Molecular epidemiology of human rotaviruses
Analysis of outbreaks of acute gastroenteritis in Glasgow and the west of Scotland 1981/82 and 1982/83

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SUMMARY

The molecular epidemiology of rotavirus infections in Glasgow and the west of Scotland during 1981/82 and 1982/83 was investigated by electron microscopy, ELISA testing and RNA migration pattern analysis. In 1981/82, rotaviruses of both the 'long' and the 'short' electropherotype (in different variants) co-circulated from the onset throughout the winter peak of the outbreak. Approximately 80 % of the children were infected during the first year of life. No differences in incidence were found between sexes. In 1982/83 the isolated rotaviruses were almost exclusively of the 'long' electropherotype (in different variants) and 36 % of the children were infected beyond the first year of life. Rotaviruses of the 'long' electropherotype serologically were of subgroup II and serotype 1 and those of the 'short' electropherotype of subgroup I and serotype 2.

INTRODUCTION

Rotaviruses have been recognized as the main cause of acute gastroenteritis in young animals and in infants and young children (Flewett & Woode, 1978; McNulty, 1978). Several subgroups (Kalica *et al.* 1981 *a*; Greenberg *et al.* 1983) and serotypes (Beards *et al.* 1980; Wyatt *et al.* 1982) have been identified by complement fixation tests (Thouless *et al.* 1977; Zissis & Lambert, 1978), ELISA techniques (Yolken *et al.* 1978; Thouless, Beards & Flewett, 1982) and neutralization assays (Beards *et al.* 1980; Wyatt *et al.* 1982).

In addition, the genomes of rotaviruses, consisting of 11 segments of double-stranded RNA, have been used to identify and differentiate rotavirus isolates. The RNA of rotaviruses can be readily isolated and segments separated by electrophoresis on polyacrylamide gels (Kalica *et al.* 1976, 1978; Espejo *et al.* 1980; Schnagl, Rodger & Holmes, 1981; Rodger *et al.* 1981; Lourenco *et al.* 1981; Flores

et al. 1982; Pereira *et al.* 1983; Follett & Desselberger, 1983*a*). RNA migration patterns ('electropherotypes', Rodger *et al.* 1981, Schnagl, Rodger & Holmes, 1981; 'electrophoretypes', Thouless, Beards & Flewett, 1982) can thus be determined. Rotaviruses of different RNA migration pattern were isolated from infected children in different areas of the world either sequentially (Rodger *et al.* 1981; Schnagl, Rodger & Holmes, 1981) or at the same time (Espejo *et al.* 1980; Rodger *et al.* 1981; Flores *et al.* 1982; Follett & Desselberger, 1983*a*).

Attempts have been made to classify rotaviruses according to their RNA migration patterns (Lourenco *et al.* 1981; Rodger *et al.* 1981; Schnagl, Rodger & Holmes, 1981). Furthermore, attempts were made to correlate RNA migration patterns with differences observed by serological means (Kalica *et al.* 1981*a*; Greenberg *et al.* 1981; Kutsuzawa *et al.* 1982; Greenberg *et al.* 1983). This turned out to be difficult, as viruses of the same subgroup or serotype were found to be genomically heterogeneous and, on the other hand, strains of different serotypes within one subgroup showed an indistinguishable RNA migration pattern (Beards, 1982).

We have recently used the rapid and sensitive technique of visualizing nucleic acid in gels by silver staining (Sammons, Adams & Nishizawa, 1981; Herring *et al.* 1982; Follett & Desselberger, 1983*a*) to monitor the appearance of rotaviruses during the onset of an outbreak of acute gastroenteritis in Glasgow and the west of Scotland in 1981. The analysis of samples collected at the beginning of the outbreak in October and November 1981 had shown that rotaviruses differing grossly in their RNA migration patterns cocirculated locally (Follett & Desselberger, 1983*a*). Both the 'long' and the 'short' electropherotypes (Rodger *et al.* 1981; Schnagl, Rodger & Holmes, 1981) were found, and within each of these two patterns further RNA migration differences were observed.

This report is aimed at documenting the extent of cocirculation over a longer period of time and attempts to draw conclusions from the changes observed on the nature of the underlying molecular events.

MATERIAL AND METHODS

Virus

Faecal specimens were obtained from children hospitalized with the symptoms of acute gastroenteritis at Ruchill Hospital, Glasgow from August 1981 to June 1983. In a number of cases, specimens taken repeatedly over several days from individual children were received. Twenty per cent suspensions of faeces in phosphate-buffered saline (PBS = 0·15 M NaCl in 0·01 M phosphate, pH 7·4) were made, thoroughly mixed and clarified by low-speed centrifugation. These crude preparations were used to test for the presence of rotavirus group-specific antigen and for determining subgroups and serotypes. For further purification and concentration virus was pelleted at 115000 g for 1 h through a 30 % sucrose cushion and the pellet resuspended in 0·3 ml of PBS. This material was used for nucleic acid analysis (see below). The Compton U.K. strain of bovine rotavirus was grown in BSC-1 and MA 104 cells and concentrates prepared from the supernatant of infected cells by ultracentrifugation as outlined above.

Electron microscopy

Electron microscopy of samples was performed as described previously (Follett & Desselberger, 1983 *a*).

