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Bacteriostasis of *Escherichia coli* by milk. V. The bacteriostatic properties of milk of West African mothers in The Gambia: in-vitro studies

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SUMMARY

Bacteriostatic activity was measured in 244 specimens of milk collected during 1977 throughout lactation of up to one year from 78 mothers; the activity varied from very good to fair and only seven were inactive. There was a wider range of activity than was found previously in milk from English mothers. Activity usually fell slowly during lactation but some of the Gambian mothers produced milk of very high activity, like that of colostrum into the second week of lactation, and two mothers did so at six and nine months; other mothers produced good-activity milk throughout lactation. The bacteriostatic activity varied little with the season but slight decreases from that expected were found after the high incidence of infant diarrhoea towards the end of the rainy season.

The bacteriostatic activity of most of the milk tested could be prevented by iron salts but that of colostrum and some of the milks with high activity could not. Only these highly active colostra and milks were inhibitory *in vitro* when the inoculum was increased from 10^4 to 10^6 organisms per ml. These and less active milks were able to inhibit the smaller, standard inoculum for longer than 3 h with the addition of bicarbonate and extra iron-binding protein at the concentrations likely to be present *in vivo*. Both commensal and pathogenic *E. coli* were inhibited to a similar degree by these milks and there was no evidence of serotype specificity.

INTRODUCTION

In developed societies breast-feeding protects against neonatal enteritis caused by *Escherichia coli*. In developing countries in which breast-feeding is universal, diarrhoea is uncommon in neonates but occurs with increasing frequency in older, breast-fed babies with the progressive introduction of weaning foods.

Bacteriostasis of human milk can be demonstrated *in vitro* for *E. coli* and other Enterobacteriaceae and is thought to be due to the action of lactoferrin, the iron-binding protein of human milk, and antibody (Bullen, Rogers & Leigh, 1972). Purified lactoferrin and IgA are bacteriostatic against enteropathogenic *E. coli*

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in vitro (Rogers & Synge, 1978; Stephens, Dolby, Montreuil & Spik, 1980). Animal experiments described by Bullen *et al.* (1972) suggested that this in-vitro bacteriostatic property of milk may be responsible for in-vivo protection against diarrhoea-producing *E. coli*. There is evidence (Dolby, Stephens & Honour, 1977) that the bacteriostatic property of human milk controls the colonization of the gut of newborn babies, but the bacteriostatic system of bovine milk does not reduce small-intestinal colonization in non-suckled piglets (Stephens, John & Cooper, 1979), nor protect baby guinea-pigs against enteric infection (Dolby, Stephens & Royston, 1980). These findings may not be relevant to human neonates being fed human milk but it is still possible that the bacteriostatic system of milk is not protective *in vivo*.

The milk of West African mothers in the village of Keneba in The Gambia was studied in order to obtain evidence on whether the in-vitro bacteriostatic system protected the human child. We wanted to determine if the in-vitro bacteriostatic activity of any of the milk for *E. coli* was lower than that of milk from mothers in England (Honour & Dolby, 1979) since this might explain why infant diarrhoea due to *E. coli* occurred in breast-fed babies in Keneba. Our tests of bacteriostatic activity and health records of the babies have been correlated and the results will be published later (Rowland *et al.* 1980). In this paper we describe the in-vitro bacteriostatic activity of individual Gambian mothers' milk for two indicator strains of *E. coli* at different stages of lactation and at different seasons.

Since milk is similarly active against commensal and pathogenic strains (Dolby & Honour, 1979), the routine assays were done with two indicator commensal strains, one, an example of a milk-sensitive strain which was inhibited directly by active milk, the other, a milk-resistant strain inhibited by active milk only in the presence of bicarbonate ions, unless the milk was of the very active colostral type. The two strains were tested in each specimen of milk alone and in milk supplemented with sodium bicarbonate in less than physiological amounts and transferrin, in order to mimic conditions in the small intestine (Dolby *et al.* 1977). On the basis of this test, milk was categorized according to activity as described.

