Bacteriostasis of *Escherichia coli* by milk. VI. The in-vitro bacteriostatic property of Gambian mothers' breast milk in relation to the in-vivo protection of their infants against diarrhoeal disease

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SUMMARY

A one-year field-study has been carried out in a diarrhoea-endemic area in West Africa to determine the relationship between the bacteriostatic activity of fresh human milk for *Escherichia coli in vitro* and freedom from diarrhoea of the infant recipients of the milk. The specific contribution of *E. coli* gastroenteritis to gastro-intestinal diseases of infants in general is not known, nor is its particular role in the Gambian infants studied. During the study period, however, both entero-pathogenic and toxigenic strains of *E. coli* were isolated.

The incidence of diarrhoea in Gambian infants of seven age-groups from 2 days to 12 months was not significantly correlated with the bacteriostatic activity of milk. This was due rather to absence of diarrhoea in babies fed on low-activity milk than illness in those receiving highly bacteriostatic milk. Indeed, very active milk appeared to protect recipients almost completely, including seven babies of over 3 months of age, five of them during the rainy season, when the risk was high. Babies receiving lower-activity milk experienced more diarrhoea. In a situation where diarrhoeal disease is multifactorial, field evaluation of the protective action by one antibacterial property of milk is difficult. A better understanding of *in vivo* protection is important, and the factors which have to be taken into account are discussed.

INTRODUCTION

Diarrhoeal disease is a major cause of growth impairment in young Gambian village children (Rowland, Cole & Whitehead, 1977) and prevalence is high, particularly in the rainy season, despite breast-feeding continued well into the second year of life (Rowland & Barrell, 1980). Breast-feeding confers protection against infantile gastroenteritis, particularly *Escherichia coli* enteritis (Mata &

Urrutia, 1971), and it is important that we understand how and when this protection fails (Mata, Urrutia & Lechtig, 1971; Rowland, Cole & McCollum, 1980).

The rainy season, when diarrhoea is most common, is a period of high energy expenditure, poor food intake and deteriorating nutritional status in the mothers (Paul, Müller & Whitehead, 1979), who produce lower volumes of breast milk (Whitehead et al. 1978) of reduced nutrient content (Whitehead, 1979) at this time. In a previous paper (Dolby, Honour & Rowland, 1980a) we described a spectrum of bacteriostatic activity of milk in vitro for E. coli. Variations within mothers are dependent more on the stage of lactation than on season, but there was some fall in the activity of early lactation milk at the height of the rainy season. Differences in activity also existed between mothers.

This paper is directed towards determining whether or not such activity measured in vitro was related to the amount of diarrhoea experienced by different babies, all of whom lived in an unhygienic, high-challenge environment (Rowland & Barrell, 1980).

METHODS

Clinical surveillance

Young children were reviewed regularly at routine monthly clinics and whenever sick. Their illnesses, including diarrhoeal disease, have been described by Rowland et al. (1977). Anthropometric measurements were regularly carried out on mothers during pregnancy and lactation (Paul et al. 1979).

Breast milk

Breast-milk intakes were measured by test weighing for 12 h periods at regular monthly intervals (Whitehead et al. 1978). The collection and testing of the bacteriostatic activity in vitro of Gambian breast milk samples has been described (Dolby et al. 1980a). Each milk was assigned a category, A being the best, B less active and E inactive; D milk would be expected to have some activity in vivo for all strains in the presence of bicarbonate and iron-binding protein in the small intestine, whereas C milk would be active only against some.

Data analysis

Infants were grouped according to their age at the time the breast-milk sample was taken and the category of bacteriostatic activity into which that milk fell. Examination of the clinical records of the children was restricted to 7-day periods beginning with the day when breast milk samples were collected and the number of days of diarrhoea experienced during these periods was recorded for each child. In this way comparisons were possible between the grading of the milk activity at different ages and the amount of diarrhoea occurring at those times.

