Biophysical Properties of a Non-cultivable 29-nm Enteric Virus

(Accepted 23 April 1979)

SUMMARY

A 29 nm non-cultivable virus (NCV) was detected in faecal extracts from children hospitalized for gastroenteritis. The NCV had a density of 1.35 g/ml in glycerol-potassium tartrate density gradients and was resistant to degradation by proteolytic enzymes, non-ionic detergents and pH extremes. The surface of these virus particles had knob-like projections which appeared to have a symmetrical arrangement. When heated to 56 °C, the virus was completely degraded to soluble components which could not be seen by electron microscopy.

Cameron et al. (1978a) recently described a survey of non-cultivable viruses (NCV) present in faecal extracts from babies in a newborn nursery in Australia. One such isolate was described as an isometric virus-like particle 28 nm in diam. which morphologically resembled both the Norwalk agent (Kapikian et al. 1972) and particles which have been termed ‘astrovirus’ (Madeley & Cosgrove, 1975). Morphologically similar 29- to 30-nm particles of uncertain pathogenicity were also found in faecal extracts from children in Venezuela (Esparza et al. 1977).

The 28 nm NCV was shed both by newborn babies who had and those who did not have diarrhoea and was sometimes shed by babies who simultaneously excreted rotavirus. This NCV was not conclusively shown to be an enteric pathogen. Although both of these viruses were similar to astrovirus in size and general appearance, neither group of workers reported finding particles with the central star-shaped surface configuration characteristic of ‘astrovirus’ (Madeley & Cosgrove, 1975), although Cameron et al. (1978b) suggested that being able to visualize such structures by electron microscopy may be dependent on staining conditions. The NCV also does not appear to be morphologically the same as minireovirus (Middleton et al. 1977), calicivirus (Madeley & Cogsrove, 1976), or minirotavirus (Spratt et al. 1978).

During a study of nosocomial rotavirus infection in the winter of 1976 in Atlanta, Georgia (Ryder et al. 1977), we detected a virus markedly similar to particles described by Cameron et al. (1978b) and Esparza et al. (1977) in faecal material from six children less than 2 years old, hospitalized for gastroenteritis. The virus was detected by direct electron microscopic examination of stool extracts. One child was also shedding rotavirus. The NCV appears to be a novel agent of the enteric tract which differs from the Norwalk agent and the astrovirus.

NCV was separated from Genetron-extracted faecal extracts by density gradient centrifugation in glycerol-potassium tartrate density gradients. We used the procedure that Martin et al. (1975) described for separating rotavirus. When Genetron-extracted faecal extracts containing both NCV and rotavirus were centrifuged to equilibrium, both viruses banded at a density of 1.35 g/ml (Fig. 1a). We were unable to separate NCV from rotavirus completely in these gradients or by centrifuging the banded viruses to equilibrium in linear CsCl gradients over the range of 1.1 to 1.5 g/ml. Velocity gradient separation of NCV from rotavirus also proved difficult because, as Cameron et al. (1978b) observed, the virus tended to aggregate, frequently in large rafts. These aggregates sedimented concurrently with rotavirus aggregates of similar size in sucrose gradients.
Fig. 1. (a) Rotaviruses and NCV following centrifugation of faecal extracts containing both viruses to equilibrium in glycerol-potassium tartrate gradients. Both viruses banded at a density of 1.35 g/ml. Virions in all parts of the figure were negatively stained with 0.5 % aqueous uranyl acetate. 

(b) Norwalk-like agent as it appears in Genetron-extracted faecal extracts. (c) Non-cultivable virus purified by density gradient centrifugation in glycerol-potassium tartrate gradients. Arrowheads point to knob-like, electron-translucent projections. Long arrow points to an ‘empty’ NCV particle. 

(d) High magnification composite of NCV showing knob-like surface projections (arrows) which appear to have a symmetrical arrangement.
Table 1. Stability of a non-cultivable enteric virus

<table>
<thead>
<tr>
<th>Reagent or treatment</th>
<th>Characteristic</th>
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<tr>
<td>EDTA trypsin (0.25 % Difco 250)</td>
<td>Stable</td>
</tr>
<tr>
<td>Chymotrypsin (200 μg/ml)</td>
<td>Stable</td>
</tr>
<tr>
<td>Protease VI (200 μg/ml)</td>
<td>Stable</td>
</tr>
<tr>
<td>Papain (100 μg/ml)</td>
<td>Stable</td>
</tr>
<tr>
<td>Deoxycholate (1 %)</td>
<td>Stable</td>
</tr>
<tr>
<td>Genesolv-D (genetron)</td>
<td>Stable</td>
</tr>
<tr>
<td>Nonidet P-40 (2 %)</td>
<td>Stable</td>
</tr>
<tr>
<td>pH 10.5</td>
<td>Stable</td>
</tr>
<tr>
<td>pH 3.5</td>
<td>Labile: completely</td>
</tr>
<tr>
<td>High salt concentration (50 % potassium tartrate)</td>
<td>Labile: completely</td>
</tr>
<tr>
<td>Storage at −20 or −70 °C for over a year</td>
<td>Stable</td>
</tr>
<tr>
<td>Storage at 4 °C for 1 month</td>
<td>Stable</td>
</tr>
<tr>
<td>Heat 56 °C, 30 min</td>
<td>Labile: completely</td>
</tr>
<tr>
<td>Heat 56 °C, 30 min in 1 M-MgCl₂</td>
<td>degraded to soluble components</td>
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NCV was prepared for electron microscopy by various methods, but among thousands of particles examined none was found that resembled astrovirus, except in size and general symmetry. Stains included uranyl acetate, phosphotungstate and silicotungstate. Grids were prepared by pseudoreplication or by the drop method of touching carbon or Formvar-coated grids to a drop of virus suspension and staining with each of the stains.