METHODS

Source of milk

Up to 1 ml of colostrum at delivery and thereafter 10–20 ml of milk were collected by manual expression from mothers visiting the clinic or in their homes and frozen at −20 °C within five minutes of collection. It was stored frozen at −20 °C for up to 6 weeks and then transported frozen by air to Harrow, transferred to −28 °C and all but 34 specimens were first tested within two months of collection. Seventy-seven mothers contributed 244 specimens; 14 provided only 1, 26 provided 2 specimens, 11 provided more than 2 up to 3 months, 9 provided more than 2 up to 6 months, and 12 provided more than 2 up to 9 months; 5 provided more than 2 up to 12 months.

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Testing of frozen specimens

A few milks were thawed and frozen repeatedly but if numerous tests were being planned, aliquots of milk were taken to avoid freezing and thawing more than two or three times. Milks which were part of a longitudinal study were tested again together after the last collection. Single specimens were re-tested as soon as possible.

Bacteriostatic test

The standard test was carried out in 0·1–0·14 ml volumes for 3 h as described by Honour & Dolby (1979) against commensal indicator strains of *E. coli*; the milk-sensitive strain V21/1 (untypable) and the milk-resistant strain VB71/1 (serotype O21 H4). The activity of each milk was tested alone against these strains and also in the presence of bicarbonate and transferrin. The milk had similar activity when tested against other pairs of indicator strains.

The routine test was carried out using a 3 h peptone–water culture; 0·02 ml of a 10^{-3} saline dilution was added to each tube containing 0·08 ml milk to give about $10^{4\cdot5}$ organisms per ml initially. Duplicate tubes were set up containing sodium bicarbonate to give 0·04 % concentration and transferrin (Sigma, Kingston-on-Thames, England) 2 mg per ml. Tubes were incubated for 3 h at 37 °C. Viable count estimations were then made on tenfold saline dilutions. Iron-reversal tests were done with ferric ammonium citrate solution, adding 0·02 ml to give the concentrations indicated.

Additional tests were carried out using 100 times the standard inoculum (0·02 ml of 10^{-1} culture dilution) to give $10^{6\cdot5}$ organisms per ml, and a longer period of incubation, namely 6 h, in order to mimic the higher in-vivo 'challenges' and long intervals between feeds that babies experienced in The Gambia during the rainy season.

Milk was also tested against enteropathogenic serotypes of *E. coli* isolated at Northwick Park Hospital or in The Gambia and other faecal and water strains from The Gambia.

The results are recorded as the number of times the inoculum increased during the test. Uninhibited inocula increased 50–100 times while active milk usually held the inocula down to an only two- to fivefold increase. Milks that allowed an increase not greater than tenfold were considered active, an 11- to 20-fold increase partially active, and an increase of more than 20-fold inactive.

RESULTS

Qualitative variations in the bacteriostatic activity of Gambian milk

Previously, in testing milk collected in England, two main kinds of bacteriostatic activity had been observed against the two indicator (commensal) strains of *E. coli* (Honour & Dolby, 1979). Early post-partum milk was active against both milk-sensitive strain V21/1 and milk-resistant strain VB71/1 without the addition of bicarbonate ions. Later post-partum milk was active against only the

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Table 1. *Examples of categories of bacteriostatic activity* in vitro *for* E. coli *of Gambian mothers' milk*

		Fold-increase of *E. coli* in 3 h at 37 °C				
		Sensitive strain			Resistant strain	
Category	Time *post partum*	Alone	With Bic/TF*	With Fe†	Alone	With Bic/TF*
A	1 day	2	3	2	3	1
	8 days	8	8	81	9	2
B	9 days	4	3	47	63	7
C	5 months	7	6	81	58	56
	11 months	38	14	100	120	41
	16 months	15	15	59	51	5
D	4 months	20	6	68	35	8
E	6 weeks	63	31	190	74	67

* 0·04% sodium bicarbonate and 2 mg/ml transferrin.
† Ferric ammonium citrate at 20 μg/ml.

milk-sensitive strain unless bicarbonate ions were added; by definition a physiological concentration of bicarbonate was required to 'potentiate' its activity against the milk-resistant strain (Category B, see below).