Bacteriostatic sensitivity to milk of Gambian E. coli

Gambian strains of E. coli were isolated from stools, food and water, identified by standard methods, and transported on Dorset-egg slopes. E. coli from stools

Table 1. *The relationship between age of infant, percentage of time ill with diarrhoea and the category of breast milk consumed*

Category of breast milk	Age of subject							All children
	2 days	2 weeks	6 weeks	3 months	6 months	9 months	12 months	
A	0 (21)	0 (12)	0 (3)	3·6±7·1 (4)	0 (1)	0 (2)	—	0·3±2·2 (43)
B	7·4±14·3 (4)	2·5±7·6 (17)	13·4±27·8 (16)	7·1±15·3 (8)	11·7±16·7 (11)	0 (1)	—	8·3±18·3 (57)
C	0 (1)	5·3±15·2 (8)	9·8±23·7 (16)	7·1±16·7 (12)	8·9±17·0 (8)	7·9±12·6 (9)	28·6±24·7 (3)	9·0±18·4 (57)
D	—	0 (2)	0 (3)	25·4±32·6 (9)	25·7±43·3 (5)	0 (5)	0 (1)	14·3±28·9 (25)
E	—	—	14·3±20·0 (2)	7·1±10·1 (2)	—	—	28·6 (1)	14·3±14·3 (5)
All milks	1·1±5·6 (26)	2·2±9·4 (39)	10·0±23·4 (40)	11·4±21·6 (35)	13·1±23·7 (25)	4·2±9·8 (17)	22·9±21·7 (5)	7·6±18·3 (187)

Figures given are mean % time with diarrhoea during 1-week periods (see text)   ±standard deviation with numbers in parentheses.

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were selected mainly from 3- to 18-month-old children and also from mothers and their babies 1 week after parturition; single-colony cultures, called 'strains' were subcultured from each source.

The sensitivity to the bacteriostatic activity of milk of about 100 of these strains to four standard milks of known bacteriostatic activity was tested as previously described (Honour & Dolby, 1979).

RESULTS

Diarrhoeal incidence and bacteriostatic activity of milk

The distribution of the incidence of diarrhoea in 2-day to 12-month-old babies receiving category A–E milk from their mothers is shown in Table 1. There was a clear trend for the bacteriostatic activity of milk to fall as lactation progressed, although a number of women showed a remarkably well sustained category A response. When the children were grouped regardless of age, as in the last column of Table 1, there was a wide range of susceptibility to diarrhoea which increased with falling bacteriostatic activity. Because decreasing antibacterial activity of the mothers' milk and increasing prevalence of diarrhoea in the children tend to go together with increasing age, at least up to 9 months (Dolby et al. 1980a; Rowland, Barrell & Whitehead, 1978a), and could be expected to correlate on that basis alone, comparisons were also made between categories of milk consumed and the diarrhoeal experience of the recipients within the various age groups studied. When this was done there was no significant difference in the amount of diarrhoea experienced by children receiving different grades of milk ($t = 1.7$, $P < 0.1$) though some of the cell numbers were small. When the 7-day clinical surveillance period was replaced by a 30-day period centred on the date of breast-milk collection, the results were unchanged.

Category A milk did, however, appear to conver greater protection than others, the only diarrhoeal episode recorded in this group being contributed by one subject on one day. On the other hand, some category-B milk was apparently less effective than milk of categories C, D and E.

Bacteriostatic activity of milk and maternal nutritional status

Two groups of women were identified for purposes of comparison: five in whom the production of high-activity milk (mainly A with some B) was well sustained over a period of many months, and eight in whom long periods of low-activity milk (categories C and D) production were observed. Nutritional status was compared using the mean of the triceps and the subscapular skinfold thickness over the period during which that milk activity was sustained. Mean weights of 12 h breast milk intake in their respective infants were also compared over the same period. The results are shown in Table 2. No significant differences were observed.

The susceptibility of Gambian-isolated E. coli to Gambian milk

Three strains of E. coli from the stools of each of seven mothers and ten 1-week-old babies were tested for sensitivity to the bacteriostatic action of standard milk. The results shown in Table 3 were similar to those in the United Kingdom; there

Table 2. *The comparison of nutritional status and category of breast milk activity between women producing sustained high- and low-activity milks*

Category of milk	Mean skinfold thickness (mm)	Mean 12-hour breast milk intake (g)
High (5)	$9\cdot7 \pm 1\cdot5$	327 ± 51
Low (8)	$10\cdot8 \pm 3\cdot4$	330 ± 48

Figures are means ± S.D. with numbers in parentheses.

