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Stool viruses in babies in Glasgow

2. Investigation of normal newborns in hospital

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SUMMARY

The stools of 37 newborn babies born in hospital were examined for the presence of virus. An extract of every stool passed by each baby was examined in the electron microscope and inoculated into cell cultures. The babies were delivered in four separate maternity units (A–D). All the babies from units A and C (9 babies) were found to be excreting rotavirus though none showed any evidence of diarrhoea. Two of the babies also excreted astrovirus. Subsequently unit A was closed for cleaning and, on reopening with more restrictions on visitors, a further 19 babies were examined. No virus was found in any of their stools, nor was virus observed in the stools of babies from units B and D, where visiting was also more restricted. Visiting restrictions in these units excluded older siblings of the babies. No virus was cultured from any stools in this study.

INTRODUCTION

In the course of a previous study (Madeley *et al.* 1977) we were asked to investigate an outbreak of enteritis in the post-natal ward in another hospital. Examination of stools from six cases by electron microscopy showed the presence of viruses in all babies. Viruses were also observed in the stools of a quarter of symptom-free babies examined as controls. This symptom-free excretion of viruses, which has also been observed by other workers (Chrystie *et al.* 1975; Murphy, Albrey & Crewe, 1977) led us to investigate symptom-free babies from birth in a number of maternity units by both electron microscopy and routine virus culture. Initially every stool passed by each baby was examined but later as the patterns became clearer daily stools only were obtained.

MATERIALS AND METHODS

Patients

In all 37 babies were investigated. Twenty-seven were from the original unit (A), 4, 3 and 2 from 3 other units respectively (B–D) and 1 was a baby delivered in hospital and discharged home on the second day (H). Of the 27 from the original unit, the first 8 were investigated before closure and redecoration of the ward and

the changing of visiting arrangements in June 1975 (see below), while the other 19 were born and investigated after the ward was reopened. Units A and C are units in district general hospitals, B is in a teaching hospital and D is a small GP hospital on the island of Bute. To enable us to investigate each baby for as long as possible the initial 8 babies from unit A and the 4 from unit B were drawn from the special care nursery rather than the general post-natal ward, but the remainder were normal full-term babies. Mostly the babies in special care were premature. There were 15 males and 22 females in the study. Thirty-five were seen in the period Feb.–Oct. 1975 and the remaining 2 in 1976. All babies were bottle fed, and at the time of the study it proved impossible to find enough breast-fed babies in the Glasgow area for a comparison to be made.

Stool specimens

Stool specimens were obtained from all babies from birth onwards. In the case of babies A1–8, B1–4, C1–3 and D1–2 a sample of every stool the baby passed, including meconium, was examined. From babies A9–27 a daily stool only was obtained and from the baby discharged home at 2 days (H1) a daily stool for the first 9 days of life was obtained. All stool specimens were examined by electron microscopy and routine cell culture. Since the babies were all symptom-free no bacteriology was carried out and a study on the acquistion of bowel bacteria has already been done in the hospital containing unit B (McAllister *et al.* 1974; Kerr *et al.* 1976).

Virology

The methods for processing and examining the stools were as previously reported (Madeley *et al.* 1977).

RESULTS

Electron microscopy

A total of 572 stools were obtained from the 37 babies in this study. The results of examination of stools from babies in unit C and from those in A up to 1 June 1975 (Group 1) are shown in Table 1 while those from units B and D and the baby discharged on day 2 (Group 2) are shown in Table 2. A comparison of the two tables showed that 10/11 babies in Group 1 excreted viruses at one time or another during their period in hospital while none of those in Group 2 did so. The duration of follow-up at 9 and 11 days respectively was similar as was the average number of stools examined from each baby, 23 and 25 respectively. From these figures it is apparent that failure to find virus in units B and D and from the home is unlikely to have been due to inadequate sampling.

None of the babies in this study had symptoms of enteritis. The babies who excreted virus in their stools all came from 2 units where there had been a number of babies with episodes of 'loose stools' or diarrhoea. The staff were therefore very sensitive to evidence of enteritis, and none was obtained. Ten babies excreted rotaviruses, starting at a mean of 4 days after birth (range 3–6 days) and continuing for a mean of four days (range 2–> 5 days, with one of the babies still

