Characteristics of Noncultivable Adenoviruses Associated with Diarrhea in Infants: A New Subgroup of Human Adenoviruses

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Virus particles morphologically resembling adenovirus were found in fecal specimens from infants and were examined for cultivability with standard cell culture techniques and for characteristics of human adenoviruses. Specimens from 13 of 15 infants could not be cultivated in cell cultures. The two adenoviruses that were cultivated, types 1 and 31, reacted in the expected manner in all tests. Counterimmunoelectrophoresis with group-specific anti-hexon serum confirmed that the observed particles in the 15 specimens were human adenoviruses. The buoyant density in sucrose of five of the noncultivable adenoviruses in original stool suspensions averaged 1.335 g/cm3 and that of the two cultivable ones averaged 1.332 g/cm3; both groups had typical adenovirus morphology by electron microscopy. Treatment of the specimens and of a variety of tissue culture cells with proteolytic and other enzymes did not improve cultivability. Examination of partially purified virus by immunoelectron microscopy did not reveal evidence of immunoglobulin A, G, or M coating on the particles, an indication that coproantibody inhibition was not the cause of noncultivability. Fluorescent-antibody studies with an antihexon conjugate and counterimmunoelectrophoresis studies of serially passaged noncultivable viruses indicated that the viruses are infecting cells but are not undergoing effective replication. Antisera to three of the noncultivable viruses demonstrated homologous reactions in counterimmunoelectrophoresis with the respective immunizing antigens but showed only low levels of hemagglutination-inhibiting and neutralizing activity to a few of the known human adenoviruses. We concluded that the noncultivable viruses in these infant diarrhea cases were indeed human adenoviruses, were not defective particles, were not bound to coproantibody, were infectious but incapable of effective replication in conventional cell cultures, were serologically related to types 11, 17, 32, and 33, and should be considered a new, distinct subgroup.

The discovery of the Norwalk agent in fecal extracts (18) and rotaviruses in duodenal mucosa (4) and fecal extracts (8) has led to increased interest in electron microscopy (EM) examination of fecal specimens. A variety of viruses, including adenoviruses (AV), have been observed by EM examination of fecal extracts (3, 7, 22, 31). Despite intensive efforts, the AV we have observed in large numbers in fecal specimens usually could not be cultivated in cell cultures, a fact noted by others as well (7, 22, 31). This was surprising, since other AV are relatively easy to cultivate and type, and since observation by EM indicates a virus concentration of ≥109 particles per g of feces (7).

In recent studies of infant diarrhea in which we were looking primarily for rotavirus, we occasionally observed particles by EM that were morphologically indetical to AV but which we could not isolate in cell culture. In this study we investigated four hypotheses which might explain our failure to cultivate these viruses in conventional cell cultures: (i) the particles were not really adenoviruses; (ii) the particles were defective viruses; (iii) the particles were coated with antibody; and (iv) the particles could not replicate in conventional cell systems. This study describes our investigation into these hypotheses for our failure to recover AV from EM-positive stools from infant diarrhea cases, and describes some characteristics of these "noncultivable" AV.

MATERIALS AND METHODS

Specimens. Stool specimens were collected from patients at Atlanta hospitals and from day care centers in Atlanta during the fall and winter of 1975, 1976, and 1977. The patients were all ≤3 years of age and had diarrhea as the predominant symptom, except for patient no. 131-7 (Table 1), who served as a nonenteritis,
**Table 1. Test results of specimens positive by EM for AV-like particles**

| Stool specimen code | Virus isolation | Crude suspension and partially purified virus CIE: precipitin lines with antiserum to: | Buoyant density (g/cm³) | HEK-HEp2, passages: FA
<table>
<thead>
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<tbody>
<tr>
<td></td>
<td></td>
<td>AV hexons</td>
<td>9801</td>
<td>131-8</td>
</tr>
<tr>
<td>Enteritis cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9801</td>
<td>–</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>131-1</td>
<td>–</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>131-2</td>
<td>–</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>131-3</td>
<td>–</td>
<td>++</td>
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<td>+</td>
</tr>
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<td>131-4</td>
<td>–</td>
<td>+</td>
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<td>131-5</td>
<td>–</td>
<td>–</td>
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<td>+</td>
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<td>–</td>
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<td>3565A</td>
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<td>±</td>
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<td>131-10</td>
<td>AV-31</td>
<td>+++</td>
<td>++</td>
<td>+</td>
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<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>131-7</td>
<td>AV-1</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>AV-2 hexons</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AV-3 culture</td>
<td>AV-3</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>AV-2 culture</td>
<td>AV-2</td>
<td>+++</td>
<td>++</td>
<td>+</td>
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<tr>
<td>Negative stools</td>
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<td>–</td>
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</table>

*a* CE test visually scored for intensity of the precipitin line, from negative (−) to very heavy (+++).