Table 3. *The milk sensitivity of* E. coli *from the stools of Gambian mothers and their 1-week-old babies*

Strain source	No. strains	Milk sensitivity		
		Sensitive	Intermediate	Resistant
7 mothers	21	9	5	7
10 babies	30	6	14	10

was little selection of strains according to milk sensitivity as both were inhibited *in vivo* and approximately equal numbers of milk-sensitive and milk-resistant strains were excreted, with slightly more in the intermediate and resistant groups than were found in Harrow (Dolby, Honour & Valman, 1977). The milk from the seven mothers was of categories A–C at the end of 8–10 days; the distribution of resistant and sensitive strains in the individual babies bore no particular relationship to the milk category.

Tests on the milk sensitivity of 21 strains of *E. coli* isolated from food and water and 30 strains from the stools of a cross-section of babies between 3 and 18 months old, with or without diarrhoea, are given in Table 4. There was a tendency for resistant strains to outnumber the sensitive ones, particularly in the older children.

The preponderance of milk-resistant strains in the environment did not seem to affect the colonization of newborns but may well have been more persistent in older children. The Gambian *E. coli* from various sources were similarly inhibited by active Gambian and Harrow milks.

The incidence of pathogenic E. coli in stools

Five strains of *E. coli* were isolated in February–March 1977, from a cross-section of children 4–12 months old with and without diarrhoea. These were sub-cultured on Dorset-egg slopes and sent to the Division of Enteric Pathogens, Central Public Health Laboratory, where *E. coli* from 57 children was investigated. The isolation of pathogenic cultures is shown in Table 5. These were of various enteropathogenic and enterotoxigenic serotypes; both were isolated from diarrhoeal and non-diarrhoeal children and a few children carried a mixture of serotypes. Enteropathogenic serotype O119 and enterotoxigenic serotype O6 were found the most often but cultures producing demonstrable toxin were isolated from only eight children, one with diarrhoea and the rest without.

Five of the seven children shown in Table 5 with diarrhoea and pathogenic

Table 4. *The milk sensitivity of* E. coli *from food, water and the stools of 3- to 18-month-old babies*

		Milk sensitivity		
Strain source	No. strains	Sensitive	Intermediate	Resistant
Food	10	3	1	6
Water	11	3	2	6
12 babies without diarrhoea	12	0	0	12
6 babies with diarrhoea	6	3	0	3
2 babies, no record	2	1	0	1

Table 5. *Potentially pathogenic* E. coli *isolated in the dry season from the stools of 57 children with or without diarrhoea*

	Pathogenic E. coli	
	Present	Absent
Diarrhoea	7	3
No diarrhoea	20	27

E. coli were older than 9 months, and seven specimens of milk from their mothers were of category C, D or E. One younger baby of 4 months in this group was receiving category E milk. The 20 symptomless children with pathogenic *E. coli* were, on the whole, younger: all but six were less than 9 months old, many 4–5 months old. Eight out of 17 milk specimens from mothers of these babies were of categories A or B; the rest were C or D.

The symptomless carriage of pathogenic *E. coli* in the younger infants may be related to the bacteriostatic qualities of the breast milk, but we must remember they are almost certainly exposed to lower challenges than older weanlings.

DISCUSSION

Correlations between category of bacteriostatic activity of milk and the amount of diarrhoea in recipient infants barely approached significance. However, category A milk appeared different from milk of categories B–E in the degree of protection conferred. This fits well with laboratory experience in which A milks were shown to hold down inocula of 10^6 organisms per ml for as long as 6 h, a substantial improvement on the performance of other categories of milk (Dolby *et al.* 1980a). This may well be relevant in The Gambia, where food given to babies over 3 months old was grossly contaminated with several species of bacteria capable of causing diarrhoea, including *E. coli* (Barrell & Rowland, 1979), especially during the rainy season. Children being weaned during the rains were often left for periods during the day-time of up to 8 h and thus deprived of breast milk while the mothers were working in the fields (Rowland & Paul, 1980). Thus, the season of highest bacterial challenges coincided with increased intervals between day-time breast-feeds.

