

as the method does not reveal the serotype. The accompanying paper discusses the value of the method for infection by reovirus-like viruses.

Electron microscopy of faeces will not be widely

applicable as a diagnostic tool until antisera of known specificity are available. Without these small isometric particles all look alike and cannot be distinguished from each other.

II Acute gastroenteritis associated with reovirus-like particles

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SYNOPSIS Virus particles resembling reoviruses or orbiviruses were found in the faeces of 40 of 73 patients under 6 years of age with acute gastroenteritis and in faeces of only two babies among 31 patients under 6 years admitted to hospital with other diagnoses. In morphology the particles resemble orbiviruses more closely than reoviruses, but differ in appearance from the orbiviruses in having a smooth, circular outline with a well marked continuous rim as seen in negatively stained preparations. They appear not to be serologically related to reovirus types 1, 2, or 3 and may be members of a new group.

Acute infectious gastroenteritis of young children is sometimes clearly associated with a bacterial pathogen, either a 'type-specific' strain of *Escherichia coli* or one of the non-lactose fermenters. From most patients, however, no pathogen can be isolated. It is generally presumed that a virus or viruses are responsible, but although viruses of various kinds have occasionally been isolated evidence of a specific viral pathogen has usually been lacking.

We have used the technique of electron microscopy of faeces described in part I (Flewett, Bryden, and Davies, 1974a) to investigate patients with acute gastroenteritis occurring during the last 10 months.

Patients

Seventy-three patients with acute gastroenteritis and 31 with other conditions, all under 6 yr of age, have been studied. Fifty-nine gastroenteritis patients over 6 years of age and 82 other patients were also examined. The patients not suffering from gastroenteritis were admitted with a wide variety of diagnoses, mostly with febrile illnesses—respiratory tract infections, meningitis, hepatitis, etc. All these had been admitted to hospital, most of them to the com-

municable diseases unit of the East Birmingham Hospital under the care of Drs M. E. Barton, E. Carr-Saunders, R. Fothergill, A. M. Geddes, E. E. Hill, or Professor H. V. Morgan. The gastroenteritis patients were of various ages (fig 1). Their illnesses in general consisted of diarrhoea of acute onset, usually with vomiting and fever, sometimes up to 39.5°C (103°F) in the infants and younger children. The duration of the illness was usually short: most children were sent home after seven to 10 days in hospital. About one quarter were admitted in a severely dehydrated state (25% or greater dehydration) and required emergency fluid replacement by intravenous drip.

Methods

Virus suspensions from faeces were prepared for electron microscopy as described by Flewett *et al* (1974a). For immunoelectron microscopy, the virus suspensions were resuspended in 1.5 ml phosphate-buffered saline (PBS) pH 7.2. One drop of serum at various dilutions was added to 0.5 ml of the resuspended virus. After standing one to two hr at room temperature and 4° overnight these suspensions were brought to 5 ml with PBS and were centri-

fuged at 50 000 rev/min for 30 minutes. The deposits were negatively stained for electron microscopy. Formvar membranes, though less stable, provided cleaner preparations than carbon membranes made hydrophilic by ionic discharge; every sort of fine particle of macromolecular dimension appeared to become attached firmly to the carbon, whereas most of this kind of fine detritus was washed off the formvar.

TISSUE CULTURE

Tissue cultures from rhesus monkey kidney cell suspensions were purchased from Flow Laboratories Ltd, Irvine, Ayrshire, and cultures were also set up from trypsinized human embryo kidneys and lungs. Mouse L cells were kindly provided by Professor Henry Harris, FRS, and a thymidine-kinase-deficient line of L cells was kindly provided by Professor D. G. Harnden. These cells were all propagated in Eagles MEM medium (Hanks' base) containing 10% foetal calf serum. Maintenance medium was Eagles MEM (Earle's base) without serum for monkey kidney cells, 2% foetal calf serum for human embryonic lung and kidney, and 10% foetal calf serum for L cells.

