Stool viruses in babies in Glasgow

1. Hospital admissions with diarrhoea

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SUMMARY

Stools from 183 babies under 2 years of age admitted to Ruchill Hospital with diarrhoea were examined by electron microscopy, virus culture, bacterial culture and light microscopy. As far as possible, several stools were examined from each patient and the results showed rotaviruses, astroviruses and other viruses in association with symptoms, as well as the expected bacterial pathogens. Examination of several stools from the same patient also showed that in this age group the viral flora of the gut changes rapidly and that the viruses seen by electron microscopy were only rarely grown in cell culture and vice versa. This phenomenon was particularly noted with adenoviruses. In 30 % of cases no microbial pathogen was identified and in the remainder the presence of the infecting organism did not always coincide with the symptoms. It is concluded that, with viruses at least, presence of the organism does not constitute proof of causation.

INTRODUCTION

Since the first observations of viruses in faeces by direct electron microscopy (Bishop, Davidson, Holmes & Ruck, 1974) reports from many parts of the world have confirmed and extended these findings. As a result rotaviruses are generally accepted as a cause of infantile enteritis (Editorial, 1975), while the role of other viruses is still being explored.

We have examined stools from babies with enteritis admitted to one ward in one hospital over a period of 16 months. The purpose was to establish what viruses, bacteria and protozoa were excreted by these babies and our findings suggest that, as others have found by cell culture methods (Bell & Grist, 1967; Stott *et al.* 1967), excretion of viruses in young children does not directly parallel symptoms, and that to regard even rotaviruses as inevitable pathogens may be too simple a view. These observations have led to further studies, to be reported

separately, and we present in this paper the results of an investigation of the stools from these babies using electron microscopy, routine virus culture, bacteriology and parasitology.

MATERIALS AND METHODS

Patients

These were children under 2 years of age admitted to one ward at Ruchill Hospital with a history of diarrhoea of less than 5 days' duration. This hospital draws patients from the northern half of the Greater Glasgow area. The period of the study was between 1 September 1974 and 31 December 1975, and 183 babies were investigated.

Stool specimens

These were obtained as soon as possible after admission. It was intended that at least two specimens should be examined but only one sample was received from half the patients (see Table 1), normally because the diarrhoeal symptoms had ceased by the time the baby was admitted to hospital. Where evidence of the presence of a virus or bacterial pathogen was obtained, further specimens were requested from the ward and the investigation continued until the baby was discharged.

Stool specimens were sent initially for bacteriological examination and a sample was removed for culture. The remainder was stored at 4 °C overnight in case a repeat test was necessary. It was then sent for virological examination. Final virological processing was usually completed within 36 h of the stool being passed.

Virology

A piece of faeces about the size of the top of an adult thumb (approx. 8 g), or an equivalent quantity of liquid faeces, was shaken by hand in a screw-capped glass bottle with 14 ml of phosphate-buffered saline containing penicillin and streptomycin, pH 7, until most of the solid matter had broken up. The mixture was allowed to stand at room temperature for 20 min for the gross debris to sediment. The supernatant fluid was transferred to a fresh screw-capped bottle and spun at 3000 rev./min for 30 min in a bench centrifuge. This normally left a slightly cloudy or almost clear supernatant, but in some cases it was necessary to recentrifuge. Four vials each containing 1 ml of the supernatant were taken for cell culture and the remainder used for electron microscopy (EM).

Cell culture. Virus isolation was attempted using our routine cell culture systems. Secondary rhesus monkey kidney (RMK) and primary human amnion (HA) were both maintained in Eagle's MEM with 0·5% fetal calf serum and antibiotics. Duplicate tube cultures of RMK and HA were each inoculated with 0·2 ml of the stool extract, or it was stored at − 70 °C until fresh cell cultures became available. The tubes were incubated stationary at 36 °C and examined microscopically on alternate days for evidence of cytopathic effects. Virus isolates were identified using equine enterovirus-neutralizing serum pools supplied by the World Health

Organization, or by individual adenovirus neutralizing sera provided by the Standards Laboratory for Serological Reagents, Colindale, London.