Enzyme-linked immunosorbent assay (ELISA)

Crude suspensions of virus (see above) were used to test for rotavirus group-specific antigen in an enzyme-linked immunosorbent assay (Rotazyme, Abbott Laboratories, Chicago; Beards & Bryden, 1981) according to the manufacturer's instructions. Simian rotavirus SA 11 was the internal positive standard.

Rotavirus isolates of different RNA migration patterns which were randomly selected from the specimens of the 1981/82 outbreak were assayed in Birmingham for serotype and subgroup specificities. All specimens were assayed under code. Type-specific antisera against human rotavirus serotypes 1, 2 and 3 were raised and used in an ELISA test as previously described (Thouless, Beards & Flewett, 1982). Subgroup specificity was assessed using monoclonal antibodies 631/9/104/56 and 255/60/125/14, kindly provided by Dr H. Greenberg, Laboratory of Infectious Diseases, National Institutes of Allergy and Infectious Diseases, Bethesda, U.S.A. (Greenberg *et al.* 1983). Flat-bottomed polystyrene microtitre plates (Dynatech M 129 B) were coated with rabbit anti-rotavirus serum no. 720 (raised against a mixture of human and calf rotaviruses of different subgroups and serotypes) which was diluted to 1 in 10000 in carbonate–bicarbonate buffer pH 9·8. For each test two plates were used, one for each subgroup. After washing in PBS containing 0·05 % v/v Tween 20 (PBS/T), the samples to be tested were diluted to 1 in 40 in PBS/T containing 1·0 % w/v bovine serum albumin (PBS/T/BSA) and added to the coated wells in duplicate. Relevant group- and subgroup-specific reference antigens and antisera were incorporated as controls in each test. After incubation at 4 °C for 18 h the plates were washed in PBS/T and 100 μl of supernatant fluid from cultured monoclonal antibody-producing hybridoma cells, diluted to 1 in 100 in PBS/T/BSA, were applied to each well. The plates were then incubated for 2 h at 37 °C, washed with PBS/T and to each well was added 10 μl of anti-mouse IgG (whole molecule) alkaline phosphatase conjugate (Sigma Chemical Co.) diluted to 1 in 100 in PBS/T/BSA. The plates were then incubated for a further 2 h at 37 °C, washed and *p*-nitrophenyl phosphate substrate solution added. Reactions were stopped after 15–30 min at room temperature with 3 M NaOH. Plates were read by measuring absorbance at 405 nm wavelength using a Dynatech MR 580 microelisa reader. Samples which repeatedly gave an OD value of greater than 0·1 OD units with one monoclonal antibody and less than 0·02 OD units with the other monoclonal antibody were assigned a subgroup status. In no case were we unable to assign a subgroup using this procedure.

Nucleic acid analysis

RNA was extracted and electrophoresis on 2·8 % polyacrylamide 6 M urea slab gels carried out as described previously (Follett & Desselberger, 1983 *a*). Gels were silver-stained as described by Sammons, Adams & Nishizawa (1981) and as used for the analysis of rotavirus by Herring *et al.* (1982) and by us (Follett &

Table 1. *Outbreaks of infantile gastroenteritis in Glasgow and the west of Scotland 1981/82 and 1982/83: association with rotavirus infections*

	Faecal extracts of children with gastroenteritis					
	Specimens			Patients		
		Rotavirus-positive[a]			Rotavirus-positive	
Month/Year	Total number	Number	Percentage of total number	Total number	Number	Percentage of total number
August 1981	60	2	3·3	45	1	2·2
September	93	5	5·4	66	3	4·6
October	114	25	21·9	70	9	12·9
November	141	28	19·9	92	13	14·1
December	158	55	34·8	105	30	28·6
January 1982	208	63	30·3	145	35	24·1
February	168	41	24·4	128	26	20·3
March	129	20	15·5	98	16	16·3
April	128	25	19·5	78	14	18·0
May	102	16	15·7	73	11	15·1
June	163	13	8·0	106	8	7·6
July	89	0	0·0	61	0	0·0
August 1981 to July 1982	1553	293	18·9	1067	166	15·6
August 1982	142	8	5·6	92	5	5·4
September	115	21	18·3	76	13	17·1
October	131	27	20·6	83	17	20·5
November	127	30	23·6	121	27	22·3
December	113	33	29·2	104	28	26·9
January 1983	91	25	27·5	84	23	27·4
February	77	16	20·8	71	14	19·7
March	98	25	25·5	68	20	29·4
April	114	14	12·3	90	10	11·1
May	162	17	10·5	130	13	10·0
June	141	16	11·4	106	13	12·3
August 1982 to June 1983	1311	232	17·7	1025	183	17·9

[a] Positive by electron microscopy and/or ELISA test. Electron microscopy was mainly used from August 1981 to December 1981. Since January 1982 samples were identified as containing rotavirus by ELISA test exclusively.

Desselberger, 1983a). The only modification from our previously described procedure was that the reducing step was followed by soaking the gels in 5% acetic acid. Destaining of overstained gels was done using the recipe given by Merril, Switzer & Van Keuren (1979). Transilluminated gels were photographed using Ilford FP 4 film.