The activity of the Gambian milk was tested in the same way and found to be much more varied; five categories of activity could be distinguished:

(A) Milk active against the milk-sensitive strain (S) and milk-resistant strain (R). Expected to be active *in vivo* against all strains.

(B) Milk active against S but active against R only in the presence of bicarbonate and transferrin. Expected to be active *in vivo* against all strains.

(D) Milk inactive against S and R except in the presence of bicarbonate and transferrin. Expected to be active *in vivo* against all strains but perhaps less so than category B milk. This category may represent a more active one than category C, below.

(C) A miscellaneous collection including milk having activity or partial activity either against strains S or R directly or in the presence of bicarbonate and transferrin but which does not fit into categories A, B or D above. Expected to be active *in vivo* only against some strains if at all.

(E) Milk having no activity against S or R even in the presence of bicarbonate and transferrin. Expected to be inactive *in vivo* against all strains.

Examples of these categories are shown in Table 1.

Effect of iron salts on the bacteriostatic activity of milk and colostra

The inhibition of the growth of *E. coli* by milk could be prevented by iron salts as shown in Table 1 which indicates that the iron-binding protein, lactoferrin, is involved. The activity of one specimen, a category A one-day colostrum, was not prevented. Other colostra and milk with A activity were tested for reversal of the bacteriostatic activity with iron which is easy with milk of most categories. The results are shown in Table 2. Colostra and category A milk varied in the concen-

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Table 2. *The effect of iron on the bacteriostatic activity of category A colostra and milks*

Colostrum or milk number	Fold-increase of *E. coli* in 3 h at 37 °C with ferric ammonium citrate in μg/ml									
	Sensitive strain					Resistant strain				
	0	20	200	2000	20000	0	20	200	2000	20000
K 415 colostrum	0	2	34	3	nd	2	nd	2	1	nd
K 540 colostrum	4	24	45	32	nd	1	nd	3	4	nd
K 838 colostrum	2	8	24	31	28	1	2	2	2	2
K 393 8-day	1	12	1	nd	nd	0	53	16	nd	nd
K 432 6-week	0	1	0	39	50	4	nd	nd	nd	nd
K 432 9-month	0	0	0	23	47	2	nd	nd	nd	nd

nd = not done.

Table 3. *The iron-reversal of bacteriostasis of category A colostra and milks for a milk-sensitive strain of* E. coli *by 20 μg/ml of ferric ammonium citrate*

Specimen	No. tested	Fold-increase of *E. coli* in presence of iron		
		<10	20–50	>50
Colostra	17	1	5	11
2–8 day milk	13	9	0	4*
6-week milk	1	0	0	1
9-month milk	1	0	0	1

* 8-day milk.

tration of ferric salt required to reverse the bacteriostatic property for the sensitive strain but this was usually achieved by 2 μg/ml. Bacteriostasis against the resistant strain was not reversed by up to 20 μg/ml, except in one sample, a one-week milk K393. We repeatedly found examples of inability of ferric salt to reverse bacteriostasis by concentrations higher than the minimal reversing one, e.g. lines 1 and 4 of Table 2. Table 3 indicates how varied was the effect of iron on the activity of the category-A colostra and milk against the sensitive strain.

The bacteriostatic activity of milk during lactation and at different seasons

A comparison of the bacteriostatic property at different times after parturition was made. The results are shown in Table 4. Milks collected up to two days *post partum* were nearly all of high, category A activity; 26% of those in the 7- to 8-day period *post partum* were also category A. This last was unexpected and found only rarely in over 100 milks collected 5 to 7 days *post partum* in Harrow. Some Gambian mothers were still producing category A milk between 3 and 12 months *post partum* (Table 4). A substantial proportion of samples collected between 1 week and 9 months *post partum* fell into category B but the longer the time since parturition the larger the number of milks that fell into categories C, D and E.