Retrospective investigation was carried out on those stools in which adenovirus was detected by electron microscopy. Duplicate cell cultures of primary human embryo kidney (HEK) were inoculated with 0·2 ml of each stool extract and incubated at 36 °C for a period of 4–6 weeks. Since this necessitated frequent (7-day interval) passages, cell culture fluids were frozen (to − 20 °C) and thawed four times before each passage to ensure maximum release of any adenovirus present in the cells.

Electron microscopy. The supernatant (4·5 ml) from the low-speed centrifugation was centrifuged in a SW 50.1 rotor in a Beckman model L3-50 ultracentrifuge at 35000 rev./min for 60 min. The supernatant was poured off, the tube plugged with a paper tissue while inverted and allowed to drain in that position for at least 10 min. The pellet was then resuspended in 2 drops (approx. 0·05 ml) of EM diluent (0·1 % bacitracin in distilled water) using a pasteur pipette. It was sometimes necessary to sonicate the suspension briefly in a bath-type sonicator to obtain an even suspension. A drop of this suspension was mixed with an equal quantity of 2 % potassium phosphotungstate (pH 7·0) on a microscope slide. If the suspension was very thick, it was diluted 1/2 or 1/4 in EM diluent before staining. A drop of the mixture was placed on a carbon formvar-coated 400-mesh copper grid and the surplus drawn off using the torn edge of a piece of filter paper. The grid was then allowed to dry in air and examined in a Philips EM 301 electron microscope. The time between preparation and examination was variable but re-examination of grids has shown that such preparations are stable for several weeks. Because of the difficulties of making exactly equivalent stool extracts, no attempt, other than the above, was made to standardize the procedure. The grids were examined for about 15 min, during which time several separate areas were inspected. When virus was present it was usually detected within the first few minutes, but where small numbers were detected they were usually 'small round viruses' (see below) interpretation of whose presence has proved difficult. The relatively short examination period has meant that a small proportion of stools were probably recorded incorrectly as negative but this limitation was accepted in order to avoid unproductive tedium and allow a high throughput of specimens. The majority of specimens were examined by one operator (BPC).

Bacteriology

Stool specimens were examined by light microscopy as a direct Gram-stained smear, and were inoculated onto MacConkey agar, deoxycholate citrate agar and into selenite F broth. Cultures were incubated at 37 °C and inspected after overnight incubation. Ten single lactose-fermenting colonies and the mass growth on the MacConkey plate were tested by slide agglutination using polyvalent antisera for the presence of enteropathogenic *Escherichia coli*. The antisera contained antibodies to the following serotypes: O18, O20, O26, O28, O44, O55, O86, O111, O112, O114, O119, O124, O125, O126, O127, O128, O142. The identity of organisms detected by slide agglutination was confirmed by tube agglutination using mono-

typic antisera and purified cultures. Non-lactose fermenting colonies were examined by standard methods to identify shigellas or salmonellas. Selenite F broth was subcultured to deoxycholate citrate agar and examined for salmonellas or shigellas after a further 24 h incubation.

Parasitology

A wet preparation of stool emulsified in saline was examined by light microscopy for the presence of parasites, particularly *Giardia lamblia*. A concentrated specimen prepared using Ritchie's formol ether technique, as modified by Allen & Ridley (1970), was also examined.

Terminology

The nomenclature of several of the viruses observed in stools by electron microscopy is not yet official. For clarity, therefore, it is necessary to define the terms used in this paper. Four morphological types of virus have been observed:

Rotavirus. Also called duovirus (Davidson *et al.* 1975), orbi-virus-like (Middleton *et al.* 1974) and reovirus-like (Kapikian *et al.* 1974), these viruses are 65 nm in diameter with an entire (complete) or spike (incomplete) outer surface (Plate 1*a*). They are distinguishable from reoviruses in both appearance and size.