*b* FA with anti-hexon conjugate; test scored for numbers of intact epithelial cells positive for nuclear or cytoplasmic fluorescence, or both: <1% of the cells present (±); 5% (+); 10% (+++); 15% (+++); 25% (1); 50% (2); 75% (3); and 100% (4).

A group of five stool specimens from infants with diarrhea in which no virus particles of any kind were seen by EM.

AV-positive control. Respiratory symptoms were not noted in any of the infants.

**Electron microscopy.** Specimens for direct EM were suspended in 4 volumes of phosphate-buffered saline (pH 7.4), centrifuged at 1,400 × g for 20 min to remove large debris, and examined by the pseudoreplica technique as described by Martin et al. (20). Specimens in which AV particles were observed were saved for further study.

**Virus purification.** Fecal suspensions that were positive for AV were taken through further steps to partial purification; 20% suspensions were clarified in a PR-2 centrifuge at 1,400 × g for 20 min, and the supernatant fluids were centrifuged at 7,000 × g for 60 min to remove bacteria. The supernatant fluids from this step were then centrifuged over 1-ml 70% (wt/vol) sucrose cushions at 149,000 × g for 60 min in an SW50.1 rotor. The pellets, suspended in 1 ml of phosphate-buffered saline per tube, were considered partially purified virus.

**Cell cultures.** Specimens consisting of both original stool extracts and concentrated, partially purified AV were inoculated onto primary human embryonic kidney (HEK), human epithelium (HEp2), human embryonic lung diploid fibroblast (MRC-5), primary rhesus monkey kidney (MK), and primary African green monkey kidney (AGMK) tissue cultures, with and without proteolytic enzymes as described below, and passaged biweekly for 12 weeks of continuous culture at 35 to 37°C. MK and MRC-5 cultures were routinely rolled; the others were stationary. Blind passaging of all cultures was done by scraping and inoculating 0.5 ml onto fresh cultures, adsorbing for 1 h at 23°C, and then adding 1 ml of appropriate maintenance medium (12).

**Enzyme treatment.** Carboxypeptidase A (bovine pancreas; Nutritional Biochemicals [NBC], Cleveland, Ohio), phospholipase C (lecitinase C, Clostridium perfringens; NBC), and pepsin (PM, hog stomach; NBC) were solubilized in Eagle minimal essential medium with 2% fetal calf serum (EMEM±FC) maintenance medium and were used in the culture tubes at final concentrations of 500 μg/ml. Trypsin-TPCK (Worthington Biochemicals Corp., Freehold, N.J.) was used at a final concentration of 5 μg/ml after the specimens were treated with a trypsin solution at 500 μg/ml, as described by Babiuk et al. (1).

**Virus identification and serology.** Cultures with cytopathic effect (CPE) were incubated 48 h beyond the point of 4+ CPE (100% of the cell monolayer...
affected) to obtain maximum titers of hemagglutinins. They were harvested by a single freeze-thaw cycle at −70°C, subgrouped by differential hemagglutination (HA) tests (11), and typed by standardized HA/he-magglutination-inhibition (HI) tests (15) with reference equine antisera (14). HI tests for AV-31 were carried out as described by Wigand and Keller (32). Identifications were confirmed by serum neutralization (SN) tests in HEK or HEp2 cell cultures.

**Counterimmunoelectrophoresis.** Counterimmunoelectrophoresis (CIE) tests were done on glass slides (25 by 75 mm) with 1% SeaKem agarose-ME (Marine Colloids, Rockland, Maine) in tris(hydroxymethyl)amino methane (Tris)-barbital-sodium barbital buffer (pH 8.8), as previously described (13). We used AV type 2 hexon antigen and mouse immune ascitic fluid to this antigen as previously described (6). Horse and rabbit antisera to AV-3 and working stock cultures of AV-2 and AV-3 came from our reference reagent collections.

