stool specimens by EM remains the single most effective laboratory test to detect the different viral agents now recognized to cause gastroenteritis in humans (Yong and Peter, 1984). Electron microscopy surveys of patients admitted for evaluation of acute diarrhea in the United States (Brandt et al., 1983; Riepenhoff-Talty et al., 1983), Canada (McLean et al., 1976; Middleton et al., 1977; Gurwith and Williams, 1977), Australia (Cameron et al., 1978), New Zealand (Goldwater, 1979),
Scotland (Madeley et al., 1977), Finland (Vesikari et al., 1981), and Tanzania (Brookfield et al., 1979) have implicated rotavirus as the most prevalent viral agent identified. In a study of 60 patients <6 yr old in New Zealand, Goldwater (1979) reported rotavirus to represent 72% of the virus-positive stools. Similarly, in a study of 1,160 infants and young children in Buffalo, NY, Riepenhoff-Talty (1983) reported rotavirus to represent 73% of the virus-positive stools. Contrary to most other parts of the world, we have found CVLPs to be the most prevalent viral-like agent identified. Coronavirus-like particles were present in 70% of our virus-positive stool specimens after an 8-yr survey of 862 patients (mostly infants and children) with gastroenteritis in Arizona. Rotavirus was second in frequency and represented 17% of all virus-positive stools. Our overall frequency of virus-positive specimens was 41% and falls within the range reported in other surveys [27% (Riepenhoff-Talty, 1983) and 58% (Madeley et al., 1977)].

Coronaviruses are pleomorphic, enveloped RNA-containing viruses (Tyrrell et al., 1978; Macnaughton and Davies, 1981) that have petal-shaped projections and contain phospholipids and glycolipids similar to that of the host cell (Pike, 1977). Coronaviruses and CVLPs are known to cause severe gastrointestinal illness in animals (Naqi et al., 1975; Horzinek and Osterhaus, 1979). Their role in human gastroenteritis, however, is somewhat controversial. Although many reports have appeared in the literature throughout the world identifying CVLPs as a possible etiologic agent of gastroenteritis in humans (Caul et al., 1975; Mathan et al., 1975; Baumeister et al., 1976; Maass et al., 1977; Moore et al., 1977; Rowland et al., 1978; Schnagl et al., 1978; Schnagl et al., 1979; Clarke et al., 1979; Maass and Baumeister, 1983; Gerna et al., 1985), CVLPs have also been identified in apparently healthy subjects (Mathan et al., 1975; Caul et al., 1975; Maass et al., 1977; Moore et al., 1977; Rowland et al., 1978; Schnagl et al., 1978; Schnagl et al., 1979; Clarke et al., 1979; Maass and Baumeister, 1983; Peigue et al., 1978; Weindling et al., 1980; Marshall et al., 1982; Puel et al., 1982; Sitbon, 1985). There are, however, two case-control studies which suggest that a relationship does exist between CVLPs and gastrointestinal illness (Vaucher et al., 1982; Gerna et al., 1985). Our current observations establish the fact that a uniquely high prevalence of CVLPs exists in the population we studied (Mortensen et al., 1985). This serves as a basis for designing a prospective, controlled study.

In 1979 we experienced an outbreak of gastrointestinal illness among neonates in the intensive care nursery of our hospital, and CVLPs were the only pathogens identified (Vaucher et al., 1982). Eight surveys of stools were conducted over a 40-wk period, and the incidence of CVLPs decreased from 69% to <10% over the study period. Many of these patients had symptoms associated with the early stages of necrotizing enterocolitis. In a later epidemiologic, clinical, and laboratory study of CVLPs, we could not experimentally produce the characteristic particles by trypsin digestion of human intestinal mucosal homogenates (Mortensen et al., 1985). These findings mitigate against CVLPs being nonspecific membranous debris. Coronavirus-like particles have now been associated with distinct clinical symptoms such as tropical sprue (Baker et al., 1982), the hemolytic-uremic syndrome (Beards et al., 1984), and necrotizing enterocolitis (Moscovici et al., 1980; Sureau et al., 1980; Chany et al., 1982; Siegel et al., 1983; Von Spencker et al., 1983). A case of fatal gastroenteritis has also recently been reported to be associated with CVLPs (Rettig and Altshuler, 1985).