Astrovirus. Viruses 28 nm in diameter with an entire outer surface (Madeley & Cosgrove, 1975). A star-shaped surface configuration which may be five- or six-pointed is seen on a proportion of particles (Plate 1*b*). Their relation to other viruses is unknown but they are distinguishable morphologically from caliciviruses (Madeley & Cosgrove, 1976).

Adenovirus. Icosahedral particles 75 nm in diameter which have been well characterized (Andrewes & Pereira, 1972). Adenoviruses have been readily cultured from stools but we and other workers (Flewett, Bryden, Davies & Morris, 1975) have observed morphologically typical adenovirus particles (Plate 1*c*) which have so far failed to grow in cell cultures.

Small round viruses. Round particles 20–30 nm in diameter and without other distinguishing features have been observed in faeces by others as well as by ourselves (Flewett, Bryden & Davies, 1974; Appleton & Higgins, 1975). They are usually present in fairly small numbers and may have an entire or a 'feathery' edge (Plate 1*d–f*). More correctly, they should be referred to as virus-like as it cannot be certain that they are truly viruses. To experienced observers they look like viruses, and in individual stool specimens are homogeneous in size and morphological appearance. Some may be bacteriophages though they are clearly different from the tailed 'phages seen frequently in stool extracts. We include them in this report as part of the observations made but whose significance is unknown. For convenience we have referred to them as SRVs.

RESULTS

From the 183 patients in this study, 357 stool specimens were obtained, an average of just under two per patient. The distribution of stools per patient is

Table 1. *Number of stool specimens per patient*

No. of stools	1	2	3	4	5	6	7	Total
No. of patients	95	47	15	13	8	4	1	183

shown in Table 1, from which it will be seen that only one stool was obtained from 95 patients (52 %). This does not conform with the original design of the study but probably reflects the degree to which social factors may influence admission to hospital from the poorer areas of the city. Nevertheless a considerable number of positive identifications were made, with rates similar to those of the study as a whole (Table 2). However, the proportion of babies with multiple isolations rises when more than one stool is examined, and the proportion of patients with no detectable stool viruses falls. As will be shown, a slightly different picture emerges from examination of more than one stool per patient and possibly erroneous conclusions can be drawn from incomplete information.

Stools from 176 patients were fully examined by all methods; the stools from the remaining 7 were not examined for viruses by cell culture. A comparison of the results of virology, bacteriology and parasitology is shown in Table 3, while the organisms identified are listed in Table 4. From these results it can be seen that no potentially pathogenic organisms were identified in approximately one third (52 = 29 %) of the patients, while in 89 (49 %) viruses detectable only by electron microscopy were found. (Table 3, cols 2–5. Total = 93 with 4 concordant results subtracted since viruses seen and morphologically different from those grown cannot be the same virus). The culture results showed the variety of viruses to be expected in children of this age group (Yow *et al.* 1966), and the absence of a predominant virus in children with similar symptoms also confirms previous results (Stott *et al.* 1967). However, infections with two or more viruses have been found in 26 patients (14 %, from Table 3, cols. 2, 3, 5). Since one cannot be certain that the virus seen was the same as that grown this total could be 30 (17 %). Further, as is shown by two examples in Table 5, a rapid turnover in gut viral flora can be detected.

The bacteriological culture results show a predominance of *Shigella sonnei* isolations but this is not unexpected. However, comparison of the dates of isolation where both viruses and bacteria were detected in the same patient (Table 6) shows some interesting findings. In 8 out of 20 occasions, the virus was detected in an earlier stool than the bacteria and in 6 of them the virus was either a rotavirus or an astrovirus. Furthermore the intervals between positive observations of bacteria and virus (and vice versa) make it unlikely that they were part of the same episode in six patients (interval of more than 7 days).

The viruses observed by electron microscopy are listed in Table 7 by the first stool in which they were seen. In the majority of cases any virus to be seen was detected in the first stool, but in 20 (11 %) a virus was seen only in the second or subsequent stool. In 12 patients (6 %) this was a second virus and shows again that new viruses are readily acquired in this age group.