Although category A milk appeared to confer protection at any age and at any

time of the year, i.e. was effective even in the high-challenge rainy season, few mothers were producing such milk after 6 weeks of lactation and only 3 after 3 months. Even so, this was better than their United Kingdom counterparts; 37 % of the 7- to 8-day post-partum Gambian milks were category A (Dolby *et al.* 1980*a*) whereas only 3 % of 5- to 7-day milks collected in Harrow were as active (Honour & Dolby, 1979).

The aetiology of diarrhoeal disease in young children is multifactorial and the protective mechanisms in breast milk equally so. Strong correlations cannot be expected between the amount of a non-specific symptom, diarrhoea, and one selected test of antibacterial activity in milk to one organism, *E. coli*. During the first half of 1977 the most frequently isolated pathogenic *E. coli* in the stools of Keneba children were of enteropathogenic serotype O119 and enterotoxigenic serotype O6, but the extent to which diarrhoeal disease in our survey subjects is due to *E. coli* gastroenteritis is not known. Experience elsewhere described in a recent review of the subject (Rowe, 1979) suggests that in our young, exclusively breast-fed survey children up to 4 months of age *E. coli* gastroenteritis may well be important. This is particularly true of the main rainy season diarrhoeal epidemic, which does not appear to be due to rotavirus (Rowland *et al.* 1978*b*).

Previously, however, in 24 6-month- to 3-year-old malnourished hospitalized children in The Gambia mostly having diarrhoea, Heyworth & Brown (1975) found that the small intestines of only half of them were colonized with *E. coli* and that not of enteropathogenic serotype. Rowland & McCollum (1977) found that only 10 % of 25 Gambian babies of 6–20 months in the main diarrhoea season had *E. coli* in the small bowel, and reported that only 3 % of the stools of Gambian babies, well or ill, contained *E. coli* of enteropathogenic serotype. In these studies, strains of enterotoxigenic serotype and toxin-producers were not tested for. We are ignorant of the pattern of small intestinal colonization in children up to 6 months of age in The Gambia. In a study in Jamaica and London on 1-month- to 2-year-old children hospitalized with diarrhoea (Ellis-Pegler *et al.* 1979) in which strains isolated from the jejunum were serotyped and tested for toxin production, there was no convincing evidence of the prime involvement of *E. coli* of pathogenic or toxigenic serotype nor of production of toxin, Although the degree of correlation, or rather lack of it, between in-vitro bacteriostasis of *E. coli* by milk and the freedom from diarrhoea of the Gambian babies is not surprising in the complex field situation studied, we had hoped to achieve a more positive indication of the protective potential of the bacteriostatic system. Knowing which properties of milk to monitor to ensure protective cover in at-risk recipients is important in developing countries for breast-fed babies and elsewhere in the context of milk banks. Animal models, for one reason or another, are inappropriate, and it is not clear whether information obtained in a guinea-pig model is relevant to human infection (Dolby, Stephens & Royston, 1980*b*).

Recent work has shown that the strong bacteriostatic activity of whole milk is non-strain-specific, in contrast to the clearly strain-specific activity of IgA and lactoferrin (Stephens *et al.* 1980). Is the specific IgA antibody for *E. coli* more important in in-vivo protection than the overall bacteriostatic property of milk?

Information about O antibodies and antitoxin in the IgA fraction of milk is already being collected in various laboratories. We hope to extend our study to cover this.

For the present, we should advise that, as well as making a valuable nutritional contribution extending into the second year of life (Whitehead *et al.* 1978), breast milk appears to confer an important protective benefit, at least in some children in high-risk environments, as late as 6 months. We found no evidence that the strength of this bacteriostatic protective mechanism was dependent on maternal nutritional status or on the amounts of breast milk produced by the mother.

REFERENCES

BARRELL, R. A. E. & ROWLAND, M. G. M. (1979). Infant foods as a potential source of diarrhoeal illness in rural West Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 73, 85.

DOLBY, J. M., HONOUR, P. & ROWLAND, M. G. M. (1980a). Bacteriostasis of *Escherichia coli* by milk. V. The bacteriostatic properties of milk of West African mothers in The Gambia: in-vitro studies. *Journal of Hygiene* 85, 347.