Table 1. *Babies in Units A and C examined up to 1 June 1975 (Group 1)*

Baby no.	Sex	Date of birth	Period of examination (days)	No. of stools examined	Symptoms	Viruses observed	Electron microscopy: First observation — Day of life	Electron microscopy: First observation — Stool no.	Electron microscopy: Duration of excretion (days)*	Electron microscopy: Range of virus qty seen†	Viruses isolated in cell culture
A1	M	12 Feb. 75	13	31	No	Rotavirus	4	10	2	sn − + +	Nil
A2	F	25 Mar. 75	7	28	No	Rotavirus	5	15	3	sn − +	Nil
A3	M	2 Apr. 75	7	22	No	Rotavirus	3	5	4	sn − + + +	Nil
						Astrovirus	7	22	> 1	+ +	
A4	M	6 Apr. 75	6	28	No	Rotavirus	3	10	2	sn − +	Nil
						Astrovirus	5	19	> 2	sn − +	
A5	F	19 Apr. 75	10	35	No	Rotavirus	3	9	3	sn − + + +	Nil
A6	M	17 Apr. 75	12	48	No	Rotavirus	3	12	5	sn − + +	Nil
A7	M	19 May 75	2	11	No	Nil	—	—	—	—	Nil
A8	M	27 May 75	5	7	No	Rotavirus	4	5	> 2	sn	Nil
						Astrovirus	4	5	> 2	+ + − + + +	
C1	F	21 Feb. 75	14	24	No	Rotavirus	6	9	5	sn − + + +	Nil
C2	M	5 Mar. 75	14	10	No	Rotavirus	4	4	3‡	+ − + +	Nil
C3	M	17 Mar. 75	7	16	No	Rotavirus	4	4	3	sn − + +	Nil
	Averages:		9 (Range 2–14)	Total 260 Av. 23			4 (Rotavirus) 5 (Astrovirus)	8 (Rotavirus) 15 (Astrovirus)	3 (Rotavirus) > 2 (Astrovirus)		

* A > sign indicates that the last stool examined was positive for virus.

† Quantities as seen on the microscope grid. + + +, 20–100 particles per grid square; + +, 5–20 particles per grid square; +, 0–5 particles per grid square; sn, small numbers only.

‡ Virus observed + in stool no. 4 (day 4) and + + in stool no. 8 (day 6) only, with three negative stools between.

Table 2. *Babies in Units B and D and from Home (Group 2)*

Baby no.	Sex	Date of birth	Period of examination (days)	No. of stools examined	Symptoms	Viruses detected: Electron microscopy	Viruses detected: Culture
B1	F	26 May 75	11	29	No	Nil	Nil
B2	M	29 May 75	7	20	No	Nil	Nil
B3*	F	7 June 75	25	32	No	Nil	Nil
B4*	F	7 June 75	18	40	No	Nil	Nil
D1	F	16 Oct. 75	5	26	No	Nil	Nil
D2	F	20 Oct. 75	5	19	No	Nil	Nil
H1	F	28 Apr. 75	9	10	No	Nil	Not done
	Averages		11	Total 176 Av. 25			

* Twins.

Table 3. *Number of babies excreting rotaviruses on each day of life*

Day	0*	1	2	3	4	5	6	7	8	9	10	11
No. of babies excreting rotavirus	0	0	0	4	8	8	7	4	2	2	2	1†

* Meconium.
† Continues to day 13.

excreting virus on discharge at 5 days). The number of babies excreting virus on each post-natal day is shown in Table 3 from which there are indications that the peak of excretion occurred at 4–5 days of life. None of the babies was found to be excreting virus at birth nor on days one or two. The amount of virus present varied considerably from small numbers (virus not present in all grid squares examined) to + + + (20–100 particles/grid square). The virus was easily recognizable although on 5 occasions the first particles seen were somewhat 'moth-eaten' (Pl. 1*a*). Particles seen in later stools were quite typical in morphology and there was no suggestion of antibody being present on the surface (Pl. 1*b*). A rotavirus from an acute case of enteritis (Pl. 1*c*) is included for comparison. As expected, no evidence of the growth of the rotaviruses in the cell cultures used was obtained.

Babies A3, A4 and A8 also excreted astroviruses starting 7, 5 and 4 days after birth respectively. All were discharged home while still excreting them but with no evidence of enteritis resulting from this second (and in all cases dual) infection.

Baby C2 excreted rotaviruses (+) on day 4 and again (+ +) on day 6 with three negative stools between. It was not possible to make exactly comparable preparations of all stools and this hiatus may indicate either variations in preparation or in amounts of excretion. A new extract of these negative stools was not made.

No virus was observed in the stools of baby A7 which was only followed up for 2 days. Six of the 10 positive babies did not begin to excrete until day 4 or later.