Reovirus types 1, 2, and 3 and monkey antisera for each type were kindly supplied by Dr Marguerite Pereira.

VIRUS ISOLATION

One drop vol of virus suspensions, concentrated as for electron microscopy, was inoculated into the tissue cultures listed above and also into suckling mice; some cultures were inoculated with 0.2 ml vol of supernatant fluids, diluted 1:10, after the first (clarifying) centrifugation.

Results

In faeces from 40 out of 73 patients under 6 yr with acute gastroenteritis, reovirus-like particles were found. In four of these, adenoviruses were also seen. Reovirus-like particles were found in only one patient over 6 yr of age (a man aged 20) with gastroenteritis. Small particles, of parvovirus or enterovirus size, were found in 30% of all faeces examined (Flewett *et al*, 1974a). Antisera were not used to identify such particles. They may well have been bacteriophages, and their prevalence did not differ significantly between the two groups of patients. These reovirus-like particles were found in faeces taken up to nine days after the onset of diarrhoea.

The distribution of the reovirus-like particles among gastroenteritis and other patients is illustrated in fig 1 and table I. The incidence during different months is illustrated in table II. We do not believe

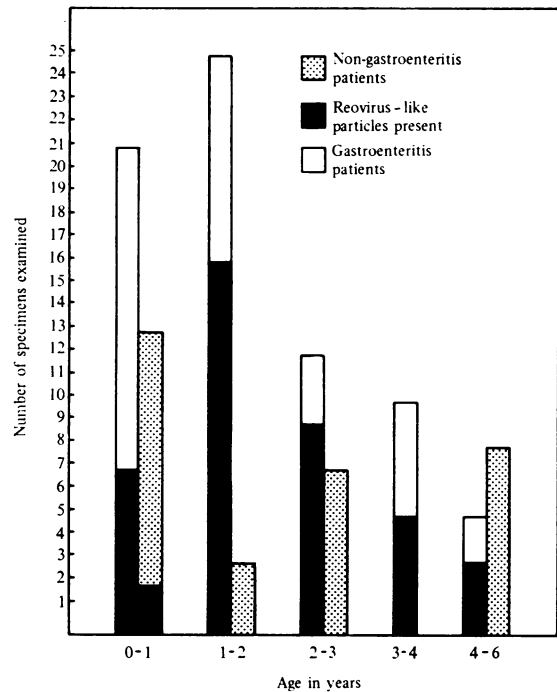


Fig 1 Left-hand columns: gastroenteritis patients. Right-hand columns: patients with other diseases. Black: patients whose faeces contained reovirus-like particles.

	Number Tested	Number Positive
Gastroenteritis < 6 yr	73	40
Gastroenteritis > 6 yr	59	1 ¹
Non-gastric < 6 yr	31	2 ²
Non-gastric > 6 yr	82	0

Table I Distribution of reovirus-like particles among patients with gastroenteritis and with other conditions

¹Man aged 20.

²One-month-old baby with 'unsatisfactory development'.

³One-month-old baby with Hirschsprung's disease. Green faeces were present, but gastroenteritis was not diagnosed clinically.

that the changes in incidence reflect an increasing expertise in detecting them because they were often present in great numbers (fig 2) so that they could hardly have been missed and were always looked for specifically after being first discovered. Furthermore, observer bias was eliminated as far as possible; deposits were prepared by A.S.B. and marked by daybook numbers only before being passed to the microscopist (H.D.), who thus did not know whether the faeces came from gastroenteritis patients or others.