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Table 4. *Variation in bacteriostatic activity of Gambian milks with time post partum*

Time *post partum*	Total no.	Percentage of milk in each category				
		A	B	C	D	E
0–2 days	24	83	13	4	0	0
7–8 days	32	37	44	16	3	0
9–14 days	10	20	30	30	20	0
3–4 weeks	6	0	68	16	0	16
5–8 weeks	39	7	44	41	5	3
9–12 weeks	31	13	23	35	23	6
4–6 months	44	5	39	34	23	0
7–9 months	26	7	19	51	19	3
10–12 months	18	11	6	34	39	11
> 12 months	14	0	7	57	36	0

Table 5. *Loss of bacteriostatic activity of the milk of two mothers during lactation and on storage at* −28 °C

Milk number	Time: Produced after delivery	Time: Tested after collection	Fold-increase of *E. coli* in 3 h at 37 °C: Sensitive strain: Alone	Sensitive strain: With Bic/TF*	Resistant strain: Alone	Resistant strain: With Bic/TF*	Category
K 41	7 days	10 weeks	4	3	5	2	A
		6½ months	10	10	48	11	B
	6 weeks	5 weeks	5	7	89	5	B
		5½ months	4	6	120	35	C
	13½ weeks	3 weeks	23	10	130	7	C
		4 months	23	17	200	46	(C)
K 84	1 day	2 weeks	4	7	2	3	A
		4 months	4	7	2	2	A
	9 days	4 weeks	4	3	63	7	B
		4 months	12	11	81	37	C
	6 months	4 weeks	4	8	57	6	B
		4 months	26	17	158	79	(C)

* Abbreviation as in Table 1.

Specific examples of the decreasing bacteriostatic activity during lactation of the milk of two individual mothers are shown in Table 5, which illustrates the general trend shown in Table 4.

Table 5 also shows the effect of storage on the bacteriostatic activity of specimens collected at different stages in lactation and of different activities. There was no loss of activity on storage up to two months; thereafter it could happen with any specimen but was by no means consistent. Freezing and rethawing exaggerated the tendency. These observations have a bearing on the interpretation of the results of 34 milk specimens (15 %) first tested between three and five months after collection. Of these, three were of category A and so could not have lost activity; five were B, fifteen C, nine D and two E, and could have done. They have, how-

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Table 6. *The variation in bacteriostatic category of milks collected at different times* post partum *with season during* 1977

Time *post partum*	No. of milks in categories A–E Feb.–Mar. A B C D E	Apr.–May A B C D E	Jun.–Jul A B C D E	Aug.–Sep. A B C D E	Oct.–Nov. A B C D E	Dec.–Jan. A B C D E
0–2 days	4 0 0 0 0	3 0 0 0 0	7 0 0 0 0	2 1 0 0 0	1 0 0 0 0	3 2 1 0 0
Totals	4	3	7	3	1	6
3–14 days	4 5 2 0 0	4 2 1 1 0	2 3 1 1 0	1 3 2 0 0	2 1 0 1 0	1 3 2 0 0
Totals	11	8	7	6	4	6
3–8 weeks	1 3 3 1 0	1 7 4 0 0	1 2 1 1 0	0 3 0 0 1	0 1 3 0 0	0 3 4 0 0
Totals	8	12	5	4	4	7
9 weeks to 6 months	0 2 5 2 1	1 1 5 3 0	1 7 3 3 0	1 5 3 3 0	2 3 2 2 0	1 5 6 1 1
Totals	10	10	14	12	9	14
7–12 months	2 5 1 4 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0	2 1 4 4 0	0 1 10 3 2
Totals	12	0	0	0	11	16