**Density gradient centrifugation.** Equilibrium centrifugation was performed in linear 35 to 71% gradients of crystalline sucrose (Sigma Chemical Co., St. Louis, Mo.) in 0.01 M phosphate-buffered saline (pH 7.2). Samples of original stool suspensions (approximately 0.5 ml each) were layered onto the 11-ml gradients and banded at 110,000 × g for 18 h at 8°C in a Beckman model SW50.1 centrifuge. The gradients were harvested dropwise from the bottom of the tube, and each 0.4-ml fraction was tested for density in an Abbé refractometer and for presence of virus particles by direct EM as above. The EM data were roughly quantitated for numbers of virus particles.

**IEM.** Immunoelectron microscopy (IEM) was carried out by mixing 0.8 ml of partially purified AV with 0.2 ml of dilutions of high-potency goat anti-immunoglobulin serum (courtesy of Charles Reimer, Center for Disease Control, Atlanta, Ga.), incubating at 4°C overnight, and pelleting at 50,000 × g for 90 min in an SW50.1 rotator. The supernatant fluids were discarded, and the pellets were suspended in 0.2 ml of phosphate-buffered saline for examination by the EM pseudoraplica technique.

**FA tests.** Direct fluorescent-antibody (FA) tests were performed on HEp2 microcultures as previously described (19). Inoculum for these cultures consisted of the HEK1, HEK2, and HEK3 passages of the stool suspensions and of the partially purified virusses; the cultures were harvested by two freeze-thaw cycles (−70°C) after 12 to 14 days of incubation at 35°C under regular maintenance medium and then were clarified by centrifugation at 1,000 × g for 10 min. Methylene blue wet mounts of the prepared inocula confirmed that no HEK cell debris was added to the HEp2 microcultures. The microcultures were read for CPE every other day and were tested for FA after 4 and 7 days of incubation at 35.5°C under 5% CO2 and 65% humidity. The slides were washed, fixed, again washed, and stained with goat anti-hexon fluorescein isothiocyanate conjugate (fluorescein/protein ratio, 2:7; Microbiological Associates, Bethesda, Md.) at a 1:40 dilution. Appropriate controls were included throughout.

**Antiserum production.** Antisera to three of the partially purified virusses were prepared in young adult New Zealand white rabbits. Virus was mixed with 2 parts of Freund incomplete adjuvant, and each rabbit was inoculated subcutaneously in the flank with 0.8 ml of the mixture at days 0 and 14; rabbits were bled at day 28. These sera were heat inactivated (56°C, 30 min) for use in all CIE, HI, and SN tests.

**RESULTS**

We examined fecal specimens from 192 infants during the acute phase of illness, and observed AV in 14 stools and rotavirus in 16. These 14 stool specimens and 1 stool specimen from a child not ill at the time of collection, all of which had particles morphologically identical to AV, constituted the subject of this study (Table 1). The 15 AV-positive specimens were negative by standard cultures for enteropathogenic bacteria and for enteroviruses. All original stool specimens that were positive by EM were also positive by CIE with AV-2 anti-hexon mouse immune ascitic fluid and with horse and rabbit antisera to AV-3, the latter being used for confirmation of the AV anti-hexon reactions (Table 1). However, only two of these specimens produced CPE in any cell culture system in 12 weeks of continuous culturing, with or without trypsin pretreatment or the presence of selected enzymes in the culture fluids. These two produced typical AV CPE in the first passage of several cultures and were readily identified by routine HI and SN tests as AV-1 and AV-31. The pretreatment of the specimens with trypsin and the presence of enzymes in the maintenance media had no obvious effect on the isolation of the AV-1 and AV-31, but both were isolated sooner from the respective partially purified preparations than from the original stool suspensions. The other 13 original specimens, their respective partially purified preparations, and the AV-negative stool controls remained negative for CPE in all systems throughout the culture period.

The buoyant densities of five of the noncultivable AV and of the two cultivated ones were determined as a test for incomplete virus particles (Table 1). The buoyant density in sucrose ranged from 1.329 to 1.339 g/cm³ for the AV in all seven stool suspensions, as monitored by EM, with no discernible difference in density between the noncultivable and cultivable strains. The morphology of the particles at their density peak again appeared to be typical of AV.

