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Stool viruses in babies in Glasgow

4. Further hospital studies

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(*Received as part of an academic tribute to A. W. Downie*)

SUMMARY

The events occurring during the time in hospital of 24 babies were recorded in detail, particularly those related to bowel function. The babies were admitted to a general paediatric unit with various diagnoses a total of 27 times during a six month period. The purpose of the study was to fill gaps in the records of a previous study (Scott *et al.* 1979) and to try to relate excretion of viruses detectable by electron microscopy to disturbances of bowel function (diarrhoea and/or vomiting). The results showed that a variety of viruses were associated with gastroenteropathy, that virus excretion could occur without disease and that hospital acquisition of virus is not uncommon. Antiobiotic treatment did not appear to precipitate diarrhoea in this small number of babies.

INTRODUCTION

Associations between viruses and diarrhoea have been documented in numerous publications but diarrhoea, particularly in infants, is difficult to define and children may be admitted to hospital with a diagnosis of 'gastro-enteritis' which is no longer apparent on the ward. In addition, a previous paper in this series (Scott *et al.* 1979) showed that the data on diarrhoea, as ordinarily recorded in the hospital notes, was insufficient to document association with the viruses found by electron microscopy in a group of babies followed throughout their admission. It was apparent that a record of 'loose stools', 'diarrhoea', etc. did not contain enough detail for any valid conclusions on the aetiological role of viruses to be drawn.

The study reported in this paper was set up to provide more detailed data and in particular to examine possible associations between viruses in the stools and diarrhoea and/or vomiting, and whether any other factors relevant to gastrointestinal disturbances could be identified.

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MATERIALS AND METHODS

Patients. These were 24 babies aged between 3 and 11 months of age admitted to the paediatric unit of Stobhill General Hospital, Glasgow between September 1978 and April 1979. They were admitted with a variety of diagnoses and were not selected as having gastro-intestinal upsets. Three of these babies were admitted twice during the period of study.

Their period in hospital was documented from the ward notes, supplemented with an extra record sheet for details of bowel function. The entries on the latter included all bowel movements, their nature and number, and all episodes of vomiting. An admission history of the babies' preceding health or illness was taken personally by T.M.S. and C.C. who also supervised the compilation of the patient's records. Consequently these were scrutinized with more informed care than usual and any alteration in bowel habit assessed against the mother's estimate of the baby's normal function. Other details of the babies' progress, including type of feeding given and drugs administered were also recorded.

Methods. As far as possible, at least one stool from each baby was obtained daily. An extract of each stool was examined by negative contrast electron microscopy using 3% potassium phosphotungstate pH 7·0. The method of preparation using high speed centrifugation of the stool extract made in phosphate buffered saline has been published already (Madeley *et al*. 1977). Attempts to culture virus were not made unless the child's illness suggested that other non-diarrhoeal viruses might have been involved. Specimens for routine bacteriology were sent to the Department of Bacteriology in the hospital and included stools, urines and throat swabs.

RESULTS

Multiplication of the number of babies by the number of days in hospital gave the total number of stools that might have been examined. This figure was 239 stools as indicated in Table 1. Of these, 181 were obtained and examined and this figure does not include the occasions when more than one stool per day were looked at. The deficiency was due to three factors – failure of the baby to pass a stool on that day, failure to obtain a stool on the day of admission and several babies where the main medical problem did not involve the gut. Failure of the baby to produce a stool occurred on 18 occasions leaving a possible total of 221. The proportion of stools examined was therefore 181/221 (81·9%). With this proportion it is fair to consider that most of the viruses excreted by this group of babies in hospital will have been recorded, particularly because the gaps in the record were rarely more than one day.