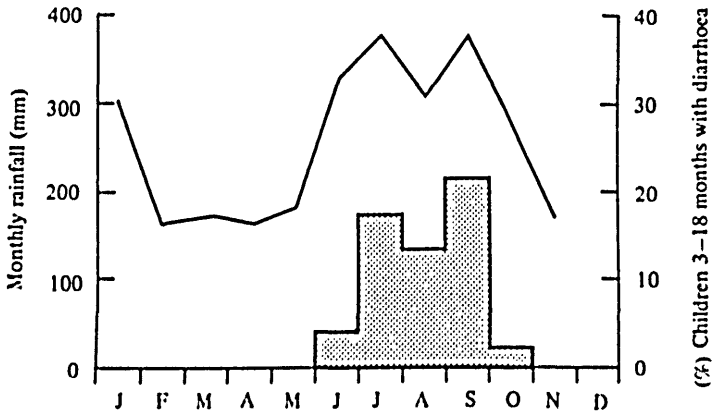

Fig. 1. The rainfall and incidence of diarrhoea in children in Keneba for the year 1977. The monthly rainfall ▨ is given in mm. The monthly prevalence of diarrhoea ●——● was in children 3 to 18 months.

ever, been included in the Table 4 data because the activity measured was much as expected from previous and subsequent samples tested within two months, from the same mothers as the long-stored specimens.

In Table 6 the bacteriostatic activity of milk is related both to time after parturition and to season. The rainfall and general incidence of diarrhoea in the infant–toddler population for the same year 1977 are shown in Fig. 1. There was

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a long period of high incidence of diarrhoea during the rains (from July to September) and another short one in the dry season (from December to January).

In the latter part of the rainy season and in the dry period at the end of the year, there was a tendency towards the production of less active milk early in lactation. Table 6 shows that not all 0- to 2-day milk was category A as at other times, and fewer 1- to 2-week milks were of category A activity. The numbers of specimens are, however, very small. This may reflect the mothers general health at a time of poor nourishment and hard physical work, but is too late to account for the high incidence of diarrhoea.

The E. coli*-specificity of the activity of milks*

The bacteriostatic activity of all milk previously tested depended only on the milk sensitivity of the strain being grown and not on its pathogenicity or serotype; no strain specificity has ever been detected. To confirm that this was so with Gambian milk, 25 specimens were tested against enteropathogenic strains of *E. coli* of serotypes O111, O128 and O119 of different milk sensitivities. The results were as for the indicator strains. (Serotype O111 was not isolated from the stools of Gambian babies in 1977 whereas O128 and O119 were. Rowe, personal communication.) Two milks were tested extensively against three milk-resistant strains isolated from London babies, seven milks were each tested against three strains of *E. coli* from the donor mother and three from the donor's baby; in all tests, the results were dependent on the milk sensitivity of the strains. One B and one D milk were tested against 20 strains isolated from Gambian babies with diarrhoea and again the results were the same as those with the indicator strains. We concluded that the activity detected against the indicator strains was likely to be shown against all *E. coli*.

The activity of milk of different categories in inhibiting large inocula and for up to six hours

In order to mimic the large challenges of *E. coli* and infrequent day-time feeding we measured the capacity of milk to inhibit larger inocula and for longer times. Milks of three categories, A, B and D or C, representing a cross-section of bacteriostatic activity, were tested. Firstly, 100 times the usual inoculum of the sensitive and resistant indicator strains was used and the results are shown in Table 7. The A milks were still active against both strains without the addition of bicarbonate and transferrin and were able to hold down the large inocula. The B milk which inhibited the sensitive strain and in the presence of bicarbonate and transferrin, the resistant strain, now only partially inhibited the sensitive strain and not the resistant strain even with the addition of bicarbonate and transferrin. Of the two D milks which were active in the presence of bicarbonate and transferrin against the smaller inocula of both strains, only one similarly inhibited a large inoculum of the sensitive strain. These results indicate that only category-A milk would be effective against a large challenge of a resistant strain although B milk and some D milk would have some activity against a sensitive strain.

To test the maintenance of bacteriostasis beyond three hours following a small

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Table 7. *Ability of Gambian milk of three categories to inhibit the growth of small and large inocula of* E. coli in vitro

			Fold-increase of *E. coli* in 3 h at 37 °C with							
			Usual inoculum				100 × inoculum			
			Sensitive		Resistant		Sensitive		Resistant	
Milk number	Time p.p.*	Category	Alone	With Bic/TF	Alone	With Bic/TF†	Alone	With Bic/TF†	Alone	With Bic/TF†
K393	6½-week	A	0	nd	0	nd	0	nd	0	nd
K2302	1-day	A	2	nd	2	nd	3	nd	2	nd
K84	9-day	B	9	nd	66	8	13	18	41	30
K479	14-month	D	23	9	27	5	19	8	20	25
K945	6-month	D	30	10	19	8	39	30	27	31

* p.p. = *post partum*. † Abbrevations as in Table 1. nd = not done.