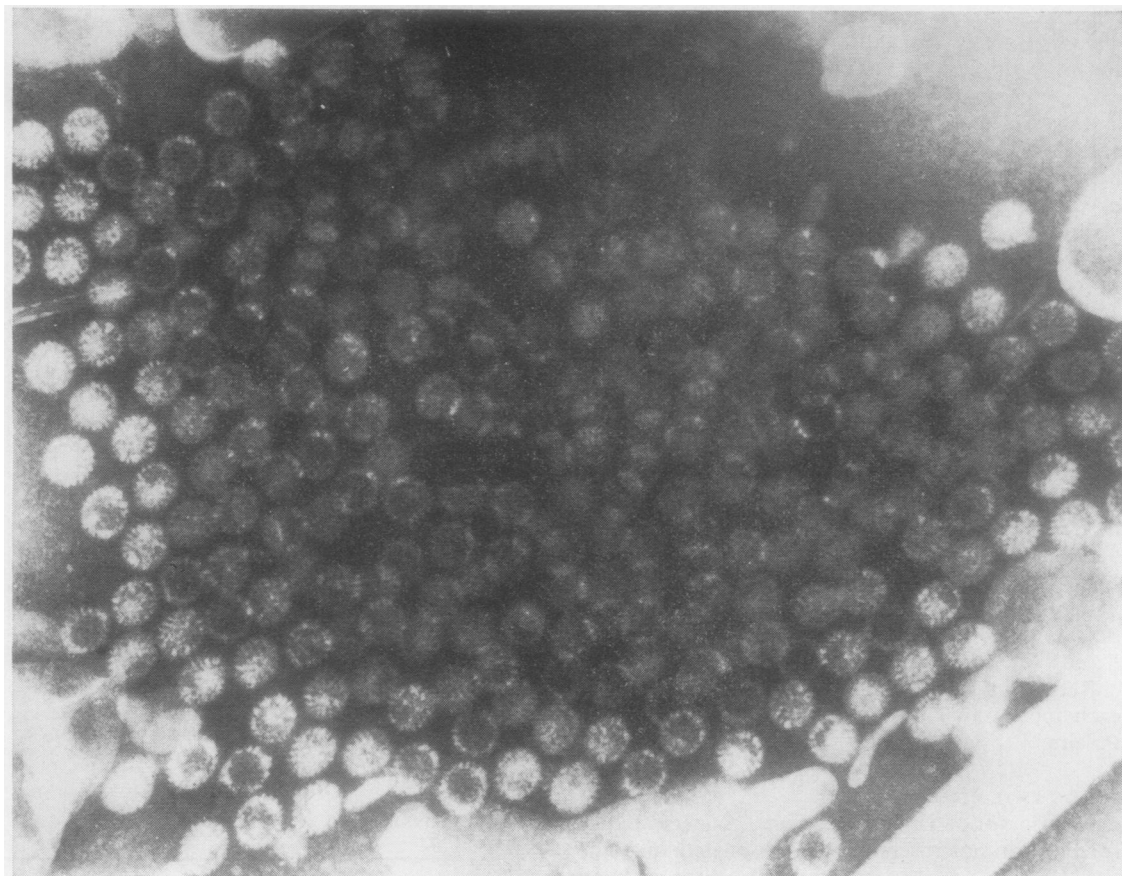


Fig 2 A group of reovirus-like particles, very numerous in this sample of faeces. $\times 119\ 000$.