The failure of certain enzymes, in particular trypsin, to enable us to isolate the noncultivable AV suggested that virus-bound antibody was not responsible for the cultivation problem. We then sought additional evidence against the virus-bound antibody hypothesis by IEM tests. IEM was set up with partially purified virus and serial
dilutions of highly class-specific human immunoglobulin A, G, and M (IgA, IgG, and IgM) goat antisera. We found no evidence that IgA, IgG, or IgM antibodies were present in any of the mixtures containing the noncultivable adenoviruses, although we observed some clumping of virus particles without indication of antibody coating. By contrast, the “positive control” preparations of the AV-1 specimen and AV-1 equine antiserum demonstrated both clumping and antibody coating (Fig. 1).

The first, second, and third HEK passages in regular maintenance medium of the 13 noncultivable specimens (both original suspensions and partially purified viruses) were tested by CIE and FA for evidence of viral replication in the absence of apparent CPE. As in the original suspensions tested directly by CIE, all 13 had positive CIE reactions in the HEK cultures, and most of them were positive in the HEK2 cultures. However, none was positive in the HEK3 cultures. This suggested that viral replication was not occurring and that the hexon antigen was simply being diluted out in the serial passages. The AV-negative stool controls were again negative throughout these tests.

By FA, most HEK1 and HEK2 passages of both the original suspensions and the partially purified viruses were positive at days 4 and 7 of incubation in HEp2 cells (Table 1); most HEK3 passages were negative. None was positive for CPE before being stained, except 131-7 (AV-1), 131-10 (AV-31), and the AV-2 and AV-3 controls. In the FA-positive cultures, including those of the AV-1 and AV-31 isolates, there was solid fluorescence in the nuclei and usually staining of aggregates in the cytoplasm (Fig. 2). An “inoculum dilution” effect was apparent here also. Whereas the positive control cultures (AV-1, -2, -3, and -31) gave 2+ to 4+ staining at 4 days, and little of the cell monolayer was left at 7 days, showing all cells infected and replication occurring, the noncultivable AV showed only 1+ staining or less in the HEK1 and only a few cells staining in the HEK2 passages. In contrast to the cultivable AV, the FA staining in the noncultivable AV progressed only slightly between day 4 and day 7. The AV-negative stools consistently showed only background staining.

We next attempted to identify the noncultivable strains in a converse manner by HI and SN tests with all 35 prototype human adenoviruses versus rabbit antisera to three of the noncultivable strains. The antisera were initially shown to contain homologous antibody by CIE tests with their respective immunizing antigens. They were also shown to possess anti-hexon antibody by CIE tests with all 15 original stool suspensions, their respective partially purified preparations, and the hexon and AV-2 and AV-3 positive controls (Table 1). By HI tests, low levels of antibody (1:8 to 1:16) were detected in one or more sera with AV types 17, 32, and 33, and similar levels of antibody were found by SN tests with types 11, 17, 32, and 33 (Table 2). Considering the strength of the homologous CIE reactions, we concluded that these low levels of antibody do not indicate identification but probably represent one-way (at least) cross-reactions between the noncultivable strains and these four prototype AV.

DISCUSSION

The association of AV with infant diarrhea has been noted for a number of years (16, 17, 21, 23, 26; J. R. Fraga, J. C. Hierholzer, A. T. Lagoc, and S. M. Sinkford, Am. J. Dis. Child., in press; P. A. Jacobsson, M. E. Johansson, and G. Wad-

![Fig. 1. Electron micrographs of IEM preparations. Stained with uranyl acetate. Bars represent 100 nm. (A) AV type 1 isolate (131-7) incubated with type 1 reference equine antiserum. Antibody coating resulted in particle aggregation and grainy appearance of particles. (B) Specimen 131-12 incubated with goat anti-IgG serum. Although the particles are clumped, there is no evidence of antibody coating on the particles.]
Fig. 2. Microcultures of HEp2 cells inoculated with the clarified HEK₂ passage of original AV-positive stools and stained with anti-hexon fluorescein isothiocyanate conjugate after 4 or 7 days of incubation. (A) Specimen 9801 at 7 days; (B) 131-6 at 4 days; (C) 131-10 (AV-31) at 4 days; and (D) 131-10 at 7 days. Similar cultures of HEp2 cells inoculated with clarified HEK₂ passages of partially purified AV from stools and stained with anti-hexon conjugate at 4 days: (E) 131-12; and (F) 131-13. Magnification, ×216.

