Diarrhea and Rotavirus Infection Associated with Differing Regimens for Postnatal Care of Newborn Babies

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Surveillance of 2,041 babies born during 4 winter months in one obstetric hospital in Melbourne, Australia, showed that 215 developed acute diarrhea during the first 2 weeks of life. Babies requiring special care from birth had a high incidence of sporadic diarrhea (36%). The incidence of diarrhea among healthy full-term babies was low if they were "rooming-in" with their mothers (2 to 3%) but high if they were housed in communal nurseries (29%). The most important factor influencing incidence of diarrhea was proximity to other newborn babies and frequency of handling by unrelated adults. Breast feeding did not always protect babies from diarrhea. Excretion of rotaviruses was temporally related to diarrhea in 61 to 76% of healthy full-term babies and in 44% of babies requiring special care. Other enteric pathogens, including enterotoxigenic *Escherichia coli*, were occasionally isolated. Calculation of the ratios of symptomatic to asymptomatic infection suggests that babies requiring special care are much more likely to develop symptomatic illness after rotavirus infection than are healthy full-term babies.

During a study of the etiology of diarrhea in a special care nursery of one obstetric hospital in Melbourne, Australia, it became apparent that sporadic diarrhea also occurred among babies in other wards of the same hospital (2). Most of these babies were full-term healthy neonates and were housed, handled, and fed differently from the babies in the special care nursery. Diarrhea seemed to be more common in babies in the special care nursery.

We decided to compare incidence of diarrhea in babies from different wards of this hospital during April to July (autumn to winter), when the incidence of diarrhea in neonates and older children was known to be high (3, 6). Etiological agents were sought by culture of feces for enteric bacterial pathogens and by electron microscopy to detect noncultivable viruses. Our aim was to determine whether babies requiring special care were more vulnerable to diarrhea than were healthy full-term babies, and, if so, whether this was influenced by differences in regimen for postnatal care.

MATERIALS AND METHODS

Three groups of babies were studied. All were born at the Royal Women's Hospital, Melbourne, Australia, during April to July 1975. They were transferred from the labor ward within 2 h of birth to wards providing either routine or special care. In addition to observations recorded by ward staff, daily surveillance of all

wards was maintained by a member of the nursing staff responsible for infection control. Group 1 comprised 251 babies housed in a special care nursery because of problems such as prematurity, low birth weight, or birth trauma. The nursery contained 80 beds distributed in 11 rooms, with 4 m² of floor space per baby. Each room was separately air conditioned, with no recirculation of air. Humidity was maintained at 55% and air temperature at 22 to 23°C. Babies were fed and attended to mainly by the nursing staff. Mothers visited their babies daily and fed them when appropriate. Other visitors viewed the babies through glass from an outside corridor. Most babies were initially fed with cow's milk formula or pooled pasteurized expressed breast milk.

Group 2 comprised 1,464 healthy babies housed with their mothers in four-bed wards. These areas were not air conditioned. A policy of "rooming-in" was practiced whereby the baby was taken out of the ward nursery when 6 h old and placed in a cot beside the mother's bed. The mother was the only person to handle her baby after the first two nights, provided she was well enough. During the first two nights of life, and also during daily visiting hours, the babies were moved in their cots to ward nurseries holding a maximum of six cots. Approximately 50% of babies in this group received colostrum from their mothers and were breast fed during their stay in hospital.

Group 3 comprised 326 healthy babies nursed in two communal nurseries. Each nursery accommodated 10 to 12 babies with approximately 2 m² of floor space per baby. The rooms were separately air conditioned. Humidity was maintained at 55% and air temperature at 22 to 23°C. Babies were wheeled to their mothers'

bedsides for feeding, but otherwise spent most of each day in the nurseries and were cared for by nursing staff common to both nurseries. Approximately 50% of this group received colostrum from their mothers and were breast fed during their stay in hospital.

Diarrhea was defined as a change in the frequency and/or character of the stools beyond that characteristic of transitional stools. An infant was accepted as having diarrhea if stools became very loose and watery or were more frequent than six per day. This judgment was made by one of us (D.J.S.C.) and the senior nursing staff. Where there was doubt, the infant was included in the nondiarrhea (control) group. Watery stools were tested for reducing sugar by the Clinitest method (11).