Statistics

Significance of differences between frequencies was calculated using the chi-square test.
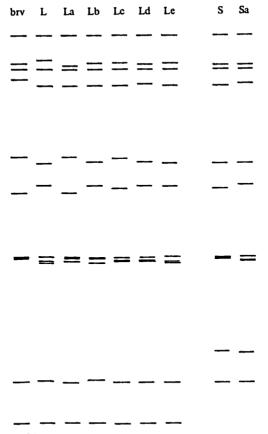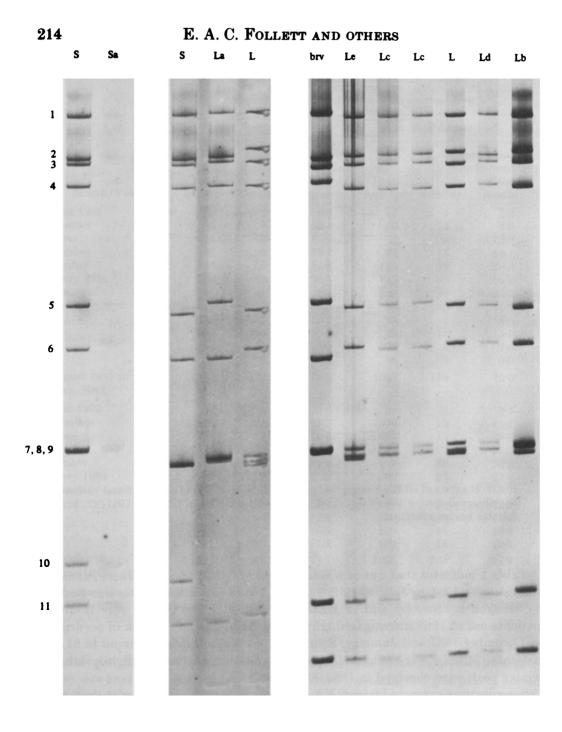
Fig. 1. Diagram of all RNA migration patterns (L and variants La to Le; S and variant Sa) observed during the outbreak of acute gastroenteritis in Glasgow 1981/82. brv denotes bovine rotavirus.

RESULTS

Table 1 indicates that out of a total number of 1553 faecal extracts examined from August 1981 to July 1982, 293 (18·9 %) were found to be rotavirus-positive by electron microscopy and/or ELISA. The positive specimens were from 166 patients out of 1067 investigated (15·6 %). Rotaviruses had a peak of occurrence in December 1981 and January/February 1982 when they were found in 91/378 patients (24·1 %). Very similar frequencies of occurrence and a slightly broader winter peak were observed in 1982/83 (Table 1).

Samples were extracted from RNA and checked for the presence of the rotavirus genome by electrophoresis in polyacrylamide gels which were silver-stained. Both the 'long' and 'short' electropherotypes (termed from the relative migration of RNA segments 10 and 11 of different isolates; Kalica *et al.* 1981 *a*) were found. Due to considerations discussed later they were named L and S, respectively. Electropherotype L has previously been described by us as RNA migration pattern 1, and electropherotype S as pattern 3 (Follett & Desselberger, 1983 *a*). Besides the preponderant L and S RNA migration patterns variants (v) were found deviating from the preponderant patterns in the relative migration of one or more

Fig. 2. Polyacrylamide (2·8 %) 6 M urea slab gels showing RNA migration patterns of human rotavirus isolates obtained during the peak of the outbreak of acute gastro-enteritis in Glasgow 1981/82. There was cocirculation of viruses of the long electropherotype L and variants La to Le, and of viruses of the short electropherotype S and variant Sa. RNAs in panels A, B and C were separated on different gels. Loening's buffer was used, migration was from top to bottom and gels were silver-stained. Segments were numbered on both sides and the electropherotypes are indicated at the top of the tracks. brv denotes bovine rotavirus.

bands. Fig. 1 gives a diagram of all RNA migration patterns observed in 1981/82 (patterns L and La to Le; and S and Sa) and Fig. 2 shows all the RNA patterns observed on three different (panels A to C) polyacrylamide gels.

Rotavirus RNA migration patterns were found in faeces of 146 out of 166 children (88 %) positive in the ELISA test in 1981/82. In no single case where RNA migration patterns were obtained from more than one stool specimen was a double infection with rotaviruses differing in the RNA migration patterns found. The longest period of shedding of rotavirus with a demonstrable RNA pattern was four days.

The sex and age distribution of rotaviruses with the RNA migration patterns observed in 1981/82 are listed in Table 2. It was found that the rotavirus infections occurred in about 80 % during the first year of life, 15 % in the second year and only 5 % beyond the age of two years. There was an even sex distribution of total numbers of isolates at different ages and no difference between the sexes was found when frequencies of occurrence of different RNA migration patterns were compared (chi-square test, $P < 0.05$). Strains of L and Lv patterns outnumbered those of S and Sv patterns by a factor of between two and three. Approximately one-third of the L pattern and about one-eighth of the S pattern isolates were variants Lv and Sv, respectively.