The kind of record obtained for each baby in the study is shown in detail in Fig. 1. This summarizes the 13-day admission of 9-month old patient number 21 (Table 1) who arrived with a diagnosis of laryngitis stridulosa and a once daily bowel habit according to his mother. During his admission his respiratory tract infection remained localized to the upper part of the tract while his bowel habit increased to a maximum of five per day on days 9 and 12 although two or more stools were recorded on every day except the first, second, eighth and last. As indicated in the diagram a proportion of these were recorded as soft, and vomiting

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Table 1. *Patient details*

Baby	Age (months)	Admission diagnosis	Viruses observed by EM on days indicated*	No. of days† in hospital	No. of stools examined
1	3	Viral infection	RV + (9); RV + (11)	18	15
2	3	Bronchitis	None	3	3
3	3	Vomiting; ?cause	None	5	5
4	3	Vomiting, social problems	AdV +, +(10), RV + +, +(11); RV + + +, +(12)	13	12
5	3	Vomiting for 1 month	None	7	7
5	3 (10 days later)	Irritable and coughing	RV + +(2), RV + (3); RV + (5); RV + +(7); AdV + (15)	15 (3)	9
6	3	Bronchiolitis	SRV + + +(4); SRV + + +(6)	8	6
6	5 (2 months later)	Battered baby	AdV + +(2); RV + +(9); RV + +, + +(10); RV + + +(11)	19 (2)	11
7	4	Wheezy bronchitis	AV + +, + + +(4); AV +, +(5)	5 (1)	4
8	4	Wheezy bronchitis	SRSV occ.(3)	8 (1)	7
9	4	Bronchiolitis	AV + + + +(8)	8	7
10	5	Bronchiolitis/Wheezy bronchitis	None	9 (3)	3
11	6	Herpetic stomatitis	SRV + + +(3); AdV + + +(7)	10	8
12	6	Measles			
		Rash	None	3	3
13	6	URTI/Diarrhoea	None	4 (1)	3
14	6	Bronchopulmonary dysplasia	AdV +(2); AdV +(3); AdV +(5); AV + + +(22); AV + + + +(23); AV + + + +(24)	24 (5)	14
14	6 (19 days later)	Rash and wheeze	None	3	3
15	7	URTI/Diarrhoea	RV + + + +(1); RV 0, +(2); RV + + +, + +, + + +(3); RV + +, +(4); RV + + + +, + + + +(5); RV + + +(6)	6	6
16	7	Febrile convulsion	None	5 (1)	2
17	8	Wheezy bronchitis	None	6	6
18	8	Wheezy bronchitis	None	4	2
19	9	Viral infection	SRSV + + +(9)	9	7
20	9	Bronchitis	SRSV + + +(4)	8 (1)	6
21	9	Laryngitis stridulosa	RV + +, + +(6); RV +(7); RV + + + +, AV + + + +(8); AV + +(9); AV + +, +(10); AV + + +(11); AV + + +(12)	12	10
22	11	Diarrhoea and Vomiting	RV + + + +(2); RV +, +(4); RV +, +(5); RV + +(6); SRSV +(17)	13	10
23	11	Viral infection (fit)	None	6	4
24	11	Diarrhoea (5 days)	RV + +(2); RV + + +, +(3)	8	8
			Total	239 (18)	181
			%	100	81·9

* AV: astrovirus; AdV: adenovirus; RV: rotavirus; SRV: small round virus; SRSV: small round structured virus. +−+ + + +: Approximate relative quantities *in stool extract.* ( ): *day after admission when stool taken.*

† Figures in brackets: number of days when no stool passed.