The first results in this study suggested that astroviruses affected younger

Table 2. *Identification of organisms in stools: patients grouped by number of stools obtained*

No. of stools per patient	No. of organisms identified	No. of patients in group	Organisms identified in each group			
			Viruses		Enteropathogenic bacteria	Protozoa
			Positive by EM	Positive by culture		
1 stool	Nil	37	—	—	—	—
	1	39	26	5	8	0
	>1	19*	12	9	17	1
>1 stool	Nil	19	—	—	—	—
	1	36	28	3	3	2
	>1	33	44	18	10	3

* Includes one patient in whose stool 3 organisms were identified.

Table 3. *Comparison of virological, bacteriological and protozoological results*

	Virus results*					Virus positive (totals)	Culture and EM negative	Total
	Culture + EM –	Culture ++ EM +	Culture + EM +	Culture – EM +	Culture – EM ++			
Negative for enteropathogenic bacteria and protozoa	8 (5)†	2 (1)	11 (6)	51 (29)	12 (7)	84 (47)	52 (29)	136 (77)
Positive for enteropathogenic bacteria	7 (4)	0 (0)	3 (2)	9 (5)	1 (0·6)	20 (11)	14 (8)	34 (19)
Positive for protozoa‡	0 (0)	1 (0·6)	0 (0)	3 (2)	0 (0)	4 (2)	2 (1)	6 (3)
Totals	15 (8)	3 (2)	14 (8)	63 (36)	13 (7)	108 (61)	68 (38)	176 (100)
Concordant results§	0 (0)	0 (0)	4 (2)					

* Culture: +, one virus isolated; ++, two viruses isolated. EM: +, one virus seen; ++, two viruses seen.
† ( ) = percentages.
‡ *Giardia lamblia* only
§ Virus seen by EM compatible with virus grown.

Table 4. *Stool organisms identified*

Type of organism	Method of detection	Result of identification	No. of patients	Total
Virus	Cell culture	Poliovirus		
		1	2	
		2	2	
		Coxsackievirus		
		B2	2	
		B4	4	
		Echovirus		
		1	1	
		7	1	
		12	2	
		14	2	
		15	2	
		21	1	
		22	2	
		Enterovirus unidentified	3	
		Adenovirus		
		1	1	
		2	4	
		5	3	
		6	1	
		7	1	
		Unidentified	2	36*
	Electron microscopy	Rotaviruses	55	
		Astroviruses	26	
		Adenoviruses	11	
		SRVs	14	106†
Bacteria	Culture	*Shigella sonnei*	21	
		Sh. flexneri 2a	2	
		Salmonella panama	1	
		Escherichia coli		
		086	2	
		0111	1	
		0114	1	
		0119	1	
		0125	1	
		0127	3	
		0128	6	39‡
Protozoa	Light microscopy	*Giardia lamblia*	6	6

Total number of organisms 187

* From 33 patients (3 double isolates).
† From 93 patients (13 double isolates).
‡ From 34 patients (5 double isolates).

babies than rotaviruses but a comparison of ages and viruses identified (Table 8) does not fully confirm this. The peak of rotavirus excretion was between 6 and 12 months while that of astroviruses may be slightly earlier. The numbers are small, the difference is not significant and no age bias in adenovirus, enterovirus or SRV excretion is apparent.

Table 5. *Comparison of virus culture and electron
microscopy results in two patients*

Patient no.	Stool no.	Viruses isolated	Viruses identified by EM*
25	1	Adenovirus 7	Rotavirus + +
	2	Adenovirus 7	Rotavirus + +
	3	Adenovirus 7	Rotavirus + + +
	4	ECHO 15	SRV
		Adenovirus untyped	
101	1	Poliovirus 2	Adenovirus + +
			Adeno satellite virus + + + +
	2	Poliovirus 2	Adenovirus +
			Adeno satellite virus + +
	3	Poliovirus 2	Nil

* No. + signs = approximately relative amounts of virus.