The outbreak of 1982/83 differed from that of 1981/82 by an almost complete absence of the S RNA migration patterns in 1982/83 (Table 3). The total numbers of L and Lv pattern isolates were virtually identical for both years (Tables 2 and 3). As in 1981/82, there was no significant difference in the distribution of rotavirus infections between sexes. But in 1982/83 a significantly higher number (chi-square test, $P < 0.01$) of children beyond the first year of life (38/104, Table 3) were infected compared to 1981/82 (28/146, Table 2).

In Fig. 3 the emergence of rotavirus strains is followed up with regard to the geographical distribution of residences of diseased children within the Greater Glasgow Area during 1981/82. It can be seen that the L and Lv types started in the east and west of the Greater Glasgow Area in October/November 1981 but spread to the south in December 1981, where also a number of Lv types were isolated in the following months. The peak of prevalence of L electropherotype rotaviruses was in January 1982. The S electropherotype rotaviruses were isolated early in September 1981 in the north and east from where they spread to the south and west in December 1981, which was also the month of the highest prevalence of these viruses, i.e. preceding the peak occurrence of electropherotype L rotaviruses (Fig. 3). In March the S electropherotype disappeared completely (with one exception found in June 1982). The viruses isolated outside Glasgow were virtually all of the L and Lv electropherotypes (11 and 12 isolates, respectively; see Table 2 and Fig. 3).

In 1982/83, rotaviruses of the L and Lv electropherotypes emerged first in the east of Glasgow, from where they spread all over the Greater Glasgow Area in October/November 1982. The three rotavirus isolates showing the S electropherotype were found in February, May and June 1983 in widely separated locations of the Greater Glasgow Area.

We had observed earlier with a small number of samples that virus particles possessing the S electropherotype reacted to higher OD values in the Rotazyme

Table 2. *RNA migration patterns of rotaviruses isolated from 146 children with acute gastroenteritis: Ruchill Hospital, Glasgow, August 1981–July 1982*

Age (months)	Female RNA migration pattern					Male RNA migration pattern					Female and male RNA migration pattern				
	L	Lv[a]	S	Sv[a]	Total	L	Lv[a]	S	Sv[a]	Total	L	Lv[a]	S	Sv[a]	Total
<6	14	6	5	2	27	9	6	6	0	21	23	12	11	2	48
6–12	21	5	7	2	35	17	4	14	0	35	38	9	21	2	70
13–24	4	4	1	0	9	4	5	2	0	11	8	9	3	0	20
>24	1	0	1	1	3	3	1	1	0	5	4	1	2	1	8
All age groups	40	15	14	5	74	33	16	23	0	72	73	31	37	5	146

[a] Variants of RNA migration patterns L and S, respectively (see Figs 1 and 2 and text).

Table 3. *RNA migration patterns of rotaviruses isolated from 104 children with acute gastroenteritis: Ruchill Hospital, Glasgow, August 1982–June 1983*

Age (months)	Female RNA migration pattern				Male RNA migration pattern				Female and male RNA migration pattern			
	L	Lv[a]	S	Total	L	Lv[a]	S	Total	L	Lv[a]	S	Total
<6	6	2	0	8	9	5	0	14	15	7	0	22
6–12	13	9	0	22	18	4	0	22	31	13	0	44
13–24	8	2	1	11	7	5	2	14	15	7	3	25
>24	4	0	0	4	4	5	0	9	8	5	0	13
All age groups	31	13	1	45	38	19	2	59	69	32	3	104

[a] Variants of RNA migration pattern L.

| Month 1981/82 | Greater Glasgow area | | | | | | | | Number of isolates of each RNA migration pattern | | | |
| | North | | East | | South | | West | | | | | |
	L, Lv	S, Sv	L, Lv	S, Sv	L, Lv	S, Sv	L	Lv	L	Lv	S	Sv
September		▲		▲					0	0	2	0
October	○		●	▲▲▲			○○○○		5	1	3	0
November		▲▲▲	●●○	▲▲▲▲			●		1	3	6	1
December	○○○●	▲		▲▲	○○○●●	▲▲▲ ▲▲▲	○○○	▲▲	9	3	12	0
January	○○○○○	▲	○○○○○●	▲▲	○○○	▲	○○○○○○	▲▲▲	19	1	7	0
February	○●			▲▲▲	○	▲	○●●	▲▲	5	2	5	1
March	○		○○○○○●		○●		○○	▲	9	2	1	0
April	○○○○		●		○●		○○		7	2	0	0
May	○●		○○		○●		○		5	2	0	0
June	●		○○●		●			▲	2	3	1	0
									62	19	37	2

Fig. 3. Temporal emergence and geographical distribution of rotaviruses isolated in the Greater Glasgow Area in 1981/82. RNA migration patterns are indicated: L ○, Lv ●, S △, Sv ▲. Each symbol represents residence of one patient and the electropherotype of the rotavirus isolate(s). Of 146 patients (see Table 2) 119 were from the Greater Glasgow Area. The other patients (not shown) lived in Lanarkshire, Ayrshire and Dumfries and Galloway; almost all rotaviruses isolated from patients outside Glasgow were of the L or Lv electropherotypes.

test than did viruses of the L electropherotype (Follett & Desselberger, 1983a). This finding was confirmed on a large scale with all the rotavirus isolates of 1981/82 (results not shown).

