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ROTAVIRUS INFECTIONS OF NEONATES

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Summary Fæcal specimens from 628 newborn babies in the nurseries of six metropolitan hospitals were examined by electron microscopy for rotaviruses. 304 babies (49%) were found to be excreting virus. All those infected were in five nurseries; viruses were not detected in specimens from the sixth nursery. Two nurseries were studied for 9 mo and another for 11 mo and rotaviruses were found consistently in 40–50% of stools examined. There was no seasonal variation. None of the neonates under the age of one day were infected but by the age of three to four days approximately 50% were excreting virus. Most of those shedding virus were symptom-free but 84 (28%) had diarrhoea. Persisting endemic rotavirus infection is apparently common in hospital nurseries in Sydney. The virus is probably transmitted by environmental spread from neonate to neonate.

Introduction

We reported the excretion of rotaviruses by neonates in the nurseries of two large metropolitan hospitals in Sydney.¹ Our studies suggested that there was a high incidence of asymptomatic infection (53% in one nursery and 26% in the other) and that the virus persisted for long periods in the nurseries. Chrystie et al.² have also drawn attention to the high infection-rate and lack of symptoms in neonates in St. Thomas' Hospital, London. On the other hand, Bishop et al.^{3,4} described outbreaks of diarrhoea in hospital nurseries which they attribute to rotaviruses.

A high percentage of cases of enteritis in infants and young children has been shown to be associated with rotaviruses,⁵ and our initial interest in neonatal infections was stimulated by the finding of rotaviruses in five babies with diarrhoea who were less than 7 days old. However, the discovery that many symptomless neonates were excreting virus raised doubts about its role as a causative agent in diarrhoea in the very young and we have therefore extended our studies to six hospital nurseries in the greater metropolitan area of Sydney.

Methods

Collection of Specimens

Fæcal specimens were collected from all babies in a particular nursery on a given day. 5 standard public hospital nurseries in widely scattered areas of metropolitan Sydney were surveyed in this manner. No baby had diarrhoea at the time of the survey. Specimens were subsequently collected from neonates with diarrhoea, a few with "loose" or "mucousy" stools, and from normal babies during outbreaks of diarrhoea. Most of the neonates were less than 7 days old. Specimens were transported to the laboratory within 24 h and stored at -20°C until processed.

Laboratory Techniques

A 20% w/v suspension of fæces was made in Hanks' balanced salt solution without antibiotics, mixed with an equal volume of Arklone P (trichlorotrifluoroethane), and homogenised thoroughly for 5 min. The homogenate was then centrifuged at 7000 *g* for 20 min and the aqueous layer removed. A portion of this aqueous layer was then centrifuged at 240 000 *g* for 30 min. For electron microscopy the pellet was resuspended in a few drops of distilled water, one drop of which was placed on a carbon-coated parlodion grid, allowed to dry, and then negatively stained with 3% phosphotungstic acid at pH 6.5. A Philips 201 electron microscope was used.

Results

Fæcal specimens from 628 neonates were processed and examined for viruses. Rotaviruses were found in 304 (49%) (table 1). The four groups of neonates (normal babies tested during surveys, normal babies tested during outbreaks of diarrhoea in the nurseries, those with diarrhoea, and those with mild gastrointestinal symptoms) gave excretion rates of 44%, 46%, 61%, and 45% respectively. Of the 6 nurseries 5 showed evidence of infection. In the sixth nursery no rotaviruses were found during the survey or later when there were cases of diarrhoea. The difference between the percentage of positive excretors in the diarrhoea group and that in the normal-baby group is statistically significant ($P < 0.05$).

Very few other viral particles were found in the deposits examined by electron microscopy. 2 myxovirus-like particles, 2 coronavirus-like particles, a small 18 nm particle (possibly a parvovirus), 4 mycoplasma-like organisms, and a number of bacteriophages were the only other microorganisms seen. Attempts to isolate viruses by standard cell-culture techniques were unsuccessful.