ell, J. Med. Virol., in press; H. Lennartz, G. Maas, and D. E. Sarateanu, Proc. 4th Int. Congr. Virol., p. 455, 1978). Tyrrell et al., who studied AV infections in the mid-1950s (29), believed that the nausea, vomiting, and diarrhea noted in some patients they studied was due to the AV infection rather than to the cough and antibiotics as previously described (5). Joncas and Pavilanas (17) noted that more than half of the viruses they isolated from diarrheic children in 1958 through 1959 were AV; association with illness was clouded, however, by a similar finding in a non-ill control group. They also found that they were not able to isolate AV from rectal swabs from five of seven children who had a fourfold or greater serological rise to AV antigen by complement fixation.

Moffet et al. (23) found a significant difference in the numbers of AV isolated from infants with diarrhea and from a control group; AV was recovered from 17% of infants with diarrhea but from only 5% of normal infants of the same age...
TABLE 2. HI and neutralization titer of rabbit antisera to three noncultivable AV tested against the prototype human AV

<table>
<thead>
<tr>
<th>AV type</th>
<th>HI titer of antisera in CPE</th>
<th>SN titer of antiserum in CPE</th>
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<tbody>
<tr>
<td>9801</td>
<td>131-8</td>
<td>131-9</td>
</tr>
<tr>
<td>1-10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
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<tr>
<td>12-16</td>
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<td>17</td>
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<td>18-31</td>
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<td>32</td>
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<td>33</td>
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<tr>
<td>34,35</td>
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<td>0</td>
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</table>

* HI titer is listed as the dilution factor of the highest titer of serum completely inhibiting HA by 4 HA units of virus per 0.025 ml in 1 h at 37°C (15).
* SN titer in MK cells is listed as the dilution factor of the highest dilution of serum causing a 2+ reduction in CPE from that observed in the working dilution (virus control) in 3 or 4 days at 35°C (14).

0 indicates titer of <8.

group admitted for elective surgery and without respiratory or gastrointestinal symptoms within a week of surgery. These authors suggested that the increased frequency of AV recovery may have been a result of the diarrhea rather than a cause, perhaps because of increased shedding of latent AV in infants with diarrhea or because of the increased inoculum size from a Watery stool. They found nine different AV types and one untyped virus; types 1 and 2 predominated.

Flewett et al. (8) described an outbreak of diarrhea involving a nurse and 6 of 19 children on the same hospital ward. AV-like particles were observed by EM of feces from four of the six children and from the nurse, but not from any of the other 13 children. Despite all efforts, they were not able to recover a virus in cell cultures of specimens taken during the outbreak. IEM results suggested that this virus was AV type 7—a type usually isolated with ease. More recently, Whitelaw et al. (31) have described two cases in one family of diarrhea and vomiting lasting 30 h and resulting in the death of one patient. AV particles were visualized within the nucleus of a mucosal cell and in scrapings of the mucosa from the patient who died. AV was reportedly isolated in MK cells from gut homogenate, but the AV type was not indicated. In the surviving patient, AV particles were visualized in stools but could not be recovered in tissue culture.

In our laboratory, many different AV serotypes have been isolated over the years from 0- to 3-year-old children with diagnoses of diarrhea, gastrointestinal illness, or gastroenteritis. These isolates, all from stool specimens, represent approximately 4% of all AV isolations made during this time regardless of site of infection or age of patient. More than half were from infants without described respiratory or generalized symptoms other than fever, and in many cases we documented seroconversion to the AV type isolated. No other virus or enteropathogenic bacterium was isolated or detected by serology in any of the patients. The isolates obtained from children from whom serum specimens were not available cannot be proven to be associated with gastrointestinal symptoms; however, because AV are shed in young children for 1 to 3 months after the initial upper respiratory or gastrointestinal infection and intermittently for even longer periods (9).

