Excretion of faecal viruses during the first year of life

JEAN HARBOUR, AP SHIPP, DK WALLER, AND PG HIGGINS

From the Department of Virology, Bristol Royal Infirmary, Bristol BS2 8HW, UK

SUMMARY Four hundred faecal samples, collected at approximately weekly intervals during the first year of life from nine babies, were examined for the presence of viruses. Only nine (2·3%) samples contained a virus detectable by electron microscopy, and on all but one occasion only one type of virus was present and that in small numbers. Thirty (7·5%) of the specimens contained an entero virus other than poliovirus, and these represented 10 infections in four of the children. All three types of poliovirus, probably vaccine derived, were excreted by each child, and one or more types were present in 87 (21·8%) of the samples. There was no evidence to suggest that any of the illnesses suffered by the children had been caused by faecal viruses. Infection with these viruses was uncommon in the first three months of life but more than 40% of faecal samples obtained from children between the ages of 3 months and 1 year contained a faecal virus.

A number of viruses which are excreted in the faeces can be detected most efficiently by electron microscopy. Some, such as the rotaviruses, are accepted as a common cause of gastroenteritis in children while the aetiological significance of others, for example, astroviruses and adenoviruses, is still a matter for debate. This paper presents the findings of a study of a small number of babies undertaken in an attempt to determine the frequency with which these, and other viruses, infect the gastrointestinal tract during the first year of life, the duration of virus excretion after infection, and the proportion of infections that prompt the seeking of medical advice.

Material and methods

The co-operation was sought of the parents of 13 children born in the local maternity hospital between October 1974 and March 1975 inclusive. All the mothers and their babies were on the register of one practice in Cirencester, England, and each mother was asked to deliver a faecal sample from her infant each week until the child’s first birthday. All attendances by the children at the surgery and all professional visits to their homes were entered on the patient's medical record kept by the practice. It was, therefore, possible to confirm if medical advice was sought at the time of any proven faecal virus infection.

A 10-20% suspension was made of each faecal specimen in medium 199, which was then clarified by low-speed centrifugation and the supernatant was inoculated into cultures of monkey kidney, human embryo kidney, Hela cells, Hep-2 cells, and human embryo diploid fibroblasts (MRC-5). The supernatant was also inoculated into suckling mice. Tissue cultures were observed for 21 days and suckling mice for 14 days after inoculation. Specimens were stored at −40°C. Viruses isolated in tissue culture were typed by neutralisation tests using specific antisera. In the event of a poliovirus being identified the faecal specimen was re-examined in four tissue cultures, each of which contained a different combination of antisera to the three poliovirus serotypes. In this way the simultaneous excretion of multiple serotypes of poliovirus, or a poliovirus and another enterovirus, could be recognised. Coxsackie A viruses isolated in suckling mice were sent to Dr DR Gamble at the Public Health Laboratory, Epsom, for typing.

Between 2 ml and 5 ml of the supernatant from each clarified faecal extract was re-centrifuged, after storage, at 56 000 g for 2 hours in a MSE Superspeed 50 or at 50 000 g for 2 hours in a Sorvall RC2-B ultracentrifuge. The deposit was re-suspended in 2 drops of distilled water and inoculated on to a formvar-coated grid before staining with 1·5% phosphotungstic acid at pH 6·5. The grids were examined in an AEI 801 or Hitachi 500 electron microscope.

Results

Seven of the 13 subjects each submitted an average of 48 samples, range 41-52, during the first year of life.
Faecal virus excretion by nine babies during the first year of life.
A further two babies provided sufficient specimens (28 and 35) to allow the results of their virological investigation to be included in the study. The remaining four children supplied an insufficient number of samples and the results obtained from the study of these specimens have been excluded from any further analysis. Five of the nine babies studied were the first child in the family but four of the children when born each had a sibling aged between 2 years and 4 years and 2 months.

The results of the examination of the 400 samples received from the nine babies during the first year of life are summarised in the Figure.