Table 4 gives the results of serological subgrouping and serotyping (Thouless, Beards & Flewett, 1982) of rotavirus isolates of L, Lv and S RNA migration patterns which were randomly selected from the 1981/82 specimens. All rotavirus isolates tested which showed the L and Lv RNA migration patterns were of subgroup II and serotype 1, whereas isolates characterized by the S RNA migration pattern were of subgroup I and serotype 2.

DISCUSSION

Rotavirus-positive samples in which the viral genome could be identified were obtained from 250 children admitted to a hospital in Glasgow during two consecutive years, 1981/82 and 1982/83, with symptoms of acute gastroenteritis. Typical winter peaks in the incidence of rotavirus gastroenteritis were observed (Kapikian *et al.* 1976; Birch *et al.* 1977; Yolken *et al.* 1978). Brandt *et al.* (1983), in their study on viral gastroenteritis in children over eight years, note 'the clockwise precision of annual waves of rotavirus infections' or 'outbreaks' with the percentage of patients showing a demonstrable rotavirus infection steadily rising from September to January and then steadily declining to August. Although

Table 4. *Subgroups and serotypes of randomly selected rotaviruses of different RNA migration patterns isolated during the outbreak of acute gastroenteritis in Glasgow, 1981/82*

Rotavirus isolate			Titres of reference sera of specificity		
Number	RNA migration pattern	Subgroup	Serotype 1	Serotype 2	Serotype 3
27082-81	L	II	< 20*	< 20*	< 20*
28309	L	II	80	< 20	< 20
28700	L	II	< 40*	< 40*	< 40*
29063	L	II	320	< 40	< 40
29186	L	II	< 40*	< 40*	< 40*
107-82	L	II	1280	40	80
1043	L	II	160	< 40	< 40
1326	L	II	320	40	40
4929	L	II	160	< 40	< 40
26660-81	Lv	II	40	< 20	< 20
28823	Lv	II	< 20*	< 20*	< 20*
28824	Lv	II	640	< 40	80
2762-82	Lv	II	160	< 40	< 40
14305	Lv	II	< 40*	< 40*	< 40*
26678-81	S	I	< 20	160	< 20
27758	S	I	< 40*	< 40*	< 40*
27841	S	I	< 40	640	< 40
28311	S	I	< 40*	< 40*	< 40*
28491	S	I	< 40	320	< 40
28565	S	I	< 20	320	< 20
28996	S	I	< 40*	< 40*	< 40*
194-82	S	I	< 40*	< 40*	< 40*
4420	S	I	< 40	320	< 40
13892	S	I	< 40	320	< 40

* Did not react in serotyping system, probably due to very low levels of serotype-specific antigen.

Testing was performed for serotypes by ELISA as described by Thouless, Beards & Flewett (1982) and for subgroups as described under Materials and Methods. The homologous titres of serotype-specific reference sera against reference antigens were: 320 for serotype 1, 160 for serotype 2, and 2560 for serotype 3. There was a weak cross-reactivity between the reference reagents of serotype 1 and serotype 3.

we are aware of the fact that the size of our sample of hospital patients is small compared to the size of the population of approximately 100000 children younger than 24 months living in the area of residence of the patients we consider the sample to be representative, as we have observed a very similar pattern of overall prevalence.

Analysis of the RNA migration patterns of the isolates has shown that strains differing in these patterns cocirculated not only at the onset of the outbreak (Follett & Desselberger, 1983a) but also throughout the whole period of observation. No difference between the sexes was found for total infections (Brandt *et al.* 1983) nor for infections with viruses of different RNA migration patterns.

During 1981/82, viruses of the 'short' (S, Sv) and of the 'long' (L, Lv) RNA migration patterns occurred side by side, but there were differences in their

geographical distribution and pattern of spread and in their temporal appearance. Among the viruses of the 'long' electropherotype the pattern L was predominant over the Lv patterns (62 as opposed to 19) within the Glasgow area and also showed a clear peak of occurrence in January 1982 (Fig. 3). Rotaviruses of the L and Lv patterns collected from children living outside Glasgow showed no clear pattern of prevalence, possibly due to the relative scarcity of the samples. The 'long' electropherotype rotaviruses overall outnumbered the 'short' electropherotype viruses by between two and three to one. Remarkably, the 'short' electropherotype viruses had less variants and had their peak of prevalence earlier (in December) than the 'long' electropherotype viruses (in January), but disappeared almost completely in March 1982. Most infections (approximately 80 %) in 1981/82 occurred during the first year of life consistent with a relatively high degree of exposure (Brandt *et al.* 1983). In 1982/83, there were only three isolates of the 'short' electropherotype out of 104 patients (compared to 42 isolates of the 'short' RNA migration patterns out of 146 patients in 1981/82). In contrast to this, Sanders, Beards & Flewett (1983) observed that 70 % of the isolates obtained from the West Midlands (UK) during the winter 1982/83 were of the 'short' electropherotype, and rotavirus isolates obtained in London during February to April 1983 were almost exclusively of the 'short' electropherotype (I. Chrystie & U. Desselberger, unpublished data). Thus, differences in the prevalence of certain electropherotypes do exist between different regions of one country during the seasonal high incidence of rotavirus infections. In the Glasgow outbreak of 1982/83 significantly more children older than one year became infected than in 1981/82. This finding could not be attributed to differences in the socioeconomic conditions nor ethnic background (Brandt *et al.* 1983) of the patient groups in the two study years.