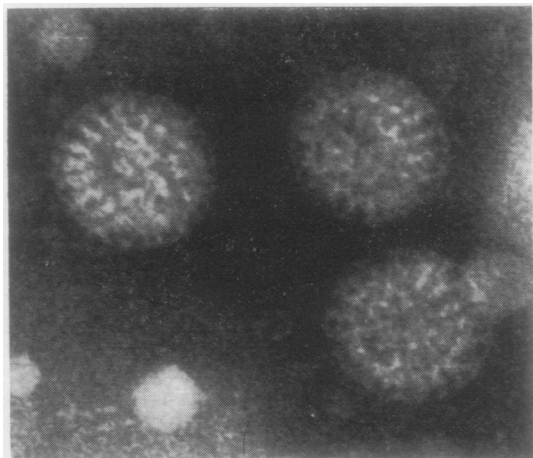


Fig 3

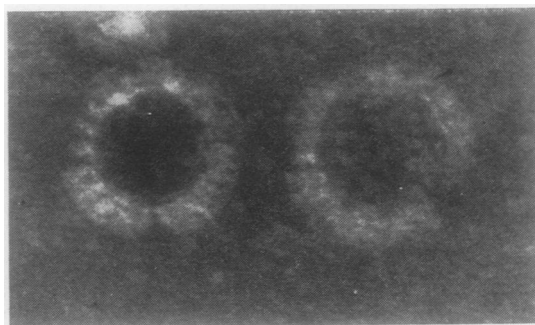


Fig 4

Fig 3 Reovirus-like particles. The rim formed by the outer layer of the capsid is smooth and well contrasted $\times 360\ 000$.

Fig 4 Two particles, both penetrated by negative stain; part of the outer layer of the capsid has become detached. $\times 360\ 000$.

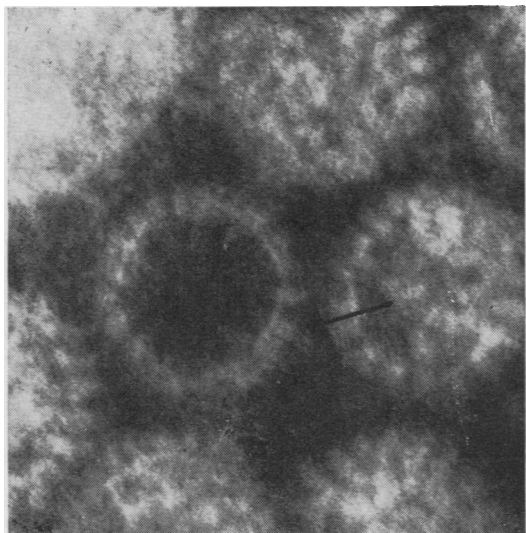


Fig 5 The outer capsid layer has been lost. The inner layer of capsomeres can be seen attached to a thin membrane surrounding a central space. One capsomere presents the appearance of a hollow cylinder (arrow). $\times 488\ 000$.

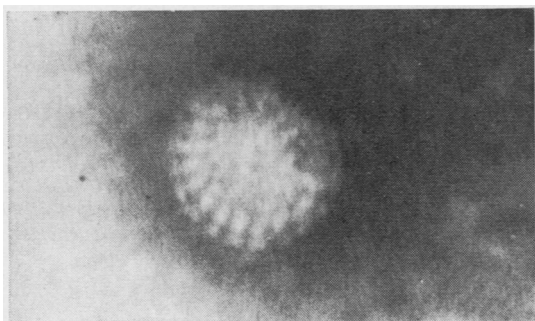


Fig 6

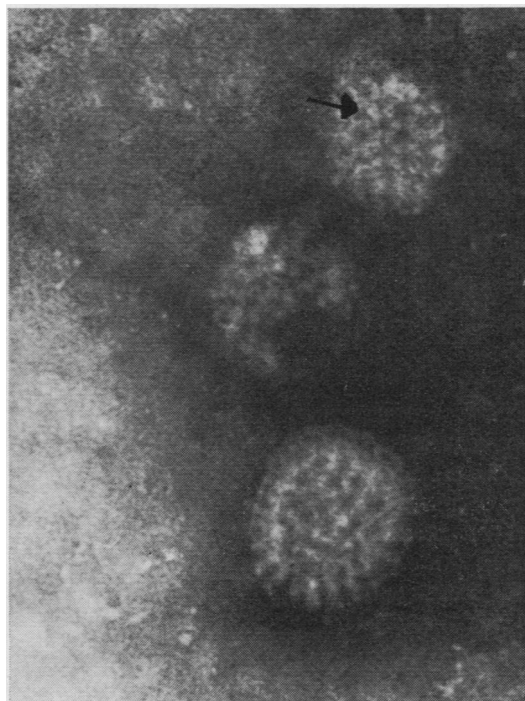


Fig 7

Fig 6 A virion distorted by surface tension near the edge of a droplet of negative stain. The subunits of the outer layer are placed directly above those of the inner layer, like the cross-pieces upon capital Ts. $\times 360\ 000$.