Where virus was observed excretion lasted at least 24 h and usually much longer in the case of rotaviruses. The duration of excretion of the astroviruses may have

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Table 4. *Babies from Unit A examined after 1 June 1975 (Group 3)*

No. of babies and nos.	Sex		Dates of birth	No. with symptoms	Average duration in days of follow-up (days) (Range)	Average no. of stools examined* (range)	Viruses detected	
	M	F					Electron microscopy	Culture
19† (A9–A27)	6	13	26 Aug. 75 –18 May 76	0	7 (6–10)	7 (4–11)	Nil	Nil

* Total number of stools examined = 136.
† Includes one set of twins (A16 and A17).

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Table 5. *Comparison of visiting regulations and virus excretion*

Unit	Period	Type of visiting permitted	No. of babies	Excreting virus (%)
A	Up to June 1975	Unrestricted	8	7(87·5%)
A	After June 1975	Restricted to adults only	19	0(0%)
B	Throughout	Restricted to fathers only, masked	4	0(0%)
C	Throughout	Unrestricted	3	3(100%)
D	Throughout	Restricted to fathers and grandparents only	2	0(0%)
H	—	Unrestricted	1	0(0%)

been as long, since all the babies excreting them were still doing so when sent home.

In contrast, no viruses were detected by electron microscopy in Group 2 (babies in units B and D and from the home). All but one of these babies were born after the last baby in Group 1 and this raises the possibility of the virus 'season' being over. However, these viruses were being detected in stool specimens from babies in other hospitals in Glasgow at this time although not with great frequency. In other respects Groups 1 and 2 are comparable.

In June 1975 unit A was closed for cleaning and redecoration and reopened in August 1975. Another 19 babies were examined in the subsequent 9 months, 16 of them being born during September and October. These babies form Group 3 (Table 4). Based on previous experience with the Group 1 babies (Table 1) it was decided that a daily stool specimen would be adequate to detect any virus excretion. Accordingly no attempt was made to examine every stool passed.

No virus excretion was detected in any of the babies of this group, though the duration of follow-up (mean 7 days, range 6–10) was such as would have detected all the rotaviruses found in Group 1. Similarly no symptoms were detected in any of the babies.

Tailed structures typical of bacteriophages were seen in the stools of 25 of the 37 babies in the study. In 2 babies they were present in the first stools received, one and two days after birth respectively. In 17 of these 25 babies no other virus was observed, and only small numbers of these phages were seen in any one stool.

Cell cultures

In all, 542 stools were cultured by the standard methods using secondary rhesus monkey kidney and human amnion cells. No viruses were isolated though isolations from stools from other subjects were made in similar cells during the same period. Neither the rotaviruses nor the astroviruses grew in the cells used.

Ward visiting

Units A and C allowed open unrestricted visiting initially and visitors of all ages were allowed without any limit in numbers (Table 5). Visitors were consequently frequent and numerous. Units B and D in contrast allowed only immediate adult close relatives. Unit A, after its closure for cleaning and redecoration, imposed

visiting restrictions similar to those in the other units when it reopened. Viruses were detected only in the stools of babies in those units allowing unrestricted visiting.

DISCUSSION

This study was initiated to find out if symptomless excretion of viruses in the stools of newborns was common. In practice it became a follow-up of 37 babies to observe any acquisition of viruses in the gut early in life analogous to studies on the acquisition of bacteria (McAllister *et al.* 1974; Kerr *et al.* 1976). Virus was only observed in 2 units, A and C, in which virtually all the babies studied were found to excrete virus at one time or another. Excretion was not transient, lasting between 2 and 11 days with a mean of 4 days in the case of rotaviruses. Astrovirus excretion started later and extended over the date of discharge of the infant. In the remaining units no virus excretion was detected, though the relative insensitivity of the electron microscope (which is unlikely to detect virus concentrations of less than 10^6 particles/g of faeces) means that these babies could have been excreting small numbers of virus undetected.

Two kinds of viruses only were observed in this study and it is interesting to note that they are both considered to be pathogens. Rotaviruses have been reported from most parts of the world and in the majority of reports they are linked with enteritis (Leading Article, 1977). Reports of symptomless excretion have been made but less frequently and usually in newborns (Chrystie *et al.* 1975; Murphy, Albrey & Crewe, 1977). Astroviruses, though less commonly reported, show a similar association with disease (Madeley & Cosgrove, 1975; Kurtz, Lee & Pickering, 1977). Our own results, to be published, show that both viruses show an approximate 80 % association with symptoms although with neither virus does the rate approach 100 %. Recent reports (Zissis & Lambert, 1978; Thouless, Bryden & Flewett, 1978) suggest that there may be more than one serotype of rotavirus. It is possible that our babies were infected with an avirulent serotype but since there were infections associated with symptoms in units A and C this may not be the explanation.