Electron Microscopy
Eight infections with viruses that could be detected only by electron microscopy were diagnosed in five of the nine babies at some time during the first year of life. Six of the infections occurred in four of the children without siblings and two in one child with an elder brother or sister. The particles seen included small, round viruses, measuring approximately 22 nm, 27 nm, and 30 nm in diameter, rotaviruses, and adenoviruses. Only nine (2.3%) of the 400 specimens contained virus particles that could be detected by electron microscopy, and on all but one occasion the number of virus particles seen was small and of a single morphological appearance. The exception was one specimen which contained both a moderate number of adenovirus particles and a large number of particles approximately 22 nm in diameter. The small round particles were still present in the subsequent specimen from this baby but only in small numbers, and this was the only instance when virus particles were seen in consecutive samples from any child. The earliest age at which a child was shown, by electron microscopy, to excrete virus particles was 4 months, and the infections occurred in February and March and between June and September.

Virus Isolation

Excluding poliovirus
Thirty (7.5%) of the specimens yielded culturable viruses excluding polioviruses. All were isolated from four of the nine children; one suffered infections with an adenovirus and an enterovirus, one with two different enteroviruses, and two with three different enteroviruses. Six of the 10 infections occurred in two of the children without siblings, and the remaining four infections were in two infants with an elder brother or sister. The viruses isolated consisted of one strain of echovirus type 6, two strains each of echovirus type 22, echovirus type 30, Coxsackievirus type A4, and Coxsackievirus type A10, in addition to one strain of adenovirus type 1. The presence of these viruses was no more common among those specimens in which virus particles were seen than in those in which virus particles were not visualised. Only two of the nine specimens shown to contain virus particles by electron microscopy were obtained from children who were infected, at some time during the study, with culturable viruses other than poliovirus. The earliest age at which infection occurred was 10 weeks; all but one of the infections occurred between May and October, and the excretion of virus commonly continued for three to four weeks.

Polioviruses
All nine babies excreted all three types of poliovirus during the first year of life. Poliovirus was detected in stools from two babies as early as 3 months of age, and the average age of first excretion of poliovirus was just over 4 months. During the first year of life poliovirus type 1 was found, on average, in more than four samples, type 2 in more than six samples, and type 3 in more than five specimens. In all, 87 (21.8%) of the 400 samples from the nine children contained poliovirus. Two of the children each received three doses of polio vaccine from their practitioner, and poliovirus was excreted after the administration of five of these doses. Coxsackievirus type A4 was present in the two faecal samples collected immediately before the third dose of vaccine was given to one of the children, and, although no virus was excreted after vaccination, Coxsackievirus type A4 reappeared in the faeces two weeks later.

Illnesses
In addition to the six visits for poliovirus vaccination, medical attention was sought on 59 occasions by the nine babies during the first year of life. Forty-one of the illnesses which prompted this action could have been infective in origin, but on only eight occasions did excretion of detectable virus coincide with the illness. The type of illness and the time relationship to virus excretion are shown in the Table.

Discussion
Considering the very demanding nature of the request, the sampling achieved must be considered to be satisfactory. Indeed, that over half the subjects approached provided, on average, 48 weekly samples over a 52-week period was far better than one would have anticipated.

Very few specimens contained virus particles that could be seen by electron microscopy, and it is difficult to be certain of the exact nature of the small, round particles seen. However, those of approxi-
Excretion of faecal viruses during the first year of life

Clinical illnesses and virus excretion

<table>
<thead>
<tr>
<th>Subject</th>
<th>Date</th>
<th>Illnesses</th>
<th>Virus excreted and dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baby 4</td>
<td>3 June '75</td>
<td>Rash on chin</td>
<td>Poliovirus excreted in all but one specimen from 16 April to 4 July '75</td>
</tr>
<tr>
<td></td>
<td>27 June '75</td>
<td>Teething, sleepless</td>
<td>Echovirus 10 excreted 26 Sept '75 to 9 Oct '75</td>
</tr>
<tr>
<td></td>
<td>3 Oct '75</td>
<td>Coryza and teething</td>
<td>Adenovirus 1 excreted 17 Oct '75 to 31 Oct '75</td>
</tr>
<tr>
<td>Baby 8</td>
<td>23 Oct '75</td>
<td>Rash on trunk, miserable</td>
<td>Coxsackievirus A10 excreted 16 May '75</td>
</tr>
<tr>
<td></td>
<td>24 Oct '75</td>
<td>Measles</td>
<td></td>
</tr>
<tr>
<td>Baby 11</td>
<td>21 May '75</td>
<td>Coryza</td>
<td></td>
</tr>
<tr>
<td>Baby 13</td>
<td>20 Oct '75</td>
<td>Teething, restless nights</td>
<td>Echovirus 22 excreted 10-31 Oct '75</td>
</tr>
<tr>
<td></td>
<td>27 Oct '75</td>
<td>Sleepless, loose motions</td>
<td>Coxsackievirus A4 excreted 21 Feb '76 (previously 19 and 24 Jan '76)</td>
</tr>
<tr>
<td></td>
<td>19 Feb '76</td>
<td>Croup, mild stridor, pus in pharynx</td>
<td></td>
</tr>
</tbody>
</table>