Table 6. *Patients in whom both viruses and bacteria were identified*

Patient no.	Virus* (V)	Bacterium (B)	Organism first observed			Interval (days) between V and B
			V	B	simul-taneous	
3	Astrovirus (EM)	*Sh. sonnei*	+	−	−	8
22	Astrovirus (EM)	*Sh. sonnei*	+	−	−	25
35	SRV(EM)ECHO(C)	*Sh. sonnei*	−	−	+	—
42	SRV (EM)	*Sh. sonnei*	−	+	−	2
59	Adenovirus 2 (C)	*Sh. sonnei*	−	−	+	—
74	Rotavirus (EM)	*Sh. sonnei*	−	+	−	6
103	Adeno + sat (EM) Poliovirus 2 (C)	*E. coli* 0128	+	−	−	1
104	Rotavirus (EM)	*E. coli* 086	−	+	−	1
120	Rotavirus (EM)	*E. coli* 0127	+	−	−	1
		Sh. flexneri 2A				
122	Adenovirus 6 (C)	*E. coli* 0127	−	+	−	3
		Sh. flexneri 2A				2
128	ECHO 1 (C)	*Sh. sonnei*	−	−	+	—
130	Coxsackie B2 (C)	*Sh. sonnei*	−	−	+	—
131	Astrovirus (EM)	*E. coli* 0114	+	−	−	9
133	Rotavirus (EM)	*E. coli* 0127	+	−	−	7
135†	Coxsackie B4 (C)	*Sh. sonnei*	−	+	−	16
136†	Coxsackie B4 (C)	*Sh. sonnei*	−	+	−	16
142	ECHO 14 (C)	*E. coli* 0128	−	−	+	—
154	Rotavirus (EM)	*E. coli* 0128	−	−	+	—
170	Adenovirus (EM)	*S. panama*	+	−	−	1
172	Rotavirus (EM)	*E. coli* 0125	+	−	−	5
		Totals	8	6	6	

* EM; by electron microscopy; C, by cell culture.
† Siblings.

With the exception of the adenoviruses, none of the viruses detectable by electron microscopy routinely produced a visible cytopathic effect in cell cultures. However, a toxic but transitory cytopathic effect in HA cultures was often detected with those stool extracts which were rotavirus-positive by electron

Table 7. *Results of electron microscopic examination of two or more stools from the same patient**

No. of stool in which virus first detected	Virus†				Negative
	Rotavirus	Astrovirus	Adenovirus	SRV	
1	34‡	10	2	6‡	0
2	3	5 (3)	0	2 (2)	19
3	4 (1)§	2 (2)	0	1 (1)	3
4	0	0	1 (1)	1 (1)	4
5	0	0	0	0	1
6	0	1 (1)	0	0	2
Totals	41 (1)	18 (6)	3 (1)	10 (4)	29

* All patients from whom only one specimen was obtained are excluded.
† Virus detected by EM.
‡ These totals include one patient in whose first stool a rotavirus and an SRV were seen.
§ ( ) = numbers of patients where the virus was the second one to be found in that patient.

Table 8. *Relation between age of patient and viruses detected*

Age group (months)	Rotavirus	Astrovirus	Adenovirus	SRV	Culture
< 1	1	4	1	1	0
1–2	2	2	2	1	Polio 2 Coxsackie B2 ECHO 15
2–3	6	6	1	4	Adeno 1, 2, 6 ECHO 12, 22
3–4	5	1	1	2	Coxsackie B2
4–6	4	2	2	1	Adeno 5 & ECHO 22, Adeno untyped
6–9	6	2	2	2	Polio 1, 2 ECHO 1, 7, 14, 22 Adeno 2, Adeno 7 & ECHO 15
9–12	9	2	0	0	Coxsackie B4 Adeno 2, 5
12–15	8	4	3	0	Polio 1 Adeno untyped ECHO 14
15–18	5	0	0	0	0
18–21	5	2	0	3	Coxsackie B4 ECHO 21 Adeno 6
21–24	0	0	0	1	0
> 24	1	0	1	0	Coxsackie B4

microscopy. To check that no virus replication was occurring undetected, cell extracts were examined by electron microscopy after culture on 73 occasions (range 1–5 passages, up to a maximum of 49 days). Rotavirus particles, for example, were seen in 47 of the stool extracts and were still detectable in 20 after passage in cell culture, up to a maximum of 29 days (2 passes). In each case the numbers