Fig 7 A group of detached capsomeric subunits. The circles are here resolved into subunits; the arrow indicates a group of six. They could not be thus resolved in the intact virion. $\times 360\ 000$.

MORPHOLOGY OF THE REOVIRUS-LIKE PARTICLES

These were found in two forms, one in which the two layers of the capsid were complete, in diameter 61-64 nm (fig 3) and the other 50-54 nm in diameter, from which the outer layer of the capsid appeared to be missing (fig 5). Both types of particles were frequently seen together in the same preparation. Occasionally, particles were seen from which only part of the outer capsid layer had become detached (fig 4). Some particles were penetrated by the negative stain; in these it appeared that the inner capsid subunits were short, narrow, hollow, parallel-sided units attached at their inner end to a thin

membrane enclosing a central space about 38 nm in diameter. These subunits were about 5 nm long, 4 nm wide, with a hole about 1 nm wide down the centre (fig 5). The outer capsid subunits appeared to be attached to the ends of these (fig 6). A detached group of these is seen, end-on, in figure 7. The outer surface of the complete double-layered virions was circular in outline, unlike reoviruses types 1, 2, and 3 from tissue culture, whose outlines are distinctly icosahedral. Also, unlike the reoviruses, the outer capsid subunits appeared to be continuous at the periphery of the virions, giving the impression of a continuous membrane surrounding the virus. The particles were easily deformed into an oval shape

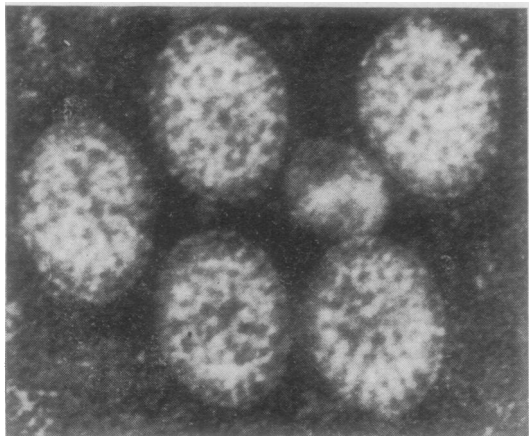


Fig 8 A group of virus particles distorted by stresses probably due to uneven drying of negative stain. $\times 300\ 000$.

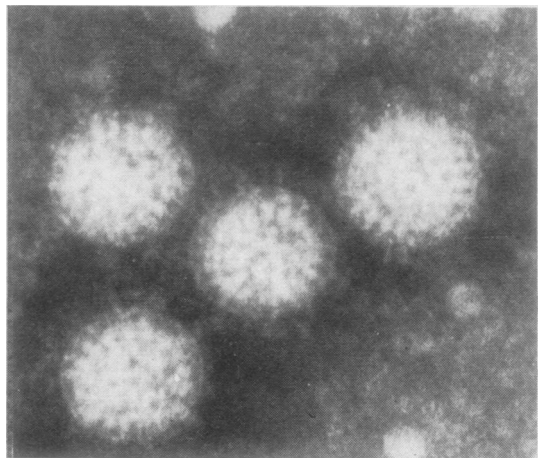


Fig 10 Virus particles agglutinated by convalescent serum. The strands of antibody connecting the particles are better visible here. $\times 300\ 000$.