Approximately 22 nm diameter present in the same specimen in which adenovirus particles were seen could well have been adeno-associated viruses. None of the electron microscopy positive specimens was from a child under 4 months of age, no infection was associated with a recorded illness, and, in all but one instance, excretion was limited to a single specimen. These findings are in contrast to some other reports where virus excretion was observed in normal babies as well as in neonates suffering from gastroenteritis but in agreement with others which failed to find virus particles in weekly faecal samples from newborn infants without diarrhoea. The accumulated evidence suggests that the excretion of these viruses by normal neonates occurs when they are placed in units where the viruses are endemic. The situation may well prove to be analogous to that found with respiratory syncytial virus, which is a much less common cause of illness in a semirural community than among children in large industrial towns.

Although many of the specimens yielded enteroviruses, mainly poliovirus, which were often present in such concentration that a marked cytopathic effect followed overnight inoculation of tissue cultures, none of these specimens was shown to contain the relevant sized particles by electron microscopy. This is a further observation that the faecal viruses that can be isolated in tissue cultures are rarely seen by electron microscopy, and those that are seen are rarely grown in tissue culture.

The findings relating to enteroviruses other than poliovirus are very much as would be expected. The seasonal incidence, duration of excretion, and the high incidence of subclinical infections reported here are typical of these viruses. The nine enterovirus and one adenovirus infections confirmed by virus isolation occurred among four of the babies, each child having at least two infections. If the spread of these viruses detected only by electron microscopy is by the faecal-oral route it would be expected that infections with them would be more common in children who experienced enterovirus infections than in those who did not. This study provides no support for this view nor for the theory that younger children acquire infection with faecal viruses from older siblings.

Only two children received poliovaccine from their practitioner, and it is assumed that the remaining infants were immunised at the infant welfare clinic as all children excreted all three types of poliovirus at some time during the year. Furthermore, the pattern of excretion of the various serotypes suggests a deliberate feeding with vaccine as opposed to infection resulting from casual contact with an excreter of vaccine or wild type poliovirus. The absence of concurrent illness, the duration of virus excretion, and the probable interference caused by other enteroviruses closely resemble the findings of the Public Health Laboratory Service study of vaccination with live, attenuated poliovirus. The conclusions reached in that report would indicate that all nine children in the present study are immune to all three types of poliovirus.

There were eight occasions when medical advice was sought for any one of the infants at a time when the child was excreting a virus. There is little evidence that infection with the virus was the cause of the symptoms as, in most instances, virus excretion was observed a considerable time before the consultation took place. The findings again illustrate the fact that most enterovirus infections are subclinical, including those with the vaccine strains of poliovirus.

The vaccination of infants against poliomyelitis, the naturally occurring enterovirus infections together with the small number of infections with viruses detectable only by electron microscopy result in the presence of virus in a high proportion of the stools collected from healthy infants during the first year of life. In this study 31% of all faecal samples contained virus and, because these infections are uncommon in the first three months of life, over 40% of specimens collected between 4 months and 1 year of age were positive. This finding warrants caution in interpreting the significance of viruses found in faecal specimens from children with symptoms in this age group.
We gratefully acknowledge the efforts of the parents in collecting and delivering the specimens and of Miss Joyce Dawson in receiving and storing them. We thank Dr RE Hope Simpson for enrolling the subjects from his practice and for making available the relevant medical histories. Finally, we thank our colleagues at the Bristol Public Health Laboratory who undertook a proportion of the electron microscopy during the delay in installing a replacement machine at the Bristol Royal Infirmary.

References


Requests for reprints to: Dr PG Higgins, Department of Virology, Bristol Royal Infirmary, Bristol BS2 8HW, UK.