None of the babies in this study showed symptoms of enteritis. The point at which a stool becomes soft enough to be significant is an arbitrary one on a continuous scale between 'hard' to 'watery'. In practice this distinction is readily made and, in unit A in particular, the staff were very worried about the possibility of more babies in the ward developing diarrhoea. This meant that from the start of this study symptoms in this unit would have been readily detected. None of the babies showed such symptoms.

The amount of virus excreted in rotavirus infection frequently reaches very high levels (up to 10^{10} particles/g of faeces or more). Levels as high as this were not observed in these babies, although the numbers of virus particles present in the stools were high enough to allow easy detection and were within the range found in association with symptoms. Intestinal hurry, by moving gut contents along faster, could flush out a quantity of virus over a shorter time and give an impression of a higher level of excretion. Such lower levels as we observed may not

therefore mean that *in toto* less virus was produced. No attempt was made to prepare exactly comparable extracts of all stools, and therefore no definite conclusions about the amount of virus seen in stools can be drawn. However, baby C2 excreted detectable amounts of virus on two occasions separated by 3 negative stools and this may reflect differences in preparation. Alternatively, it may represent variable flushing out.

In half the babies in which virus was seen the first rotavirus appeared to be damaged, with irregular surface structure and frayed outlines. This atypical appearance seemed to be due to a loss of structure rather than any coating. No babies excreted virus in the stools passed on the first and second days of life but the damaged particles could have been lying within the gut for a period and, if so, an intrauterine infection cannot be ruled out. No direct evidence to support this possibility was obtained. The outline of the virus particles did not suggest the presence of antibody and surface structure was visible clearly. In some babies the appearance of virus improved in subsequent stools, and it seems unlikely that the amount of antibody present, if secreted locally, would diminish in the presence of the antigen. Maternal antibody (if present and secreted into the gut) would be more likely to prevent infection altogether, and none of the children was breast-fed.

In this study, then, symptomless excretion of two viruses which are often associated with symptoms has been found with high frequency in 2 units and not at all in 2 more. This poses two questions:

1. Where did the virus come from?
2. Why was it found in only 2 of the units?

McAllister *et al.* (1974) found rapid colonization of newborns with bacteria at all body sites and, later, Kerr *et al.* (1976) found that in one third the probable source was the mother's gut. Symptomless gut carriage of any viruses cultivable in cell cultures in adults is uncommon and little is known about the epidemiology of rotaviruses or astroviruses, particularly in adults. There was close contact between mothers and their babies in all units. Virus excretion was found only in two of the units and this makes a maternal source seem unlikely.

Von Bonsdorff *et al.* (1976) found evidence of excretion of rotaviruses by adult hospital staff with symptoms in Finland and it is possible that there were excretors among the nursing or medical staff of units A and C, though no symptoms were reported. There were no changes among the senior staff of unit A after the period of closing for cleaning and redecoration, although the junior nursing and medical staff were changed by rotation.

The only association with virus excretion that we found was the type of visiting permitted in the ward. When open visiting was permitted virus excretion was observed and no virus was found in wards where visiting was restricted to close adult relatives. After redecoration of unit A the rules were changed from open to restricted visiting and no viruses were observed thereafter. No record of visitors to the ward was kept but children were among them. Rotaviruses are most frequently found among children under 5 years old and serological evidence obtained by Kapikian *et al.* (1975) suggested that, in the USA at least, exposure to the virus reaches over 90 % by this age. Children, then, could have been the

source of the infection and this would lend some support to the traditional view that children should be kept out of post-natal wards. However, the baby discharged home early had 4 older siblings, and no virus excretion was detected. Since these viruses were excreted with no symptoms it may be difficult to demonstrate that such infection was harmful to the baby and, if it could be shown to induce a local gut immunity, it might even be thought to be beneficial.

Definite answers to the questions posed above have not been provided by this study, but it is tempting to believe that virus may come from the baby's older siblings or other children visiting the ward. Proving this will be difficult.

We are grateful to Drs Barr, Fox, Fyfe, Kerr, Patrick and Riley for allowing us to study the patients under their care, to the nursing staff of the 4 units and the district midwife (Sister N. J. Graham) who between them patiently collected, labelled and dispatched the numerous stool specimens, and to the Scottish Hospitals Endowment Research Trust for a grant (HERT no. 484) to one of us (C.R.M.).