The increased study of fecal specimens by EM in recent years has led to the observation that, although particles of obvious AV morphology are seen in some fecal specimens, sometimes in concentrations of ≥10^8 particles per g of feces, they frequently cannot be cultured in vivo (7, 22, 31; Jacobsson et al., in press; G. Wadell, M. L. Hammerskjöld, G. Winberg, P. A. Jacobsson, M. Johansson, and T. Hovi, Proc. 4th Int. Congr. Virol., p. 471, 1978). As opposed to the considerable data on AV isolations in general in cell culture, there are few data relating EM observation to success of isolation. We have, however, always isolated AV from eye and lung specimens when the particles had been seen by EM. From these experiences we would expect to recover the fecal AV as well. But such was not the case, and hence in this study we investigated the possible reasons for our failure to isolate these viruses in cell culture.

We tested four main hypotheses concerning the noncultivability of these viruses. The first hypothesis was that the particles seen by EM resembled AV by gross external morphology but were in fact not members of the AV group. The CIE test with anti-hexon antisera readily confirmed that these uncultivable viruses were human AV. The anti-hexon sera react only with mammalian AV hexons (of any type), because these sera are AV group specific as in complement fixation tests (6). CIE was decidedly the more practical test in this case, because stool suspensions and concentrates containing sucrose are often anti-complementary in complement fixation tests, and CIE tests consume only 5 μl of sample per well. FA tests performed with an anti-hexon conjugate supported the CIE data entirely.

The second hypothesis was that the particles were AV but were defective. But defective AV particles and infectious virions have different
morphological appearances by EM, and the defective particles have significantly lower buoyant densities (10, 24, 28). All of the AV we studied appeared typical by EM examination and had densities in sucrose of around 1.33 g/cm³.

The third hypothesis was that the particles were intact AV but were rendered noninfectious by coproantibody coating. Watanabe et al. (30) found antibodies of the three major classes in feces of infants and young children with rotavirus infection. They believed that many of these antibodies had been damaged or degraded by intestinal enzymes, resulting in low avidity. However, they also thought that secretory IgA whose avidity was more stable in the intestine might bind firmly to the rotavirus particle, covering the virus and assisting in its elimination. Our IEM results indicate that the AV particles were not coated by IgA, IgG, or IgM. Indeed, evidence from the many instances in which AV have been isolated from stools seems to preclude the probability that certain AV are inhibited to a greater degree than others. Our study and that of Watanabe et al. (30) are not completely comparable, however, because they involved different viruses, and because IEM and radioimmunoassay have different levels of sensitivity.

Previous reports indicating that trypsin enhances infectivity of reoviruses (27) and rotaviruses (1) have prompted us to examine certain enzymes as infectivity enhancers. Carboxypeptidase A, phospholipase C, pepsin, and trypsin did not effect the isolation of the previously noncultivable AV, nor did these enzymes influence in any way the culture of the two AV isolated in this study. These enzymes are apparently not capable of altering the virus or the cells sufficiently to allow isolation of the noncultivable AV. By contrast, Spendlove and Schaffer (27) and Babiuk et al. (1) studied viruses previously shown to be recoverable in cell cultures, even though Babiuk et al. found that trypsin treatment made the difference between isolation and nonisolation of calf rotavirus in their study (1).

The fourth hypothesis was that the particles were intact infectious AV but were incapable of productive infection in the tissue culture systems we employed. FA and CIE results both demonstrated a physical dilution effect of the starting inoculum in serial tissue culture passages, resulting in decreasing positivity from the first through the third passage. Although the degree of positivity by FA was lower in the noncultivable AV than in the cultivable ones, the FA staining in both groups was typical of AV in epithelial cells (2, 19, 25) and showed that the virus was taken into the cells, was degraded, and migrated to the nucleus but did not replicate effectively. We feel, for reasons stated above, that this "nonproductive infection" is not in any way related to defective or defective-interfering particles. Thus the fourth hypothesis appears to frame the problem of noncultivability of these EM-positive stools.

Whether the viral genome is insufficient for replication in the cells used or whether the cells are unsuitable for replication of the virus is not known. However, the methods we used are effective if not ideal for propagating all previously known human AV. We believe that these data are consistent with the suggestion of Wadell et al. (Proc. 4th Int. Congr. Virol., p. 471, 1978) that these noncultivable viruses are distinct from the other human AV subgroups. Evidence for a causal relationship with gastroenteritis is increasing but remains inconclusive at the present time.

LITERATURE CITED