Table 9. *Adenoviruses: comparison of culture and electron microscopic findings*

	Culture + EM −	Culture + EM + *	Culture − EM + *	Culture − EM + + †	Total
No. of patients	9 (4)‡	2	4	6	21

* Small numbers present – ≤ 5 particles/grid square.
† Large numbers present – approximately 5–100 particles/grid square.
‡ ( ) = number of patients excreting other viruses, detected by culture or EM.

Table 10. *Comparison of excretion of rotaviruses, astroviruses and SRVs alone or in combination*

	Rotavirus alone	Rotavirus + astrovirus	Rotavirus + SRV	Astrovirus alone	SRV alone
No. of patients	42	8 (2)*	5 (2)	18	9

* ( ) = number where one stool was positive for both viruses simultaneously.

Table 11. *Comparison of astrovirus seen by electron microscopy with viruses cultured from the same stool*

	No. (%)	Viruses cultured
Stool positive for astrovirus and by culture	5 (19)	⎧Poliovirus 1 ⎪Echo 14 ⎨Echo 22 ⎪Adenovirus 2 ⎩Adenovirus 6
Stool positive for astrovirus only. Negative by culture	21 (81)	
Total	26 (100)	

seen were small and after allowing for the small amount of dilution in passage it is likely that the virus seen was input virus carried over passively and no definite evidence of growth was obtained.

The adenoviruses detected in this study presented a more subtle problem. As can be seen from Table 9, 21 patients were shown to be excreting adenovirus; 9 were detected only by culture (4 of these were also excreting other viruses), and 10 only by microscopy. Only 2 were detected by both methods and, since more than one type of adenovirus may be excreted by a single patient (for example patient no. 121 excreted types 2 and 5), it is not certain that the virus seen was identical with that grown. Therefore with these 2 exceptions, the chances of growing an adenovirus from a stool appeared to be inversely related to the amount of virus seen. In addition to this, all the adenoviruses detected only by microscopy showed some evidence of growth in culture (either RMK, HA or HEK and usually all three). This cytopathic effect was typical of adenoviruses, appearing 1–10 days after inoculation (mean 4·5 days) but could not be passaged nor could the virus be typed. Furthermore, electron microscopy of the cell cultures did not suggest

virus multiplication. In comparison, where an adenovirus was isolated and typed, the typical cytopathic effect developed more slowly (3–15 days, mean 9 days). These phenomena are the subjects of further study to be reported later.

The finding of astroviruses or SRVs in association with rotaviruses, not infrequently in the same stool, suggested that either might be satellite viruses to the rotavirus. However a direct comparison (Table 10) shows that no significant association was detectable and each virus was found alone more frequently than in association. Similarly the possibility that astroviruses are an atypical form of a better-known virus does not withstand examination. Astroviruses were found in the stools of 26 patients in this study and in only 5 (Table 11) were associated with positive culture results. In each case the virus was different and clearly incompatible in two (adenovirus types 2 and 6 respectively).

DISCUSSION

The purpose of this study was to discover if a particular virus, bacterium or protozoon was a predominant cause of enteritis in the babies admitted to hospital in this area. If no such predominance was found, the secondary aim was to find out which agents were associated with the condition and, by taking more than one specimen from each patient, to obtain information about the viral flora of these children. For this purpose several stools were necessary and it was disappointing that in 52 % only one specimen was obtained. This reflects the rapid improvement in symptoms which is often observed in patients whose admission has been determined to some extent by social factors. However it should be strongly emphasized that a rapid turnover of viral flora can occur in babies under 2 years of age (see Table 5, for example) and this will only be detected by examining multiple stools from each patient.