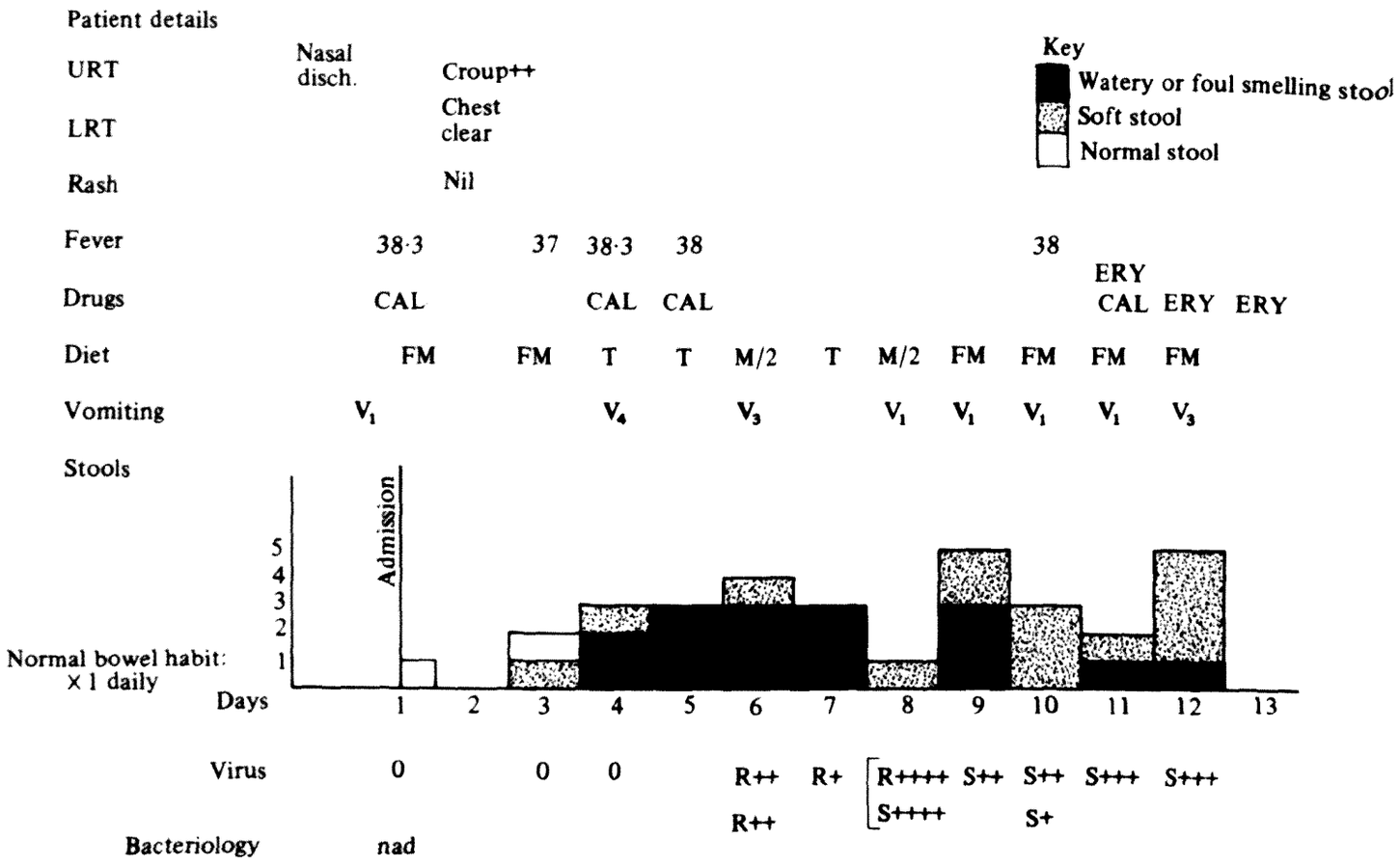

Fig. 1. Hospital record of patient number 21. URT, Upper respiratory tract; LRT, Lower respiratory tract. Drugs: CAL, Calpol; ERY, Erythromycin. Diet: FM, Full cream milk; M/2, Half cream milk; T, Clear fluids. Vomiting: Bold type, significant vomiting; Normal type, insignificant regurgitation. Number indicates number of episodes. Virus: R, rotavirus; S, astrovirus; 0, no virus seen. Number of plusses indicates approximate amount of virus: + + + +, > 100 particles/400 mesh grid square. + + +, 20–100 particles/400 mesh grid square. + +, 5–20 particles/400 mesh grid square. +, < 5 particles/400 mesh grid square. Bacteriology: nad, no pathogens isolated from stool.

that we felt was greater than normal posseting was noted on six days (days 1, 4, 6, 9–11). Viruses were found in his stools from day 6 onwards, initially rotavirus but changing to astrovirus on day 8. He was febrile (38·3°) on admission and remained febrile during most of his stay. He was put on clear fluids after a soft stool (day 3) and considerable vomiting. There was no further vomiting on day 5 and half strength milk was re-introduced on day 6. This coincided with further vomiting and a soft stool and the first observation of viruses. Even with a particular interest in saving stools, none was kept for examination on day 5. This would have been one of the most valuable from this baby and its absence highlights the problems of investigating gastroenteropathy thoroughly. An antibiotic (erythromycin) was not thought necessary until day 11 and by this time the gut disturbance had already occurred. It does not look as if the antibiotic played any part in causing it and this point is considered in more detail below.