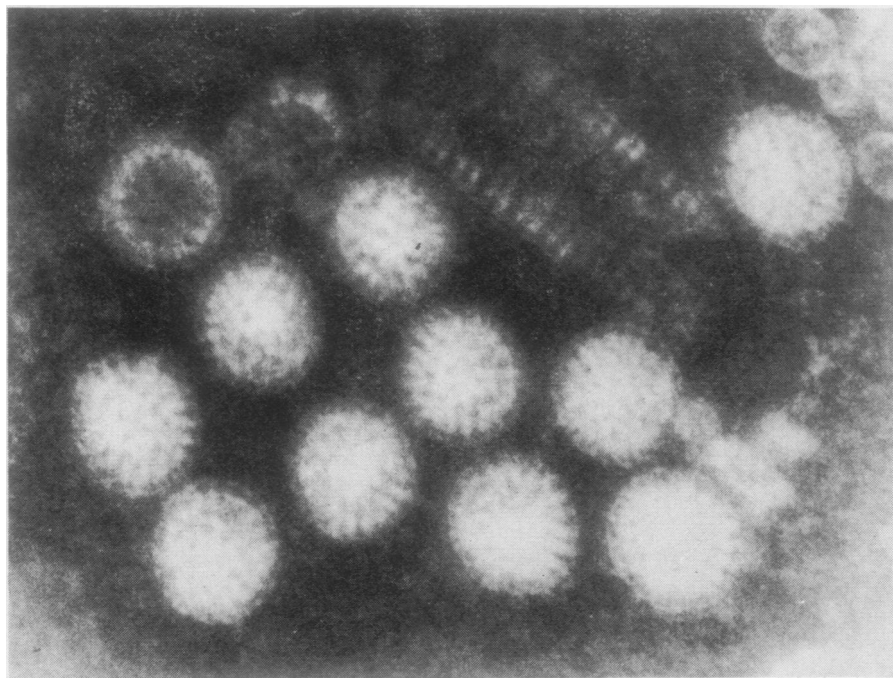


Fig 9 Virus particles agglutinated by convalescent serum. A cylindrical structure, probably composed of capsid protein, is attached. Its diameter is similar to that of the spherical particles. $\times 300\ 000$.

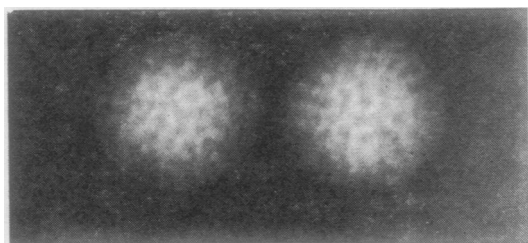


Fig 11 These particles show large-diameter capsomeric groupings, which in certain orientations of the particle appear as rings on the surface; in this these particles resemble the orbiviruses, especially blue-tongue virus. $\times 360\ 000$.

by surface forces during drying (fig 8). Here, the 'surface membrane' effect is clearly visible. The capsomeres of the outer layer are placed directly upon those of the inner layer.

On the surface, both of the double-layered and single-layered particles, the spaces between capsomeres (or holes in 'megameres') were somewhat larger than they appear in reoviruses; the appearance resembled more closely that of the published pictures of blue-tongue, Irituia, or haemorrhagic epizootic disease of deer (Murphy, Borden, Shope, and Harrison, 1971; Verweerd, Els, de Villiers, and Huismans, 1972) than that of Colorado tick fever virus. Radial division of surface or capsomeres on the surface was never clearly apparent, as in Colorado tick virus on whole virions, though individual capsomere subunits were visible when detached (fig 7).

Sometimes the particles, when numerous, were aggregated into clumps. No strands could be found connecting them to suggest that they might have been linked by globulin molecules, although in the thick layer of negative stain surrounding 60-80 nm particles fine strands might be difficult to discern, especially in the comparatively crudely purified material under investigation. A tubular structure was found in a clump of virions aggregated by convalescent serum; this was presumably an aggregate of capsid protein, similar to those described in preparations of blue-tongue, Tribec, and other orbiviruses (fig 9). Aggregates of what may have been capsid protein were described by Fernelius, Ritchie, Classick, Norman, and Mebus (1972) in material from tissue cultures infected by the newborn calf diarrhoea agent, but these appeared as sheets of protein rather than tubules.