No one organism was found to be predominant. Bacterial pathogens (particularly *Sh. sonnei*) were found, together with several enteropathogenic strains of *E. coli*. The latter were not screened for the production of enterotoxins and recent work has shown that not all such strains produce toxins detectable by available methods (Rowe, Gross & Scotland, 1975). The association between enteropathogenic strains of *E. coli* and enteritis is similar to that found with rota- and other viruses and complete proof of causation by either may be difficult to obtain. It is interesting to note that in 5 babies a virus, adenovirus + satellite/polio (1 case), rotavirus (3 cases) and astrovirus (1 case) was found before the bacterial pathogen. In 3 cases the interval was 5, 7 and 9 days respectively. It is possible that the bacteria were present but undetected in the earlier specimen and if this was so it would undermine the role of the viruses in initiating disease. Hence there is a need to understand more fully the ecology of viruses and other agents in the gut and improve the methods of detection.

As it is, in 52 (28 %) of those stools examined by all methods no specific organism was identified. If the small round viruses are excluded the figure rises to 59 (32 %). Malabsorption due to various factors (genetic, inappropriate feeding, etc.) could account for some of these negatives and in these cases no infective agent need be

involved. In the remainder an infective agent may have been present and either not detected or not recognized. In addition to those we have recorded as SRVs there are numerous membrane-bound objects that are not convincingly virus-like as we recognize the term but some of which may still be viruses none the less. If so, we have not been able to identify features consistent enough to arouse suspicion. A reliable catch-all culture system would, of course, be a great help in this but is not yet available, and virtually all the viruses seen by electron microscopy do not grow in routine cell cultures. Further, as an almost invariable rule any virus seen in the electron microscope was not grown in culture and any viruses grown were not seen. There were 4 exceptions to this paradoxical rule. In 2 an enterovirus was grown and an SRV compatible in size was seen. In the others an adenovirus was seen and one was grown, but in none of these can it be certain that the same virus was both grown and seen; double isolations are not unknown. However, the adenoviruses pose a particular problem in that apparently the more virus there is detectable by electron microscopy the more difficult it is to grow, although all showed some cytopathic effect in culture which usually occurred too late to be a simple toxic effect by the input virus. On the other hand, it also developed more quickly than with those adenoviruses which grew, were passaged and typed.

The small round viruses are another problem. Even the name we have used may be misleading as some may not be viruses at all. It is a term of convenience and, while it can be argued that there is no value in collecting such vague information, we think that it is important to remember that our catalogue of stool viruses is probably not complete and it is worth retaining this information whose significance can be assessed later. The Norwalk agent (Kapikian *et al.* 1972), the small virus of Paver, Caul, Ashley & Clarke (1973) and that of Appleton & Higgins (1975) all fall into the category of small round viruses and underline the need to keep our lists open. Serological studies by immune electron microscopy may help to divide them into groups, but we have yet to do this.