NCV and the Norwalk agent were not morphologically identical when prepared for electron microscopy by the same procedures. Both viruses have surfaces which appear to consist of organized structures suggestive of capsomeric arrangement but NCV has more pronounced electron-translucent knob-like projections (Fig. 1b and c, arrowheads). At higher magnification the knob-like projections are more clearly seen (Fig. 1d) and appear to have a symmetrical arrangement. This morphology has not been described for astrovirus. Empty NCV particles have an isometric structure with an electron-dense centre surrounded by an electron-translucent membrane (Fig. 1c, arrow). The average diam. of 500 particles was 29.1 nm. Thus, NCV is slightly larger than the 27-nm Norwalk agent. The NCV more closely resembles the 29- to 30-nm particles described by Esparza et al. (1977) and also has most of the characteristics in the 28-nm particles described by Cameron et al. (1978b). The difference in size may be explained by experimental conditions and the difficulty in determining true margins of these small viruses. Even when catalase crystals (Luftig, 1967) were used as an internal calibration standard, measurements of NCV particles varied by at least 1 nm. However, antigenic relatedness of NCV and the above viruses could not be established because these viruses were not available to us during this study.

The stability of the morphology of NCV to enzymic, chemical and physical stress was also determined. Virions were stable to enzymic degradation after 2 h at 37 °C, to degradation by lipid solvents, to long-term storage at low temperature, to high salt concentration and to pH extremes. Virus was stable to overnight dialysis against pH 3 and pH 10.5 glycine buffer. However, virions were very labile to heat. They were completely degraded to soluble products which could not be visualized by electron microscopy after a few minutes at 56 °C. Heat lability at 56 °C for 30 min was not stabilized by 1 M-MgCl₂. Results of these tests are shown in Table 1.

Density gradient banded virus was passaged in Vero, BHK 21, human embryonic lung fibroblasts and HEp-2 cells. Each was inoculated with about 50 particles per cell. Virus was adsorbed at 37 °C for 1 h and the cultures then covered with Eagle's minimum essential medium supplemented with 2% calf serum. After 24 h, fresh medium was substituted for the original. Inoculated cells were incubated at 37 °C and examined daily for cytopathic
effect (c.p.e.). The culture fluid was examined daily for virus by electron microscopy. No c.p.e. developed and no virus was observed in supernatant fluids or in fluids from cells scraped from inoculated tubes and lysed by freeze-thaw. Blind passage in most of the cells was also attempted, but inoculated cells failed to show evidence of virus replication. Crude stool filtrates were passaged in human rhabdomyosarcoma cells, primary monkey kidney cells, human embryonic lung fibroblasts and suckling mice. No virus was detected in any of the systems. In addition, no antibody to NCV was detected by immune electron microscopy (IEM) in sera from randomly selected rabbits, guinea pigs, horses, dogs, cats, rats and mice from the Center for Disease Control animal facility.

Antibody to NCV was detected in randomly selected sera of 10 human adults and 12 children by IEM. The presence of antibody to the virus was also shown in delipidized colostrum from six human mothers by IEM. All six colostrum samples tested had anti-NCV antibody, which supports the observation of Cameron et al. (1978a) that breast-fed babies which they studied in their nursery in Australia excreted a similar virus much less frequently than did infants fed with artificial formulae. It is pertinent to add here that there is some difficulty in determining the presence of antibody to NCV by IEM because the virus has a tendency to aggregate even in the absence of antiserum. However, the use of preparations containing large numbers of virus particles (about 10^9/ml) allowed us to document the presence of aggregates of thousands of particles not seen in control preparations.

With several paired human sera obtained from patients in outbreaks of gastroenteritis in the United States, we tested for seroconversion to NCV by IEM; none was detected. Further, a pair of sera with titres of 100 and 10,000 to the Norwalk agent by radioimmune assay both had low stable titres to NCV by IEM. Therefore we could not conclusively link the virus with disease even though five of the six children who excreted the virus had gastroenteritis of unknown aetiology. Nevertheless, NCV appears ubiquitous, being shed by children, and antibody to the virus appears widespread in human adults. Whether NCV is a common commensal of the human enteric tract or plays a role in enteric disease of humans is not yet clear.

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REFERENCES
Short communications


(Received 6 December 1978)