Similar records were kept of the other 26 admissions, but space does not permit publishing each. They have been summarized in Tables 2 and 3 and, to make

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Table 2. *Viruses and diarrhoea, vomiting or upper respiratory tract infection*

	Diarrhoea*			Vomiting			Febrile			Diet altered	
Baby	Days	Virus (days)	Causal relationship?	Days	Virus (days)	Causal relationship?	°C	(Days)†	URTI‡	Days	To what§
1	2–6, 9–12	RV (9, 11)	Doubtful	1 (11)	RV (11)	Doubtful	40	(1–5, 11)	No	5	IV infusion
2	1	None	No	1 (–1)	None	No	39	(1)	D	No	—
3	None	None	—	'From 2/52'	None	No	No	—	No	No	—
4	B3, 4, 5, 11, 12‖	Ad (10)	Doubtful	4 (1, 3, 5, 6)	None	No	38	(5)	No	No	—
5	None	None	—	'For 2/12'	None	No	No	—	No	No	—
5	None	(RV (2, 3, 5, 7))	—	No	(RV (2, 4, 5, 7))	—	39	(4)	D	No	—
6	None	(SRV (4, 6))	—	No	(SRV (4, 6))	—	38	(1–5)	B	No	—
6	9–11	RV (9–11)	Yes	4 (5, 10–12)	RV (9–11)	Yes	39·4	(8–12)	D	No	—
7	?4, 5	AV (4, 5)	Yes	2 (4, 5)	AV (4, 5))	Yes	No	—	B	No	—
8	3, 4, 5	SRSV (3)	Yes	No	(SRSV (3))	—	38	(1)	B	No	—
9	8	AV (8)	Yes	No	(AV (8))	—	38	(2)	B	No	—
10	None	None	—	2 (5, 6)	None	No	39	(1, 6)	Yes	No	—
11	3	SRV (3)	Doubtful	3 (3, 4, 10)	SRV (3)	Doubtful	No	—	B	No	—
12	None	None	—	((–1)	None	—	No	—	Yes	No	—
13	B2‖	None	—	1 (4)	None	—	37·5	(2, 3)	Yes	1½	CF
14	None	(Ad (2, 3, 5), AV (22–24)	—	2 (8, 22)	AV (22)	Yes	No	—	B	No	—
14	None	None	—	No	None	—	No	—	Yes	No	—
15	1, 2, 3, 5	RV (1–6)	Yes	4 (–1, 1, 2, 3)	RV (1–6)	Yes	39	(1)	D	4	M/2
16	None	None	—	1 (–1)	None	—	39·5	(1, 2)	No	No	—
17	2, 3	None	—	None	None	—	39·5	(2)	Yes	No	—
18	None	None	—	1 (–2)	None	—	38	(1)	Yes	No	—
19	–2, –1	None	—	5 (–2, 1, 3, 8, 9)	SRSV (9)	Doubtful	38·5	(3–5)	B	2	CF
20	3, 6	SRSV (4)	Yes	1 (5)	SRSV (4)	Yes	38·5	(1)	B	No	CF
21	6, 9, 12	RV (6–8), AV (8–13)	Yes	6 (–1, 4, 6, 9–11)	RV (6–8), AV (8–13)	Yes	38	(1, 4, 5, 10)	B	5	CF, M/2
22	4, 5	RV (2, 4, 5, 6)	Yes	7 (–1, 1, 2, 5, 8, 9, 11)	RV (2, 4, 5, 6), SRSV (11)	Yes (2)	40	(1, 2, 5)	No	6	CF, M/4
23	None	E5	—	3 (2, 3, 5)	E5	No	40	(1–4)	D	No	—
24	–2, –1, 2	RV (2, 3)	Yes	1 (1)	RV (2, 3)	Doubtful	No	—	B	2	M/2

* Diarrhoea defined as three or more stools per day.