This study has not identified any agent as the sole cause of infantile enteritis, but has indicated that the interplay between infant and communicable agent is complex. In the 2 cases quoted in Table 5, can it be said with certainty which of the agents identified, if any, was the cause of the symptoms? In showing that gut viruses may be exchanged frequently our work confirms previous results by culture, and shows that with viruses whose target organ is the gut, we need to know very much more about their ecology before we can understand the causation of infantile enteritis and consider ways of prevention. Further studies are being carried out to try to obtain some of this information.
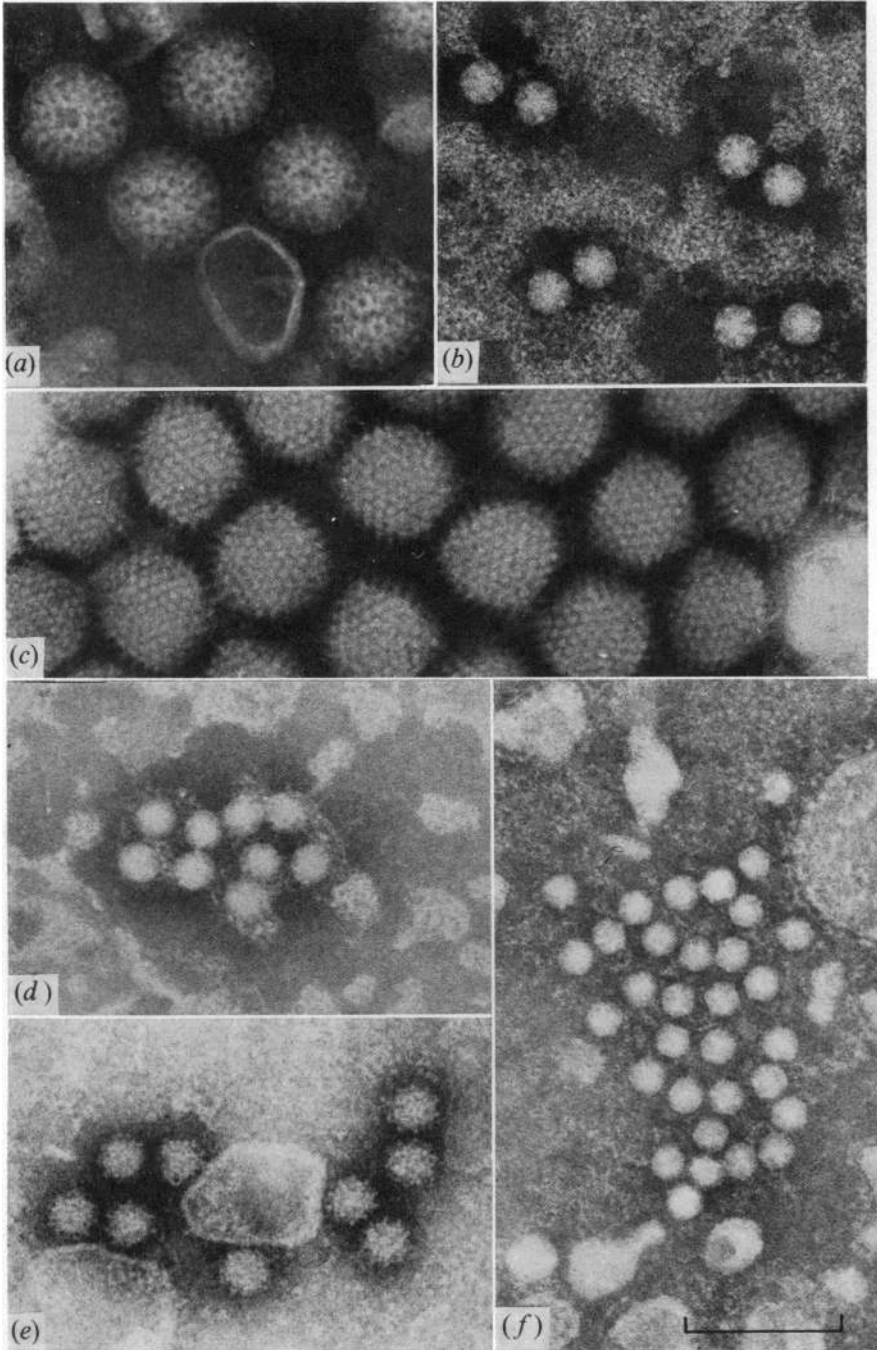
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EXPLANATION OF PLATE

Viruses and virus-like objects seen in faeces by direct electron microscopy, negatively stained with 2% potassium phosphotungstate, pH 7·0. (a) Rotavirus. (b) Astrovirus. (c) Adenovirus. (d–f) 'Small round viruses' (SRVs). All are printed at a final magnification of × 200000. Scale bar = 100 nm.