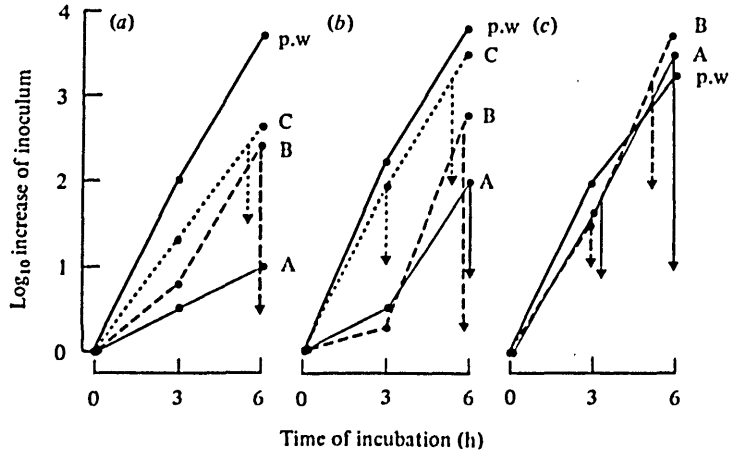

Fig. 2. The inhibition of growth over 6 h of three strains of pathogenic *E. coli* of varying milk-sensitivity, by Gambian milk of categories A, B, C at the time of testing. The inoculum was the smaller one of 10^4 organisms/ml. The curves show the growth of *E. coli* in the milk alone: ●—●, K838, 5-week, category A milk; ● – – ● K432, 9-month category B milk; ● · · · ● K432, 12-month category C milk. Growth in 1% peptone water is shown as ——— and vertical lines show the increased activity and decreased growth at 3 and 6 h of *E. coli* in bicarbonate (0·04%) and transferrin (2 mg/ml) potentiated milk. For *E. coli* (a)–(c), see text.

inoculum three other milks were incubated for up to six hours with the usual 10^4 inoculum of enteropathogenic *E. coli*, namely an O111 strain (b) of intermediate sensitivity and one sensitive (a) and one resistant (c) strain of type O128 (Fig. 2). Only the A milk could prevent the growth of all strains to below a tenfold increase; for any but the sensitive strain this happened only in the presence of bicarbonate and transferrin. The B milk was inhibitory in the presence of bicarbonate and transferrin for the sensitive strain and strain of intermediate resistance only. The C milk was ineffective against all strains even in the presence of bicarbonate and transferrin.

It can thus be assumed that, to be effective against larger numbers of organisms or for up to six hours, the milk must either be category A (for all strains) or category B for strains of no more than intermediate milk sensitivity. Thus, milks of categories A and B may be capable of bacteriostatic effect *in vivo* against a small amount of ingested *E. coli* for a period equivalent to the transit times through the small intestine.

DISCUSSION

Before considering the implications of our findings, two aspects of our routine screening of the milk specimens for bacteriostatic activity will be discussed: the addition of bicarbonate and transferrin, and of iron salts.

The reason for assuming that the addition of bicarbonate and extra iron-binding protein to milks under test for inhibitory properties to *E. coli in vitro* has some bearing on *in vivo* activity has been discussed previously (Dolby *et al.* 1977). The

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categorization is based on differences in performance without and with these two additives, category A being so bacteriostatic alone that no bicarbonate or transferrin is required. This method of categorizing bacteriostatic activity may obscure differences between milks due to lactoferrin content and be based on differences in antibody content only. Concentrations of lactoferrin and IgA, and bacteriostatic activity all tend to fall together, however, (Spik, Stephens & Dolby, unpublished data), so that it seems valid and useful to categorize the bacteriostatic activity of milk as we have done.