† Days of admission when febrile.

‡ URTI: Upper respiratory tract infection. B: Before virus excretion. D: During virus excretion. Yes: URTI in a baby with no virus detected in the stools. No: No URTI.

§ CF: Clear fluids; M/4: Quarter strength milk; M/2: Half strength milk.

‖ B3, B2: Diarrhoea 3 and 2 days before admission respectively.

Table 3. *Viruses and illness*

Virus	Associated with: Diarrhoea	Vomiting	URTI	No illness	Total
Adenovirus	(1)*	0	(1)	3	5
Astrovirus	2	4	0	0	4
Echovirus	0	(1)	(1)	0	1
Rotavirus	5 (2)	3 (2)	5	1	8
SRSV	2	2 (1)	2	0	4
SRV	(1)	(1)	2	0	2

* Figures in brackets indicate a doubtful association.

particular points, those of patients 1, 4, 6, 15, 21 and 22 have been illustrated in Fig. 2.

Viruses and diarrhoea

Some form of diarrhoea was associated with 15 of the admissions. The episodes varied from one or two loose stools and vomiting on two days (patient number 7) requiring no special treatment (and associated with astrovirus excretion) to a more severe and prolonged biphasic diarrhoea extending over about 12 days with two febrile episodes coinciding with the peaks of diarrhoea (patient number 1). Only the second bout of diarrhoea was associated with a virus (rotavirus) and the amounts of virus seen were small. Viruses were associated in time with these episodes on nine occasions (4 rotaviruses, 2 astroviruses, 2 small round structured viruses (SRSV) and one dual infection with rotavirus and astrovirus). There were three rather more doubtful associations (patients 1, 4 and 11) in two of which the virus was found only after the main episode of diarrhoea (patients 1 and 4). In the third patient (number 11) a virus was seen at the peak of diarrhoea (six stools in 1 day) but it showed some substructure which was similar to that seen on some cubic bacteriophages.

In four more patients (numbers 5, 6, 14 and 23) viruses were detected which had no associated diarrhoea; rotavirus (1), small round virus (SRV) (1), a fastidious adenovirus (1) and an echovirus type 5. Of these, all but the echovirus have been found in diarrhoeal stools in other studies.

In the case of patient number 1 (Fig. 2), the main episode of diarrhoea occurred in the 6 days after admission with a peak of 19 bowel movements on day 3 and the child was on an intravenous drip for all 6 days. The stools were all watery but examination yielded no pathogens, bacterial or viral, although initially it was thought that a cytopathic agent (? virus) was present in one of the stools. The cytopathic effect could not be passed to fresh cultures and no agent was identified. The second episode coincided not only with excretion of moderate amounts of rotavirus but also with the re-introduction of full cream milk from day 8. He was changed to clear fluids on day 11 and settled rapidly thereafter. Rotavirus was seen in his stools only on days 9 and 11, not before and not after.

Ten admissions lasted 9 or more days. Without exception all these ten babies began to excrete a previously undetected virus in the period from 6 days after admission onwards (Table 4). These are probably all hospital-acquired, with the possible exception of the adenoviruses. Seven of these 'late' infections were