DOLBY, J. M., HONOUR, P. & VALMAN, H. B. (1977). Bacteriostasis of *Escherichia coli* by milk. I. Colonization of breast-fed infants by milk-resistant organisms. *Journal of Hygiene* 78, 85.

DOLBY, J. M., STEPHENS, S. & ROYSTON, J. P. (1980b). The effect of freezing and pasteurizing bovine milk on its ability to protect neonatal guinea-pigs against colonization of the small intestine by *Escherichia coli*. *British Journal of Experimental Pathology* 61, 8.

ELLIS-PEGLER, R. B., LAMBERT, H. P., ROWE, B. & CROSS, R. J. (1979). *Escherichia coli* in gastroenteritis of children in London and Jamaica. *Journal of Hygiene* 82, 115.

HEYWORTH, B. & BROWN, J. (1975). Jejunal microflora in malnourished Gambian children. *Archives of Disease in Childhood* 50, 27.

HONOUR, P. & DOLBY, J. M. (1979). Bacteriostasis of *Escherichia coli* by milk. III. The activity and stability of early, transitional and mature human milk collected locally. *Journal of Hygiene* 83, 243.

MATA, L. J. & URRUTIA, J. J. (1971). Intestinal colonization of breast-fed children in a rural area of low socio-economic level. *Annals of the New York Academy of Sciences* 176, 93.

MATA, L. J., URRUTIA, J. J. & LECHTIG, A. (1971). Infection and nutrition of children of a low socio-economic rural community. *American Journal of Clinical Nutrition* 24, 249.

PAUL, A. A., MÜLLER, E. M. & WHITEHEAD, R. G. (1979). The quantitative effects of maternal dietary intake in pregnancy and lactation in rural Gambian women. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 73, 686.

ROWE, B. (1979). The role of *Escherichia coli* in gastroenteritis. *Clinics in Gastroenterology* 8, 625.

ROWLAND, M. G. M. & BARRELL, R. A. E. (1980). Ecological factors in gastroenteritis. In *Disease and Urbanization, SSHB Society Proceedings*, vol. 7 (ed. E. G. Clegg and J. P. Garlick), p. 21.

ROWLAND, M. G. M., BARRELL, R. A. E. & WHITEHEAD, R. G. (1978a). Bacterial contamination in traditional Gambian weaning foods. *Lancet* i, 136.

ROWLAND, M. G. M., COLE, T. J. & McCOLLUM, J. P. K. (1980). Weaning diarrhoea in The Gambia: implications of a jejunal intubation study. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 74 (in the Press).

ROWLAND, M. G. M., COLE, T. J. & WHITEHEAD, R. G. (1977). A quantitative study into the

role of infection in determining nutritional status in Gambian village children. *British Journal of Nutrition* **37**, 441.

ROWLAND, M. G. M., DAVIES, H., PATTERSON, S., DOURMASHKIN, R. R., TYRRELL, D. A. J., MATTHEWS, T. H. J., PARRY, J., HALL, J. & LARSON, H. E. (1978b). Viruses and diarrhoea in West Africa and London: a collaborative study. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **72**, 95.

ROWLAND, M. G. M. & McCOLLUM, J. P. K. (1977). Malnutrition and gastroenteritis in The Gambia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **71**, 199.

ROWLAND, M. G. M. & PAUL, A. A. (1980). Factors affecting lactation capacity: implications for developing countries. In *Infant and Early Childhood Feeding* (ed. J. T. Bond, L. J. Filer Jr, G. A. Leveille, A. M. Thomson and W. B. Weil). Nutrition Foundation Monograph Series. New York: Academic Press. (In the Press.)

STEPHENS, S., DOLBY, J. M., MONTREUIL, J. & SPIK, G. Differences in inhibition of the growth of commensal and enteropathogenic strains of *Escherichia coli* by lactotransferrin and secretory immunoglobulin A isolated from human milk. *Immunology* (in the Press).

WHITEHEAD, R. G. (1979). Nutrition and lactation. *Postgraduate Medical Journal* **55**, 303.

WHITEHEAD, R. G., ROWLAND, M. G. M., HUTTON, M. A., PRENTICE, A. M., MÜLLER, E. M. & PAUL, A. A. (1978). Factors influencing lactation performance in rural Gambian mothers. *Lancet* ii, 178.