We had already observed that iron salts did not reverse the inhibitory activity of colostra against milk-resistant strains (Honour & Dolby, 1979). This was also true of Gambian *milk* of category A and the earlier in lactation the specimen was collected, the more difficult it was to reverse the bacteriostatic activity with iron. By definition, category A colostrum or milk is that which inhibits all strains directly without additives. The reversal of bacteriostatic activity by iron salts was introduced routinely to check that extraneous antibacterial activity, e.g. antibiotics or disinfectants, was not being measured. Yet, in defining category A, it would seem that we are ignoring a possible explanation of activity. As shown in Table 2, however, activity against the milk-sensitive strain *is* reversed by higher concentrations of iron-salt and non-reversing activity has always been found in colostrum or milk having concentrations of IgA and lactoferrin more like that of colostrum than milk (Spik & Dolby, unpublished data). We believe, therefore, that the antibacterial activity of category A colostrum and milk is due to a natural mechanism.

The bacteriostatic activity of Gambian milk so measured has been shown to be no lower than that of United Kingdom milk and often better. Specimens collected in England were almost entirely of categories A and B; colostrum was almost entirely category A but only 2% of early post-parturition milks were in this category (two out of eighty at 5–7 days *post partum*) compared with 26% Gambian milk in category A at 7–8 days. On the other hand, milk collected in England tended to remain in category B; there was a sudden decrease in activity just before the end of lactation in three mothers (Honour & Dolby, 1979).

Only category A colostrum or milk was really effective *in vitro* in holding down large inocula of *E. coli* and for up to six hours. Category B milk was capable of doing so for smaller inocula for six hours for the more milk-sensitive strains; bicarbonate and transferrin potentiated the activity. With large inocula, however, milk other than A was ineffective and the addition of bicarbonate and transferrin made no difference to the performance.

Thus, the quality of Gambian milk with regard to its bacteriostatic property for *E. coli* is as good as elsewhere. The quantity late in lactation, particularly during the rainy season, is lower than at other times and for this there is no comparison in the United Kingdom (Whitehead *et al.* 1978). From our in-vitro results, only category A milk might be expected to be effective *in vivo* against large challenges. Our findings in the field are described by Rowland *et al.* (1980).

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REFERENCES

BULLEN, J. J., ROGERS, H. J. & LEIGH, L. (1972). Iron-binding proteins in milk and resistance to *Escherichia coli* infection in infants. *British Medical Journal* i, 69.

DOLBY, J. M. & HONOUR, P. (1979). Bacteriostasis of *Escherichia coli* by milk. IV. The bacteriostatic antibody of human milk. *Journal of Hygiene* 83, 255.

DOLBY, J. M., STEPHENS, S. & HONOUR, P. (1977). Bacteriostasis of *Escherichia coli* by milk. II. Effect of bicarbonate and transferrin on the activity of infant feeds. *Journal of Hygiene* 78, 235.

DOLBY, J. M., STEPHENS, S. & ROYSTON, J. P. (1980). The effect of fre zing 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.

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.

ROGERS, H. J. & SYNGE, C. (1978). Bacteriostatic effect of human milk on *Escherichia coli*: the role of IgA. *Immunology* 34, 19.

ROWLAND, M. G. M., COLE, T. H., TULLY, M., DOLBY, J. M. & HONOUR, P. (1980). 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. *Journal of Hygiene* 85, 405-413.

STEPHENS, S., DOLBY, J. M., MONTREUIL, J. & SPIK, G. (1980). 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.)

STEPHENS, S., JOHN, M. & COOPER, J. E. (1979). The effect of *in vitro* anti-bacterial properties of bovine milk diets on the natural colonization of newborn piglets with coliform bacteria. *Research in Veterinary Science* 26, 21.

WHITEHEAD, R. G., ROWLAND, M. G. M., HUTTON, M., PRENTICE, A. M., MULLER, E. & PAUL, A. (1978). Factors influencing lactation performance in rural Gambian mothers. *Lancet* ii, 1078.

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