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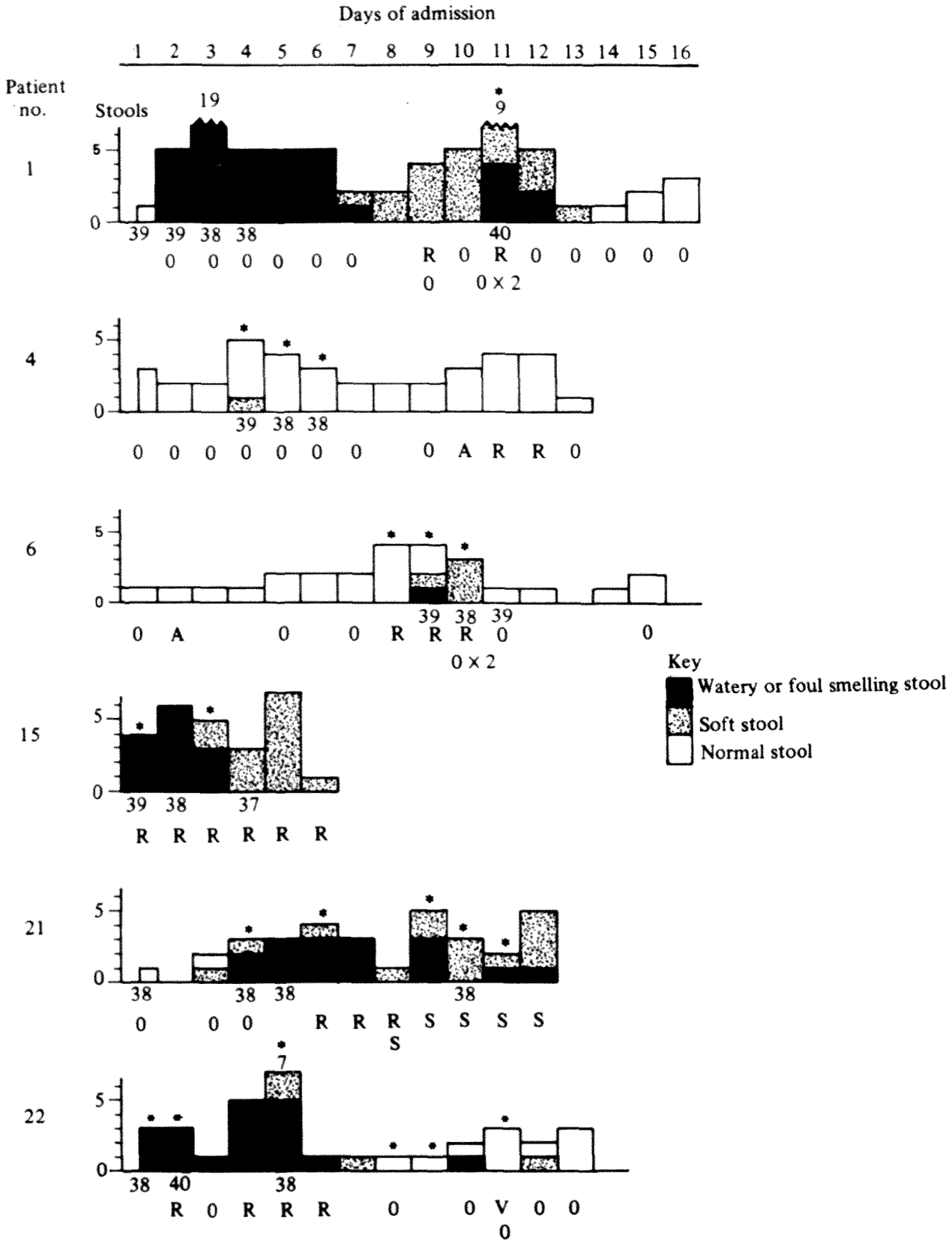

Fig. 2. Stool and virus records of six patients. Asterisk: Significant vomiting during the day. Figures below the line: Temperature higher than normal. Letters below the line: Viruses found by electron microscopy: A, adenovirus; R, rotavirus; S, astrovirus; V, small round virus; 0, No virus seen.

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Table 4. *Late acquisition of viruses in hospital*

Patient No.	Duration of admission (days)	Virus excreted (days excretion started)	Gastro-intestinal upset?	1st or subsequent virus
1	18	RV (9)	Yes (see text)	1st
4	13	AdV (10, RV (11)	Doubtful	1st
5	15	AdV (15)	No	2nd
6	19	RV (9)	Yes	1st
9	9	AV (8)	Doubtful	1st
11	10	AdV (7)	Doubtful	1st
14	24	AV (22)	No	2nd
19	9	SRSV (9)	No	1st
21	13	RV (6), AV (8)	see Fig. 1	1st
22	13	SRSV (11)	Doubtful	2nd

accompanied by some alteration of bowel habit, but in only two (one of them patient 1) was this more than a slight increase in the number of motions passed.