In 1982/83, rotazyme-positive faeces of only 104 out of 183 patients contained enough rotavirus RNA to give detectable migration patterns. This success rate is much lower than that achieved in 1981/82, when rotazyme-positive faeces of 146 out of 166 patients contained enough rotavirus RNA to obtain detectable patterns. We have reexamined rotazyme-positive faeces for which the RNA migration pattern could not be determined from the 79 patients in 1982/83 and found that in 60 % of these cases O.D. values of $0.05 < 0.2$ had been observed and that in 32 % of the low O.D. value samples no rotavirus particles could be detected by electron microscopy. Thus we conclude that the lower success rate in 1982/83 with regard to obtaining RNA migration patterns from rotazyme-positive samples was largely due to a relative higher number of faeces containing low concentrations of rotavirus.

Cocirculation of different electropherotypes or replacement of one by another have been found in many parts of the world (Espejo *et al.* 1980; Rodger *et al.* 1981; Schnagl, Rodger & Holmes, 1981; Lourenco *et al.* 1981; Flores *et al.* 1982; Pereira *et al.* 1983; Follett & Desselberger, 1983*a*). At present it is not quite clear how these differences emerge and what their significance is. We have produced oligonucleotide maps from the RNAs of a number of isolates of the 1981/82 collection (Follett & Desselberger, 1983*b*) and it was found that RNAs of different isolates indistinguishable in their RNA migration pattern (of the S electropherotype) showed minor differences in their large oligonucleotides which could be characterized as

sequential point mutations. Their frequency in time was similar to that observed in sequential influenza A virus isolates obtained during an epidemic (Young, Desselberger & Palese, 1979) and thus in our opinion could reflect a comparable degree of circulation in the human population. On the other hand it was observed that RNAs differing in their RNA migration pattern like the predominant L and S electropherotypes showed extensive differences in the positions of their large oligonucleotides, and that the oligonucleotides of the L and Lv electropherotypes also differed extensively (Follett & Desselberger, 1983b). This finding was also the reason for naming the different RNA patterns observed L and S and their pattern variants without further prejudicing their genetic relatedness.

Besides the predominant 'long' and 'short' electropherotypes L and S smaller numbers of at least five genomically different 'long' variants (La to Le) and of at least one 'short' variant (Sa) were found in the collection of 1981/82 rotavirus strains. This finding makes the analysis of genomic relatedness more difficult than was the interpretation of results obtained from closely related isolates of identical RNA migration pattern. The mechanisms leading to the higher degree of genomic diversity in the electropherotype variants of human rotaviruses remain to be elucidated. Possible mechanisms are accumulation of sequential point mutations (Follett & Desselberger, 1983b) in different populations of viruses (genomic drift), reassortment events (Street et al. 1982) or contributions of rotavirus strains normally circulating in other species to the pool of rotaviruses strains circulating in man. An investigation on the degree of genomic relatedness of the L, Lv and S electropherotype strains of the 1981/82 season is ongoing at present, and the results will be reported elsewhere (Desselberger & Follett, in preparation).

It was established serologically that viruses of electropherotype L were of subgroup II, whereas those of the S electropherotype were of subgroup I. This confirms the findings of the literature (Kalica et al. 1981a; Kutsuzawa et al. 1982; Greenberg et al. 1983). All subgroup II rotaviruses were of serotype 1, and all subgroup I rotaviruses of serotype 2, the pattern most often found in the U.K. in recent years (Sanders, Beards & Flewett, 1983). Some of the rotavirus isolates showing an Lv RNA migration pattern reacted serologically like roataviruses of the L electropherotype, thus demonstrating genome diversity within one subgroup and serotype (Beards, 1982). Vice versa, it was found, that viruses of different species belonging to the L electropherotype differed in their subgroup (Thouless, Beards & Flewett, 1982). This is conceivable, as the antigen defining subgroup specificity is coded for by RNA segment 6 (Greenberg et al. 1981; Kalica et al. 1981b) so that mutational changes (or reassortment) in only this segment could affect subgroup specificity. An analogous statement can be made for serotypes whose specificity is dependent on the protein product of segment 9 (Both, Mattick & Bellamy, 1983). Beards (1982) described two rotavirus strains with different serotypes but indistinguishable RNA migration patterns.

Antiserum directed against the common group-specific antigen of SA 11 virus which is used in the Rotazyme test to detect human rotavirus reacted with human rotaviruses of the S RNA migration pattern to higher O.D. values than with human rotaviruses of the L electropherotypes. Our earlier observation (Follett & Desselberger, 1983a) was confirmed by a larger number of samples. As SA 11 virus possesses the serological characteristics of subgroup I, serotype 3 of human

rotaviruses (Hoshino *et al.* 1983) it seems likely that anti-SA 11 antiserum will contain not only anti-group antibodies, but anti-subgroup (and possibly anti-serotype) antibodies as well and that subgroup specificities possibly contribute to the group reaction resulting in higher O.D. values for subgroup 1 (S electropherotype) viruses than for subgroup II viruses (L electropherotype).