Note added in proof. Since this paper was submitted, Cameron *et al.* (*Journal of Medical Virology* (1978) **2**, 7–13) have found similar patterns of excretion of these two viruses in 10 premature infants. Eight of these developed diarrhoea with viral excretion usually preceding it by 12–72 h.

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EXPLANATION OF PLATE

Rotaviruses seen in faeces. A. Particles seen in an early stool. B. Particles seen in later stool but not from the same patient as in A. C. Particles from the stool of a baby with diarrhoea. All printed at a final magnification of ×200000. Stain: 2% potassium phosphotungstate pH 7·0. Scale bar = 100 nm.

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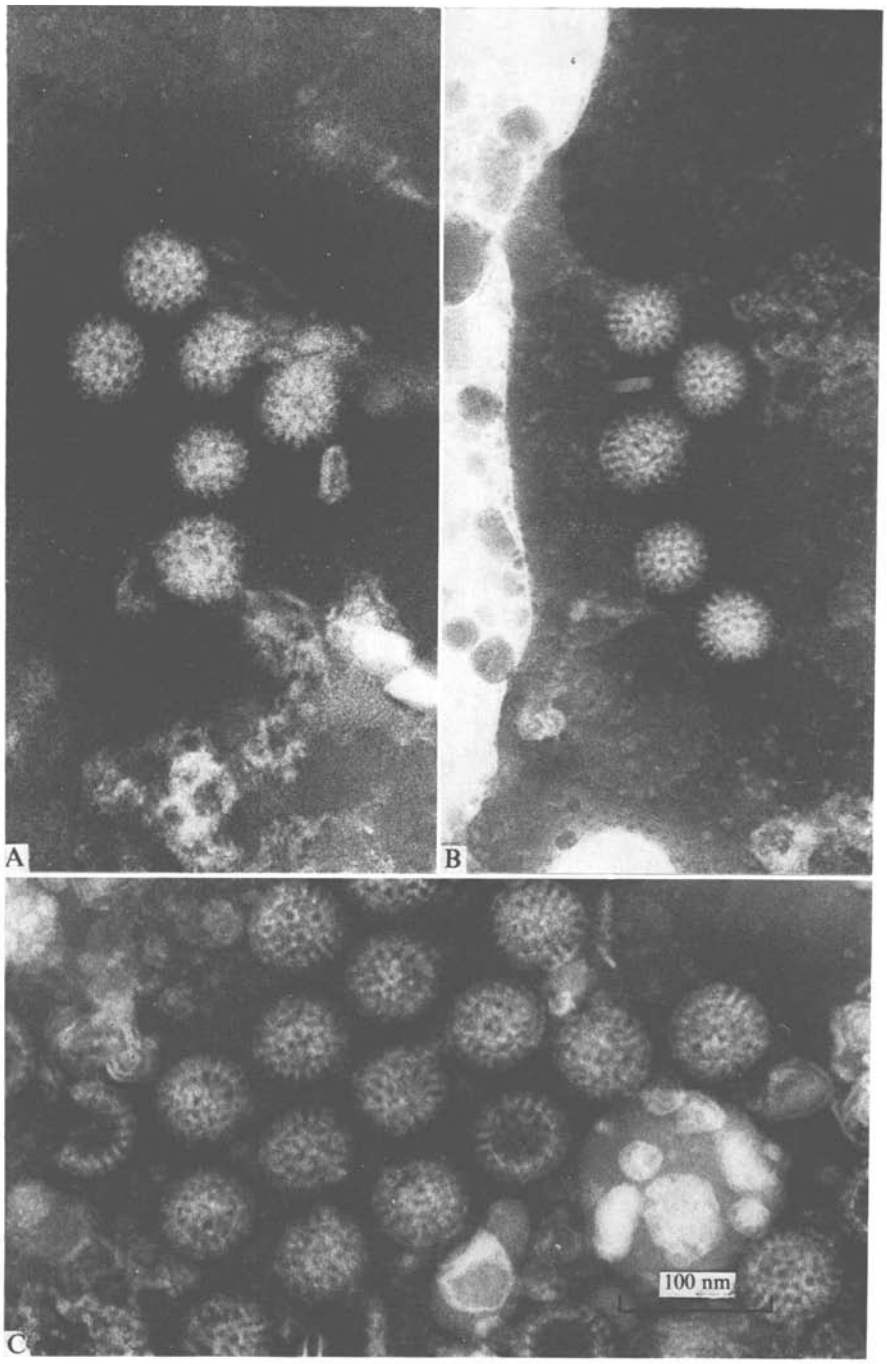

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