Viruses and vomiting

All young children are liable to regurgitate part of their feeds, particularly when they swallow air. When this becomes abnormal is even more difficult to decide than what is or is not normal bowel function. Twenty one of the admissions were associated with vomiting. Of these six occurred before admission (anything up to two months in duration) but were not confirmed in hospital. Another four started prior to admission and continued at intervals in hospital. These were all associated with a virus, two of them from the first or second day onwards (2 rotaviruses) and in the other two virus was not found until 6 days (rotavirus) or 9 days (SRSV) after admission.

Of the remaining eleven episodes, viruses were found in the stools of eight of them at the same time. These included three rotaviruses, two astroviruses, one SRV, one SRSV and the echovirus type 5. With three of these (one rotavirus, one SRV and the echovirus) we were not convinced that the virus was likely to be the sole cause of the vomiting. In four babies virus was seen in the stools with no associated vomiting (one rotavirus, one astrovirus, one SRSV and one SRV).

Specimens of vomitus were not examined directly. There have been few reports of virus being found and it is difficult to make satisfactory electron microscopy preparations from it.

Respiratory tract infections

It has been reported that an upper respiratory tract infection (URTI) often precedes a rotavirus infection of the gut (Lewis *et al.* 1979). Twenty one of the admissions were associated with an URTI and in ten of these, it occurred before a virus was seen in the stools. The viruses included one rotavirus, three astroviruses, three SRSV, two SRV and one dual infection with rotavirus and astrovirus. In five there was a respiratory component which occurred at the same time as the virus excretion, and six had a respiratory tract infection but did not excrete detectable virus.

Three episodes of rotavirus excretion (patients 1, 4 and 22) were not associated with a respiratory tract infection. In one of these (patient number 22) an SRSV was also observed.

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Fever

Eight of the babies were afebrile throughout but the remainder had at least one episode. Of these, seven lasted more than one day but none exceeded 40 °C. On only four occasions did the fever accompany virus excretion.

Other pathogens identified

Herpes simplex virus was isolated from the stomatitis of baby 11 on day 2. *Str. pneumoniae* and *H. influenzae* were isolated from a throat swab taken from baby 12 on the first day of admission. Respiratory syncytial virus was identified by immunofluorescence (Department of Virology, Belvidere Hospital, Glasgow) in the nasopharyngeal secretions of babies 17 and 18 on the second and first days of admission respectively. Echovirus type 5 was isolated from a stool from baby 23 taken on the day of admission. Although admitted with a febrile convulsion, he did not have meningitis.

Diet

During seven of the admissions the normal diet (usually full cream milk without solid supplements) was altered to reduced strength or to clear fluids. Only one child required intravenous fluids. Of those who required an altered diet, four were excreting rotavirus at the time or a day or so later. The child who needed intravenous fluids was patient number 1 referred to earlier. He excreted rotavirus later and was put on clear fluids for 1 day.

Antibiotics

Fourteen of the babies were given antibiotics either in the few days before admission or in hospital. They were prescribed usually for the respiratory component of the child's illness and included (with the number of patients in brackets) penicillin (two), ampicillin (three), amoxycillin (three), cloxacillin (one), co-trimoxazole (three), erythromycin (four), cephradine (one) and gentamicin (one). In some cases they were given in combination. It has been suggested that antibiotic use could precipitate diarrhoea, particularly with broad spectrum antibiotics, and we looked at the individual records for evidence to support this. We found no regular pattern; in four patients diarrhoea accompanied antibiotic treatment but in two of them viruses were also present. Antibiotic-induced diarrhoea is more likely to follow treatment and diarrhoea occurred in three patients. It was usually mild and the interval between the end of treatment and diarrhoea varied from two days to more than a week.

DISCUSSION

The number of babies in this study is too small for many conclusions to be drawn, and was terminated because those concerned moved to posts elsewhere. Nevertheless some comments can be made that could be explored further in other studies.