TABLE 1—RESULTS OF TESTING FÆCAL SPECIMENS FROM NEONATES FOR ROTAVIRUSES

Site of hospital	Surveys of nurseries		Normal neonates during outbreak of diarrhoea		Neonates with diarrhoea		Neonates with minor intestinal symptoms		Total		
	No. tested	No. +ve	No. tested	No. +ve	No. tested	No. +ve	No. tested	No. +ve	No. tested	No. +ve	% +ve
Inner City	57	28	10	2	30	25	13	7	110	62	56
Southern Metropolitan	80	43	22	13	5	2	15	2	122	60	49
Northern Metropolitan	16	0	11	0	2	0	11	0	40	0	0
Western Metropolitan ⁽¹⁾	20	12	—	—	1	1	2	2	23	15	65
Western Metropolitan ⁽²⁾	21	3	30	16	8	5	2	0	61	24	39
Western Metropolitan ⁽³⁾	Not surveyed		26	14	100	56	146	74	272	144	53
	198	86 (44%)	99	45 (46%)	146	89 (61%)	189	85 (45%)	628	305	49

ful. More than 100 specimens were tested but no viruses were cultivated. Likewise bacteriological investigations did not reveal any pathogens.

Age of Infection

Table II shows the number of babies tested and the number and percentage positive according to age. Babies with and without diarrhoea are included. In addition to

TABLE II—RELATIONSHIP BETWEEN AGE AND EXCRETION OF ROTAVIRUSES

Age	No. tested	No. +ve	% +ve
<24 h	22	0	0
1 day	35	3	9
2 days	65	24	37
3 days	131	73	56
4 days	123	64	52
5 days	147	70	48
6 days	77	48	62
7+ days	50	22	44

the specimens already reported a further 22 were collected from newborn babies less than 24 h old.

Viruses were not found in babies less than 24 h old, but 9% of those tested 24–48 h after birth were excreting virus. By the third and subsequent days, about half the babies had become infected.

Continuing Presence of Virus and Seasonal Incidence

2 nurseries were studied continuously for 9 mo and another for 11 mo. Rotaviruses were regularly found in

TABLE III—SEASONAL INCIDENCE OF NEONATAL ROTAVIRUS EXCRETION

Season	No. of specimens tested	No. +ve	% +ve
Spring, 1975	93	49	53
Summer, 1975/76	140	68	49
Autumn, 1976	144	57	40
Winter, 1976	251	130	52

40–50% of all stools. There was little variation in the percentage of positive specimens at different times of the year (table III).

Discussion

These studies indicate that about half the newborn babies in the nurseries of five major metropolitan hospitals in Sydney were infected with rotaviruses before they were discharged. The great majority of these infections were asymptomatic and apparently quite transient in some babies. One surprising feature was the rapidity with which the neonates became infected after they entered the nursery. 3 out of 35 babies tested were found to be excreting virus one day after birth and 24 out of 65 (35%) two days after birth. From the third day, approximately 50% were excreting virus. The neonatal gut is colonised by lactobacilli and *Escherichia coli* within a few days of birth and it is not unreasonable to assume that rapid infection can take place with rotavir-

uses. Mebus et al.⁶ have shown that bovine rotavirus will produce diarrhoea in newborn calves in 15 h after artificial inoculation. Excretion of viruses 24–48 h after birth by the human neonate is therefore not improbable.

How the virus is acquired is not known but the few available facts strongly suggest environmental spread within the nursery. Adults seldom excrete rotaviruses and although a few cases of gastroenteritis and asymptomatic infection have been recorded^{7,8} it is unlikely that individual infection is acquired directly from mother or nurses.

It is most likely that the virus enters the nursery through a visiting child or the rare adult excretor and then passes readily from neonate to neonate; the result is a persisting endemic infection as susceptible babies enter the nursery.

We found no seasonal variation in the incidence of infection. About half the babies in the nursery seem to be consistently excreting virus throughout the year, unlike the infants and young children in whom the prevalence of the virus increases in the late winter and early spring.⁹

Because of the high infection-rate among the newborn babies without symptoms, the role of rotaviruses as a cause of enteritis is not clear. Only 84 out of 304 babies (28%) excreting virus had diarrhoea. However, there were significantly more neonates with diarrhoea excreting virus than the symptom-free group ($P < 0.05$) and in the absence of any other pathogen it seems that these viruses do in fact cause diarrhoea in some neonates. It was noted that viruses from some babies were coated with an antibody-like material; this feature may be an indication of the antibody level of the mother's milk or perhaps the non-antibody virus inhibitor recently described in human and cow's milk by Matthews et al.¹⁰ We did not find "antibody" coated viruses in cases of diarrhoea. The possible existence of rotavirus serotypes of differing pathogenicity could also explain the variable response to infection, but the determination of minor antigenic differences is not possible with the techniques available at present.

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