Collection and examination of specimens. Feces were obtained within 48 h of onset of diarrhea. All specimens were cultured by routine methods used to detect enteric bacterial pathogens, including slide agglutination tests to determine OB antigens of *Escherichia coli* using commercially available antisera (Baltimore Biological Laboratories). In addition, strains of *E. coli* were sent to the Salmonella Reference Laboratory, Institute of Medical and Veterinary Science, Adelaide, Australia for more detailed typing. Strains of *E. coli* isolated from babies with diarrhea were tested for ability to produce heat-labile and heat-stable enterotoxins using monolayers of Y₁ adrenal cells and intragastric inoculation of infant mice. Examination of stools for visible blood was used as an estimate of the presence of invasive strains of *E. coli*.

A portion of each specimen of feces was stored at 4°C and later prepared for electron microscopy by homogenizing 1 to 2 g in 10 ml of phosphate-buffered saline, followed by thorough mixing with 10 ml of trifluorotrichloroethane. This homogenate was centrifuged at $10,000 \times g$ for 20 min at 4°C. The supernatant fluid was further centrifuged at $100,000 \times g$ for 60 min at 4°C. The deposit obtained was resuspended in 3 to 5 drops of tris(hydroxymethyl)aminomethane buffer and examined after negative staining with 2% potassium phosphotungstate (pH 7.0).

RESULTS

Clinical. During the 4 months studied, 215 of the total 2,041 babies under surveillance developed diarrhea. Age at onset, severity, and duration of symptoms did not differ between the three groups. The majority of babies (144 of 215) developed diarrhea when aged 3 to 6 days. Thirteen showed symptoms during the first 2 days of life. Stools contained abnormal amounts of reducing sugar (>0.5%) in 80% of all babies with diarrhea. Treatment consisted of the substitution of normal diets with 5% glucose in water for 12 to 24 h, followed by the gradual reintroduction of normal or lactose-free diets. Intravenous fluid therapy for dehydration was required by fewer than 5% of affected babies. Fewer than 20% of babies who developed diarrhea required long-term lactose-free diets. Diarrhea was not a direct cause of death in any of the babies.

Table 1 lists the incidence of diarrhea in each group. During the 4 months studied, 36% of babies in group 1, 2% of babies in group 2, and 29% of babies in group 3 developed diarrhea. Diarrhea developed sporadically throughout the 4 months in babies in groups 1 and 2. Within group 1, diarrhea developed sporadically throughout the 11 rooms of the nursery and with equal frequency in babies in humidicribs and those in open cots. Babies in group 3 suffered three epidemics of diarrhea. The first epidemic affected 41 of 58 babies exposed during a 3-week period and stopped when the area was closed to new admissions. The area was reopened a month later, and within a week the second epidemic began: 22 of 39 babies exposed during a 3-week period developed diarrhea. The area was again closed and disinfected as thoroughly as possible by scrubbing all surfaces with soap and water, followed by application of sodium hypochlorite solution. Three weeks later the communal nurseries were reopened. Twenty-seven of the 49 babies admitted during the next 3 weeks developed diarrhea. Between epidemics, when babies were rooming-in with their mothers, there was a low incidence rate of diarrhea (3%).

Information about type of food received before onset of diarrhea was available for 182 of the 215 babies who developed diarrhea (Table 2). Breast-fed babies in group 1 received pooled pasteurized expressed breast milk. Breast-fed babies in groups 2 and 3 received colostrum from their mothers and were fed at approximately 4-h intervals. An occasional supplementary feeding (20 to 40 ml) of artificial milk formula was given during the night. The majority of babies in groups 1 and 2 who developed diarrhea were fed artificial milk formula from birth. The majority of babies in group 3 who developed diarrhea were breast fed (57%), and eight of these babies were known to have been exclusively breast fed.

Microbiological. No *Salmonella* sp. or *Shigella* sp. were isolated. *E. coli* O6H31, O6H36, O18acH20, and O91H14 were separately isolated from four of the babies. Heat-labile enterotoxin-positive strains of *E. coli* were isolated from 6 of 51 babies in group 1 and from 2 of 29 babies in group 2. Heat-stable enterotoxin-positive strains of *E. coli* were isolated from 3 of 51 babies with diarrhea in group 1. No enterotoxigenic strains of *E. coli* were isolated from 46 babies examined from group 3. None of the stools was visibly blood stained.