ATTEMPTS AT ISOLATION

At the time of writing only one reo-like virus has been isolated in tissue culture, though many specimens have been inoculated. This virus was detected in the third blind subculture in human embryo kidney (HEK) by electron microscopy of ultrasonically disrupted cells. In further subcultures in both HEK and MK cells a granular cytopathic effect resembling that of reoviruses appeared. Its haemagglutinin is neutralized to titre by antiserum to reovirus type 1. Other viruses were isolated from a few patients whose faeces contained the 'gastroenteritis virus': adenoviruses, types 2 and 6, and 2 untyped; Echo 11 virus and *Escherichia coli* serotype 026 were both isolated from another.

Serological reactions

The particles could be aggregated easily by convalescent sera, but only feebly by acute phase sera

from two patients (fig 10). However, strands resembling globulin fibres could be discerned on some virus particles mixed with acute phase sera, taken three to five days after the onset of disease. The virions were not agglutinated by antisera, derived from hyperimmunized monkeys, to reoviruses types 1, 2, and 3. These sera agglutinated their corresponding serotypes of reovirus particles; a heavy deposit of globulin could be seen upon them by electron microscopy.

Discussion

Most young children with acute diarrhoea and vomiting in recent months in and around Birmingham have had characteristic virus particles in their faeces, detectable by electron microscopy. As the period during which the virus is detectable is only a few days (in some patients at least) others may well have been missed. As virus particles must be very numerous to be detectable by electron microscopy, they may have been missed in some patients because they were too scanty. The occurrence of reovirus-like particles in faeces could be used by the laboratory, on the results so far obtained, for the diagnosis of viral gastroenteritis in young children.

	Number Examined	Positive	Negative
March—July 1973	16	5 (31%)	11
November 1973	15	9 (60%)	6
December 1973	22	16 (73%)	6
January 1974	19	9 (47%)	10

Table II Percentage of faeces from gastroenteritis patients with reovirus-like particles from March 1973 to January 1974

It will be interesting to see whether they are present or absent in outbreaks of acute gastroenteritis associated with type-specific *E. coli* infection.

We agree with Bishop *et al* (1973) that the reovirus-like particles are probably not true reoviruses; if we had been dealing with reovirus infection it seems unlikely that intensive attempts at isolation would have been so unsuccessful. Furthermore, virus particles could not be agglutinated by antisera to reovirus types 1, 2, and 3. The single isolation of a reovirus type 1 is probably merely an incidental finding. Simultaneous infection by two or even more viruses in young children is not very rare. Morphologically, the particles are different and resemble blue-tongue virus more than the reoviruses types 1, 2, and 3 (fig 11). But we hesitate to place them among the orbiviruses (Borden, Shope, and Murphy, 1971) without more evidence: for orbiviruses are all

acid-labile, and though acid-labile enteric pathogens, eg, *Vibrio cholerae*, are not unknown, most are acid-stable. Furthermore, in their distinct and characteristic smooth, circular outline, with well defined rim, the human viruses exactly resemble the gastroenteritis virus of calves (Fernelius *et al*, 1972; Woode *et al*, 1974), which is known to be acid-stable (Welch and Thompson, 1973) and differs from the appearance of orbiviruses (Murphy *et al*, 1971). Acid resistance may, of course, not be necessary for an enteric virus of young children, whose intestinal mobility is brisker and gastric acid weaker than that of adults.

These gastroenteritis viruses may well be members of a new group of double-stranded RNA (diplorna) viruses.

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Addendum

Since this paper was submitted for publication, Middleton, Szymanski, Abbott, Bortolussi, and Hamilton (1974) have published similar evidence that orbivirus-like particles were associated with acute gastroenteritis of infancy in Canada and were not found in patients with non-enteric symptoms. Although their estimate of size is rather larger than ours, their picture leaves little doubt that the virus is the same.

Flewett, Bryden, Davies, Woode, Bridger, and Derrick (1974) have suggested that the closely related viruses of acute diarrhoea in children and calves are distinct from the orbiviruses and have proposed that they should be called rotaviruses.

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