REFERENCES

BEARDS, G. M. (1982). Polymorphism of genomic RNAs within rotavirus serotypes and subgroups. *Archives of Virology* **74**, 65–70.

BEARDS, G. M. & BRYDEN, A. S. (1981). Evaluation of a new enzyme linked immunosorbent assay test for rotavirus antigen in faeces. *Journal of Clinical Pathology* **34**, 1388–1391.

BEARDS, G. M., PILFOLD, J. N., THOULESS, M. E. & FLEWETT, T. H. (1980). Rotavirus serotypes by serum neutralization. *Journal of Medical Virology* **5**, 231–237.

BIRCH, C. J., LEWIS, F. A., KENNETH, M. L., HOMOLA, M., PRITCHARD, H. & GUST, I. D. (1977). A study of the prevalence of rotavirus infection in children with gastroenteritis admitted to an infectious diseases hospital. *Journal of Medical Virology* **1**, 69–77.

BOTH, G. W., MATTICK, J. S. & BELLAMY, A. R. (1983). Serotype-specific glycoprotein of simian 11 rotavirus: coding assignment and gene sequence. *Proceedings of the National Academy of Sciences of the United States of America* **80**, 3091–3095.

BRANDT, C. D., KIM, H. W., RODRIGUEZ, W. J., ARROBIO, J. O., JEFFRIES, B. C., STALLINGS, E. P., LEWIS, C., MILES, A. J., CHANOCK, R. M., KAPIKIAN, A. Z. & PARROTT, R. H. (1983). Pediatric viral gastroenteritis during eight years of study. *Journal of Clinical Microbiology* **18**, 71–78.

ESPEJO, R. T., MUÑOZ, O., SERAFIN, F. & ROMERO, P. (1980). Shift in the prevalent human rotavirus detected by ribonucleic acid segment differences. *Infection and Immunity* **27**, 351–354.

FLEWETT, T. H. & WOODE, G. N. (1978). The rotaviruses. *Archives of Virology* **57**, 1–23.

FLORES, J., PEREZ, I., WHITE, L., PEREZ, M., KALICA, A. R., MARQUINA, R., WYATT, R. G., KAPIKIAN, A. Z. & CHANOCK, R. M. (1982). Genetic relatedness among human rotaviruses as determined by RNA hybridization. *Infection and Immunity* **37**, 648–655.

FOLLETT, E. A. C. & DESSELBERGER, U. (1983a). Cocirculation of different rotavirus strains during a local outbreak of infantile gastroenteritis. Monitoring by rapid and sensitive nucleic acid analysis. *Journal of Medical Virology* **11**, 39–52.

FOLLETT, E. A. C. & DESSELBERGER, U. (1983b). Cocirculation of different rotavirus strains in a local outbreak of infantile gastroenteritis. Monitoring by rapid and sensitive nucleic acid analysis and by oligonucleotide mapping. In *Double-stranded RNA Viruses* (ed. R. W. Compans and D. H. L. Bishop), pp. 331–342. New York: Elsevier-Biomedical.

GREENBERG, H. B., KALICA, A. R., WYATT, R. G., JONES, R. W., KAPIKIAN, A. Z. & CHANOCK, R. M. (1981). Rescue of non-cultivable human rotavirus by gene reassortment during mixed infection with ts mutants of a cultivable bovine rotavirus. *Proceedings of the National Academy of Sciences of the United States of America* **78**, 420–424.

GREENBERG, H., MCAULIFFE, V., VALDESUSO, J., WYATT, R., FLORES, J., KALICA, A., HOSHINO, Y. & SINGH, N. (1983). Serological analysis of the subgroup protein of rotaviruses using monoclonal antibodies. *Infection and Immunity* **39**, 91–99.

HERRING, A. J., INGLIS, J. F., OJEH, C. K., SNODGRASS, D. R. & MENZIES, J. D. (1982). Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained polyacrylamide gels. *Journal of Clinical Microbiology* **16**, 473–477.

HOSHINO, Y., WYATT, R. G., GREENBERG, H. B., KALICA, A. R., FLORES, J. & KAPIKIAN, A. Z. (1983). Serological comparison of canine rotavirus with various simian and human rotaviruses by plaque reduction neutralization and hemagglutination inhibition tests. *Infection and Immunity* **41**, 169–173.

KALICA, A. R., GARON, C. F., WYATT, R. G., MEBUS, C. A., VANKIRK, D. H., CHANOCK, R. M. & KAPIKIAN, A. Z. (1976). Differentiation of human and calf reovirus-like agents associated with diarrhoea using polyacrylamide gel electrophoresis of RNA. *Virology* 87, 86–92.

KALICA, A. R., GREENBERG, H. B., ESPEJO, R. T., FLORES, J., WYATT, R. G., KAPIKIAN, A. Z. & CHANOCK, R. M. (1981*a*). Distinctive ribonucleic acid patterns of human rotavirus subgroups 1 and 2. *Infection and Immunity* 33, 958–961.

KALICA, A. R., GREENBERG, H. B., WYATT, R. G., FLORES, J., SERENO, M. M., KAPIKIAN, A. Z. & CHANOCK, R. M. (1981*b*). Genes of human (strain Wa) and bovine (strain UK) rotaviruses that code for neutralization and subgroup antigens. *Virology* 112, 385–390.