Electron microscopy revealed two noncultivable viruses, easily distinguished from each other and identified by morphology (Fig. 1). These were rotavirus particles and a 28-nm vi-

TABLE 1. *Diarrhea in newborn babies in different wards of one Melbourne obstetric hospital during April to July 1975*

Group no.	Care	No. of babies at risk	Total no. of babies with diarrhea (% of babies at risk)	Incidence rate of rotavirus diarrhea
1	Special care, communal nursery	251	90 (36)	14% (36/251)
2	Routine postnatal care, rooming-in	1,464	29 (2)	1.5% (22/1,464)
3	Routine postnatal care, communal nursery			
	Epidemic 1	58	41 (71)	47% (27/58)
	Epidemic 2	39	22 (56)	36% (14/39)
	Epidemic 3	49	27 (55)	18% (14/49)
	Between epidemics	180	6 (3)	2% (4/180)
	Total	326	96 (29)	17% (54/326)

TABLE 2. *Method of feeding 182 babies who developed diarrhea*

Group no.	No. of babies with diarrhea	Type of diet	
		Breast milk	Artificial milk
1	81	6 ^a	75
2	26	8 ^b	18
3	75	58 ^b	17

^a Pooled pasteurized expressed breast milk.^b Colostrum and breast milk obtained by suckling.

rus-like particle (4). This small virus failed to grow when inoculated into cell cultures of primary human fetal kidney cells, primary monkey kidney cells, human fetal fibroblasts, and HeLa cells. Specimens containing this small virus were sent to H. Greenberg, Laboratory of Infectious Diseases, Bethesda, Md. Radioimmunoassay showed that the particle is not antigenically related to Norwalk agent.

Rotavirus particles were seen in feces from 44% of babies with diarrhea in group 1, 76% of babies with diarrhea in group 2, and 61% of babies with diarrhea in group 3 (Table 3). The percentage of babies with diarrhea in group 3 who excreted rotavirus particles was much higher during the first two epidemics (75 and 67%) than during the third epidemic (33%).

The overall incidence rates of diarrhea associated with rotavirus infection in groups 1, 2, and 3 were 14, 1.5, and 17%, respectively (Table 1). Incidence rates of rotavirus diarrhea in group 3 varied from 2% between epidemics to 47% during the first epidemic.

The 28-nm virus was identified in diarrheal feces from some babies in all three groups (Table 3). Twenty-four of the 31 babies who excreted this small virus were simultaneously excreting rotavirus particles.

FIG. 1. *Rotavirus particles and 28-nm virus-like particles in diarrheal feces from a newborn baby. $\times 97,000$.*

Excretion of rotaviruses and the 28-nm virus was temporally related to symptoms of diarrhea in this and another study (3, 4). Excretion often preceded symptoms of diarrhea, but neither virus was detected in fecal specimens obtained 3 to 30 days after cessation of diarrhea. Once diarrhea developed, excretion rates of each virus were not influenced by type of diet. Rotaviruses were excreted by 57% of breast-fed babies and 57% of artificially fed babies with diarrhea. The 28-nm virus was excreted by 17% of breast-fed babies and 19% of artificially fed babies with diarrhea.

TABLE 3. Occurrence of two noncultivable viruses in diarrheal feces from newborn babies (April to July 1975)

Group no.	No. of patients with specimens examined	Electron microscopy		
		Rotavirus (% patients)	28-nm virus	Both ^a
1	81/90	36 (44)	14	10
2	29/29	22 (76)	3	3
3				
Epidemic 1	36	27	3	3
Epidemic 2	21	14	7	4
Epidemic 3	27	9	4	4
Between epidemics	5	4	0	0
Total	89/96	54 (61)	14	11

^a Included also in total for each virus.

DISCUSSION

Throughout the 4 winter months of this study, 215 of the total 2,041 babies under surveillance developed diarrhea during early postnatal life. Babies requiring special care from birth showed a high incidence of sporadic diarrhea (36%). The incidence of diarrhea in healthy full-term babies varied according to conditions of housing. Only 2 to 3% of babies rooming-in with their mothers developed diarrhea, even though contact often occurred with adult relatives and siblings during visiting hours. By contrast, 36 to 71% of babies housed in communal nurseries and cared for mainly by nursing staff developed diarrhea during the same period.