The present study provides some new ultrastructural observations that strongly implicate human CVLPs as a viral agent. First, many of our CVLP-positive stools contain homogeneous populations of similarly sized particles and resemble viral preparations purified on sucrose gradients (Caul and Egglestone, 1982). This appearance would not be consistent with a nonspecific degenerative process affecting...
the mucosal lining of the intestine. Second, small CVLPs were observed in the process of budding-off from larger fringed particles by a narrow stalk. This process has not been described in solubilized fractions of intestinal brush border (Maestracci, 1976), nor have we observed it in our jejunal homogenates (Mortensen et al., 1985). Third, CVLPs were noted to be morphologically different from nonspecific membranes. In addition to the previously noted greater width of the surface fringe (Caul et al., 1977), CVLPs' envelopes appeared more flexible and had a tendency to collapse onto the grid surface. Flexible envelopes were also a noted feature of the CVLPs of bluecomb turkeys (Naqi et al., 1975). Coronavirus-like particles also tend to exclude the negative stain, whereas nonspecific membrane profiles are penetrated by stain (Eugster and Sneed, 1980). Fourth, “natural immune” aggregates can be observed in many of our CVLP-positive stools. These aggregates are morphologically indistinguishable from the immune aggregates of fringed viruses experimentally produced in vitro (Berry and Almeida, 1968). Fifth, in some CVLP profiles, distinct holes that were indistinguishable from those seen in complement lesions (Berry and Almeida, 1968) were identified. The dimensions of the ring-shaped structures were similar to those of the terminal complement C5b-9 membrane attack complex (MAC) that is lytic for erythrocytes, nucleated cells, and bacteria (Biesecker, 1983). It is probable that viruses that are enveloped and contain a lipoprotein component such as avian infectious bronchitis virus (Berry and Almeida, 1968), herpes simplex virus (Yoshino and Tanziguchi, 1965), and human enteric coronavirus (present study) may be similarly attacked by complement. The presence of natural immune aggregates and complement-like lesions indicate that the host's immune system has most probably been activated by the CVLPs. Although we initially reported a serum antibody response to CVLPs in some patients (Vaucher et al., 1982), no seroconversion could be demonstrated with other patients in a later study (Mortensen et al., 1985). Perhaps the development of local immunity (Kapikian et al., 1976) plays an important role in the host defense to coronavirus enteric infections.

The seasonal distribution of viral diarrhea and its possible association with specific climatic factors has been most intriguing and relates to important questions of epidemiology. Rotavirus epidemics or peak incidence of excretion have been noted to occur worldwide during the winter months or cooler parts of the year (Middleton et al., 1977; Kapikian et al., 1976; Birch et al., 1977; Maiya et al., 1977; Hieber et al., 1978; Black et al., 1980; Brandt et al., 1982). A high prevalence of rotavirus excretion has also been noted during the dry months in Africa (Sitbon, 1985; Rowland and McCollum, 1977), India (Maiya et al., 1977), Bangladesh (Black et al., 1980), Costa Rica (Hieber et al., 1978), and in regions with low relative humidity (Dossetor et al., 1979; Paul and Erinle, 1982). The unique desert environment of the southwestern United States provides an opportunity to study the effects of weather on the seasonal distribution of viral diarrhea. In Arizona, we similarly found a peak incidence in enteric virus excretion in the cooler months of the year; 50% of all virus-positive stools occurred between the months of September and December, and peak rotavirus and adenovirus excretion occurred in the month of December alone. There was also a strong association between virus excretion and precipitation. The lowest percentage of virus-positive stools occurred in the month of July, the beginning of our summer rainy season. No adenoviruses, small round viruses, and only 2% of the rotavirus-positive cases occurred at this time. Soenarto et al. (1981) found peak rates of rotavirus excretion in both dry and wet months in Indonesia, but similarly noted a decline with the onset of the wet season. Our findings in Arizona are therefore similar to other warm climate countries where temperature and humidity show minor fluctuations but where there is a definite rainy season (Sitbon, 1985; Maiya et al., 1977; Hieber et al., 1978; Black et al., 1980; Rowland and McCollum, 1977; Soenarto et al.,
1981). We did have a second peak of rotavirus excretion in spring similar to the findings of Schnagl et al. (1979) in Australia. This second peak cannot simply be explained on the basis of climatic conditions, but it may be related to declining antibody levels in the general population. It is intriguing, however, that we should experience such marked seasonal distribution of viral excretion in association with precipitation, when our highest average monthly rainfall was only 2.2 in. in July compared with a low of 0.2 in. in April and June. The precipitation during our summer rainy season usually comes daily in the form of a torrential downpour over a very short period of time and occurs after the high heat of the day. Perhaps this phenomenon can contribute to low aerosol formation (Brandt et al., 1982), which can minimize the spread of rotavirus, adenovirus, and small round viruses at this time of the year. The peak incidence of viral excretion with the onset of cooler weather may be related to crowding conditions (Brandt et al., 1982), as cooler temperatures (especially after sundown in the desert) drive people indoors (Brandt et al., 1982), and also coincides with the beginning of the school season.

The only virus group that did not show a statistically significant seasonal distribution throughout our observation period was the CVLPs. Although there was a peak incidence of CVLP excretion in September that showed a gradual decline through December, there were no months where CVLPs were not detected. There was also no marked decline in CVLP excretion in July as observed with the other viral groups. Schnagl et al. (1979) in Australia and Maass and Baumeister (1983) in Germany similarly could not define a definite seasonal pattern to the incidence of CVLP excretion. Sitbon (1985), however, found that the CVLP prevalence in diarrheic stools was highest during the dry season in Gabon, Africa. Contrary to our findings, Sitbon (1985) detected no CVLPs in diarrheic stools in the months of March and April (the middle of their long rainy season). It is possible that CVLP transmission may be different from other enteric viruses, and contact spread (Chany et al., 1982) may be more important than aerosol formation. On the other hand, the peak incidence of CVLP excretion may be obscured by the fact that CVLP can be shed up to 85 wk (Caul and Egglestone, 1982) after an episode of acute diarrhea. Further biochemical, immunologic, ultrastructural, tissue culture, and epidemiologic studies will be necessary before a clear understanding of the role of CVLPs in human gastrointestinal illness is obtained.

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