KALICA, A. R., SERENO, M. M., WYATT, R. G., MEBUS, C. A., CHANOCK, R. M. & KAPIKIAN, A. Z. (1978). Comparison of human and animal rotavirus strains by gel electrophoresis of viral RNA. *Virology* 87, 247–255.

KAPIKIAN, A. Z., KIM, H. W., WYATT, R. G., CLINE, W. L., ARROBIO, J. O., BRANDT, C. D., RODRIGUEZ, W. J., SACK, D. A., CHANOCK, R. M. & PARROTT, R. H. (1976). Human reovirus-like agent as the major pathogen associated with 'winter' gastroenteritis in hospitalized infants and young children. *New England Journal of Medicine* 294, 965–972.

KUTSUZAWA, T., KONNO, T., SUZUKI, H., EBINA, T. & ISHIDA, N. (1982). Two distinct electrophoretic migration patterns of RNA segments of human rotaviruses prevalent in Japan in relation to their serotypes. *Microbiology and Immunology* 26, 271–273.

LOURENCO, M. H., NICOLAS, J. C., COHEN, J., SCHERRER, R. & BRICOUT, F. (1981). Study of human rotavirus genome by electrophoresis: attempt of classification among strains isolated in France. *Annales de Virologie (Institut Pasteur)* 132 E, 161–173.

MCNULTY, M. S. (1978). Rotaviruses. *Journal of General Virology* 40, 1–18.

MERRIL, C. R., SWITZER, R. C. & VAN KEUREN, M. L. (1979). Trace polypeptides in cellular extracts and human body fluids detected by two-dimensional electrophoresis and a highly sensitive silver stain. *Proceedings of the National Academy of Sciences of the United States of America* 76, 4335–4339.

PEREIRA, H. G., AZÉREDO, R. S., LEITE, J. P. G., CANDEIAS, J. A. N., RÁCZ, M. L., LINHARES, A. C., GABBAY, Y. B. & TRABULSI, J. R. (1983). Electrophoretic study of the genome of human rotavirus from Rio de Janeiro, São Paulo and Para, Brazil. *Journal of Hygiene* 90, 117–125.

RODGER, S. M., BISHOP, R. F., BIRCH, L., MCLEAN, B. & HOLMES, I. H. (1981). Molecular epidemiology of human rotaviruses in Melbourne, Australia, from 1973 to 1979 as determined by electrophoresis of genome ribonucleic acid. *Journal of Clinical Microbiology* 13, 272–278.

SAMMONS, D. W., ADAMS, L. D. & NISHIZAWA, E. E. (1981). Ultrasensitive silver-based color staining of polypeptides in polyacrylamide gels. *Electrophoresis* 2, 135–141.

SANDERS, R. C., BEARDS, G. M. & FLEWETT, T. H. (1983). A shift in prevalent human rotavirus strains. *Communicable Diseases Report* 83/14, p. 3.

SCHNAGL, R. D., RODGER, S. M. & HOLMES, I. H. (1981). Variation in human rotavirus electropherotypes occurring between rotavirus gastroenteritis epidemics in Central Australia. *Infection and Immunity* 33, 17–21.

STREET, J. E., CROXSON, M. C., CHADDERTON, W. F. & BELLAMY, A. R. (1982). Sequency diversity of human rotavirus strains investigated by Northern blot hybridization analysis. *Journal of Virology* 43, 369–378.

THOULESS, M. E., BEARDS, G. M. & FLEWETT, T. H. (1982). Serotyping and subgrouping of rotavirus strains by the ELISA test. *Archives of Virology* 73, 219–230.

THOULESS, M. E., BRYDEN, A. S., FLEWETT, T. H., WOODE, G. M., BRIDGER, J. C., SNODGRASS, D. R. & HERRING, J. A. (1977). Serological relationships between rotaviruses from different species as studied by complement fixation and neutralization. *Archives of Virology* 53, 287–294.

WYATT, R. G., GREENBERG, H. B., JAMES, W. D., PITTMAN, A. L., KALICA, A. R., FLORES, J., CHANOCK, R. & KAPIKIAN, A. Z. (1982). Definition of human rotavirus serotype by plaque-reduction assay. *Infection and Immunity* 37, 110–115.

YOLKEN, R. H., WYATT, R. G., ZISSIS, G., BRANDT, C. D., RODRIGUEZ, W. J., KIM, H. W., PARROTT, R. H., URRUTIA, J. J., MATA, L., GREENBERG, H. B., KAPIKIAN, A. Z. & CHANOCK, R. M. (1978). Epidemiology of human rotavirus types 1 and 2 as studied by enzyme-linked immunosorbent assay. *New England Journal of Medicine* 299, 1156–1161.

YOUNG, J. F., DESSELBERGER, U. & PALESE, P. (1979). Evolution of human influenza A viruses in nature: sequential mutations in the genomes of new H1N1 isolates. *Cell* 18, 73–83.

ZISSIS, G. & LAMBERT, J. P. (1978). Different serotypes of human rotaviruses. *Lancet* i, 38–39.