Comparison of these differing regimens of postnatal care suggests that an important factor influencing incidence of diarrhea is proximity to other newborn babies and frequency of handling by adults who are not members of the family. These results agree with the historical observation that epidemics of diarrhea in newborns accompanied the change from home to hospital confinement (8). Aggregation of babies in communal nurseries not only increases the likelihood of infection entering and spreading through a nursery but increases the likelihood that babies will be vulnerable to infection, since they may possess little passive immunity against infectious agents not acquired from their mothers.

Human colostrum and breast milk contain rotavirus antibodies (14), and breast feeding is associated with decreased incidence of detectable rotavirus excretion (5). It was not possible in this study to assess whether breast feeding decreased the incidence of diarrhea or of rotavirus infection, or both, since the numbers of breast-fed and artificially fed babies in the total population at risk in each group was not known. It is clear that breast feeding did not always protect

against infection with rotavirus. Many of the babies with diarrhea had been fed colostrum and breast milk (supplemented by minor amounts of artificial milk formula), and eight had been exclusively breast fed since birth. Once diarrhea occurred, there was no difference in incidence of rotavirus excretion between breast-fed and artificially fed babies.

Sporadic diarrhea has persisted among babies admitted to the special care nursery and shows seasonal variation with a high incidence during winter months (3). Susceptibility to diarrhea of babies requiring special care has been noted by others (8, 10). Many factors may contribute to this susceptibility, including physiological immaturity of the gut (9), diarrhea of parenteral origin, communal nursing, and methods of feeding. Most babies are fed artificial milk formula or pooled and sterilized expressed breast milk. It would be valuable to assess the incidence rate of diarrhea in premature babies fed unprocessed colostrum and breast milk expressed by their mothers.

It is considered that most of the diarrhea observed in babies during this study was due to intestinal infection, and that rotavirus infection was a major cause of sporadic diarrhea during the period of surveillance. Two of the three epidemics were associated with high incidence of rotavirus excretion by babies with diarrhea. This virus can cause severe enteritis in older children and in the newborns of many other animals (7). Other enteric pathogens were occasionally isolated from babies with diarrhea. The 28-nm virus excreted by 10 to 17% of babies may have contributed to development of diarrhea, but its etiological role is still uncertain (3, 4). A similar particle has been described in stools from babies with mild diarrhea in the U.K. (1).

Many rotavirus infections in newborn babies, particularly healthy full-term babies, are asymptomatic (2, 3, 5, 13). The incidence of asymptomatic infection in the three groups during the period of surveillance is not known, but a later prospective study has shown that approximately 50% of babies in all groups excrete detectable amounts of rotavirus during their first week of life. This agrees with the incidence rate of rotavirus infection observed during winter in one U.K. maternity hospital (5). Calculation of the ratio of symptomatic to asymptomatic rotavirus infection, based on the assumption that 50% of babies were infected with rotavirus during the period of surveillance, gives a low ratio (1:30) in healthy babies rooming-in and a high ratio (1:2) in babies requiring special care.

An intriguing analogy exists between the behavior of rotavirus infection in babies in this study and observations by Lecce et al. (12) on

the relation between rearing regimen and diarrhea in piglets. Approximately 70% of piglets reared in a communal nursery and fed with artificial formulae developed diarrhea after being in the nursery for about 1 week. The incidence of diarrhea decreased when piglets were placed in freshly fumigated quarters. Diarrhea increased in severity with prolonged use of the nursery. Lecce et al. postulate that rotavirus is prevalent in pigs, that a small amount of virus or a virus of low infectivity is transmitted postpartum from dam to young, and that colostral antibody causes an asymptomatic infection of little consequence unless the nursery contains susceptible piglets capable of amplifying the infection. Weaned piglets continually added to the nursery allow infection of increasing severity to pass from piglet to piglet.

The results described here and in an earlier study (3) show that rotavirus is ubiquitous in newborn babies in Melbourne, Australia, and that diarrhea is more common in communal nurseries. Weaning may be decisive in leading to symptomatic infection in babies requiring special care. An understanding of mechanisms that protect the majority of healthy newborn babies from disease due to rotavirus infection might lead to effective measures for controlling infection in more vulnerable babies.

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