The study was begun because previous investigations into the ecology of stool viruses (Madeley *et al.* 1977; Madeley, Cosgrove & Bell, 1978; Scott *et al.* 1979) had suggested that virus excretion in stools was very common in Glasgow babies, that the virus excreted changed frequently and unpredictably and that diarrhoea was an ailment which routine hospital records were not precise enough to define. It was particularly to improve on the last that led to the design of this latest study.

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Even if the number of stools passed and their nature is recorded, a definition of diarrhoea remains elusive. Clearly 19 stools in a day is abnormal but with others the borderline is less clear. With patient 6 an increase to 4 stools a day is more likely to be regarded as significant when accompanied by virus excretion but other children excreted several stools a day without virus (e.g. patient 4) while virus excretion was not accompanied by much alteration in bowel habit. Although virus excretion could be associated with an increase in number of stools passed or a change in their consistency there were a number of episodes not accompanied by viruses at all. Examples are found in the records of patients 1 and 4 but only the former was severe enough to require specific treatment.

Although considerable efforts were made to obtain daily stools from all these patients, some were missed and these amounted to some 18%. Their absence has left some queries and the interpretation of the onset of diarrhoea in patient 21 (Fig. 1) would depend very much on whether a stool had been examined on day 5. There could have been three possible results of such an examination and the interpretation would have been different with each. No virus would have left the rotavirus as something of a late arrival, raising the question of how long after the onset of diarrhoea can one expect the precipitating micro-organism to appear in the stool. In the case of bacterial enteritis or colitis the organism is normally found in the first stool obtained – what about one seen two or three days later? The second is of a virus other than the rotavirus and astrovirus seen later. This would have cast some doubt on the role of rotavirus as causative agent whilst the third possibility of a rotavirus would have strengthened the evidence. The time of virus excretion in relation to the signs and symptoms of disease has not been explored to any depth. In an age group where the virus excreted changes frequently, it is an aspect that merits further study.

Most of these babies were in hospital for several days and in several of them there was an interval of several days between admission and the start of virus excretion. This suggests that these infections were hospital acquired and confirms other evidence that these viruses are denizens of paediatric wards, although the damage they do is not usually very great (Totterdell, Chrystie & Banatvala, 1976).

A number of viruses were associated with diarrhoea and/or vomiting. The numbers were, again, small but they included six rotaviruses, three astroviruses, two SRSVs and one patient had rotavirus and astrovirus in rapid succession including some overlap with both viruses and signs of illness (patient number 21). Four of the rotavirus infections had a straightforward association with diarrhoea but the other two, in patients 1 and 4, were more doubtful. In the case of patient 1 the main diarrhoeal episode had already occurred and the virus excretion coincided with re-introduction of full cream milk. Which of these events actually precipitated the second episode of diarrhoea isn't clear but it might have been either, or both.

All the kinds of virus found in the stools of these babies were associated with some alteration of bowel habit and all probably have a potential for causing illness, although it is not exercised on every occasion. It is noteworthy that a hospital stay of nine days or more was always accompanied by evidence of a hospital acquired infection. Evidence of long-term excretion of faecal adenoviruses was obtained in the previous study (Scott *et al.* 1979) and these may have been brought in by the baby concerned. The others would appear to have been acquired in the ward.

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No evidence to suggest that antibiotics played any role in causing diarrhoea was obtained. A thorough exploration of this subject would require more patients than we had but we found no evidence to suggest that the results would justify the effort.

This brief study has shown that a more objective criterion of diarrhoea would be valuable. The clinician will want to know whether the bowel is functioning normally, not what it does with surplus contents. A measurement of absorption would allow a better estimate of the damage due to viruses to be made. Secondly, the data suggested that rotavirus infection was more likely than any other virus to cause a disease severe enough to require modification of the child's diet, although rotaviruses did not always cause disease. Whether differences in any disease induced are due to different 'serotypes' was not explored due to a lack of reliable markers. A full investigation of the effects of viruses on children is still awaited.

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