J. Hyg., Camb. (1979), **83**, 255
Printed in Great Britain

Bacteriostasis of *Escherichia coli* by milk. IV. The bacteriostatic antibody of human milk

BY JEAN M. DOLBY AND PAULINE HONOUR
The Clinical Research Centre, Harrow, Middlesex HA1 3UJ

(*Received* 16 *October* 1978)

SUMMARY

Bacteriostatic activity for milk-sensitive and milk-resistant strains of *Escherichia coli* is reduced when IgA is removed from milk by precipitation. Lysozyme is not involved in bacteriostasis and can be removed without loss of activity; heavy bentonite absorption however removes some lactoferrin causing partial loss of activity.

The heat-labile antigen eliciting bacteriostatic antibody for *E. coli* is present in milk-sensitive and milk-resistant strains and in some other Enterobacteriaceae, e.g. salmonella; it cross reacts with the antigen in others, e.g. proteus and enterobacter. The antibody is therefore likely to be present in all human milk as a result of the normal commensal gut flora and with widespread activity.

INTRODUCTION

Lactoferrin and transferrin, the iron binding proteins found in milk, have a bacteriostatic effect on the growth of *Escherichia coli* by depriving the bacteria of iron (Oram & Reiter, 1968). Milk antibody, which alone has no effect on the growth rate of bacteria, increases the bacteriostatic effect of lactoferrin or transferrin. Bullen, Rogers & Leigh (1972) demonstrated this for horse-serum IgG and human milk lactoferrin, Rogers & Synge (1978) for human milk IgA and lactoferrin and Spik *et al.* (1978) for human and bovine immunoglobulin from milk with lactoferrin and serum transferrin.

This paper brings together some further observations on the antibody involved in bacteriostasis by human milk against strains of differing susceptibility to the bacteriostatic system: i.e. milk-sensitive strains which are inhibited directly by milk and milk-resistant strains which are inhibited by milk in the presence of bicarbonate, and explores the specificity of participating antibody.

METHODS

Milk specimens

Milk was collected and stored at −28 °C, raw, or at 4 °C after heating to 56 °C for 30 min. Collection was made from local mothers mainly in Northwick Park Hospital and from mothers in the Gambia. Milk was heated as previously

0022-1724/79/0123-1978 $01.00 © 1979 Cambridge University Press

https://doi.org/10.1017/S0022172400026048 Published online by Cambridge University Press

described and dilutions where indicated made in 'milk 100°' as before (Honour & Dolby, 1979).

The bacteriostatic test

This was as described previously (Honour & Dolby, 1979).

Bacterial strains

The strains of *E. coli* used as standard indicator strains in the bacteriostatic test were V21/1 isolated from the stools of a mother 1 week after delivery and VB71/1 from the stools of a 1-week old, breast-fed baby. Other commensal strains were from similar sources, locally and from Keneba, the Gambia. *E. coli* was also isolated from well-water and food in the Gambia. Strains of enteropathogenic serotype were from the Microbiology Department, Northwick Park Hospital. Strains were kept either on Dorset-egg slopes or in nutrient agar stabs.

Other Enterobacteriaceae used for absorption experiments were isolated from the stools of bottle-fed babies of varying ages and were *Klebsiella* sp. M4/3, *Enterobacter* sp. VB84/2, *Pseudomonas* sp. AM26/1, *Proteus* sp. AM755B. *Salmonella newport*, *Staphylococcus albus*, *Streptococcus viridans* and *Neisseria pharyngis* were from hospital specimens.

Absorption of milk with bacterial suspensions

The bacteria were grown in 10 or 20 ml volumes of brain–heart infusion-broth overnight at 37 °C. The cultures were heated to 63 °C for 45 min or 100 °C for 15 min, centrifuged and the bulk of supernatant discarded. The sediments, resuspended in culture supernatant, were collected in deposits of 1×10^9 to 5×10^{10} cells in $10 \times 0{\cdot}5$ mm glass tubes. To each mass of packed cells was added 1 ml of milk which was absorbed for 2–3 h at 37 °C. This was repeated two to three times with fresh bacterial cells which were centrifuged out at about 3000 *g*. Washing the heated organisms with saline reduced their absorbing capacity.

Absorption of milk with bentonite

The lysozyme was absorbed from milk by bentonite (Hopkin & Williams, Chadwell Heath, Essex, England); 0·5 g amounts were shaken with 50 ml of distilled water and the large particles allowed to settle by gravity for 15–30 min. The supernatant was decanted off and divided into five portions. Additional water up to 25–50 ml was added to each portion, the suspensions shaken and then centrifuged to collect the finer particles. The washed sediments of bentonite were each used to absorb 1 ml of milk for 30 min at room temperature.

Lysozyme assay

Lysozyme (Sigma, Kingston-on-Thames, England) was made up in 0·5 mg/ml stock solutions in phosphate buffer at pH 6·4 and diluted in buffer as required to give 500, 100, 20 and 4 μg/ml solutions. The assay was carried out by the method of Osserman & Lawler (1966). The clearing in agar of a suspension of heat-killed *Micrococcus luteus* NCTC 2665 (National Collection of Type Cultures,

https://doi.org/10.1017/S0022172400026048 Published online by Cambridge University Press

Central Public Health Laboratories, Colindale, London) by standard lysozyme solutions and unabsorbed and absorbed milk was compared and the amount of lysozyme absorbed by bentonite estimated.

Precipitation of IgA from milk

Milk samples of known IgA content were depleted of IgA by direct addition of anti-IgA. Specifically purified rabbit anti-IgA (Platts-Mills & Ishizaka, 1975) was added to achieve optimal precipitation (approximately equal quantities of anti-IgA and IgA). In one experiment anti-IgA was dialysed against three changes of unbuffered saline over 24 h prior to precipitation. Precipitates were allowed to form overnight at 4 °C and were removed by centrifugation at 15000 *g* for 20 min. IgA concentrations in milk samples were measured by double antibody inhibition radio-immunoassay.

RESULTS

The dependence of bacteriostasis on antibody

IgA immunoglobulin was precipitated from human milk by rabbit anti-IgA. The experiment was done on two individual, 1-week *post partum* milks and a pool of milk collected later in lactation with similar results. Bacteriostasis was measured before and after precipitation against the milk-sensitive indicator strain V21/1 and the milk-resistant one VB71/1.

Table 1. *The dependence of bacteriostasis for a milk-sensitive strain of* E. coli *on IgA*

	Maximum bacteriostatic dilution*	
	Milk V97	Pool milk 29376
Untreated milk + 2 mg/ml transferrin	1/18	1/27
Milk after precipitation with rabbit anti-human IgA + 2 mg/ml transferrin	1/3	1/3

* The dilution which allowed the growth of less than three times that of the untreated milk. The result for V97 was the average of four similar assays, that for the pool milk the average of two similar assays.

Against the milk-sensitive strain, undiluted, precipitated milk still had some activity. The maximum bacteriostatic dilutions were determined by diluting in milk inactivated for 5 min at 100 °C. Transferrin was added to each tube to ensure that iron-binding protein was not the limiting factor; 0·2–0·5 mg/ml is needed for bacteriostasis and lactoferrin becomes quickly diluted out from the 2–4 mg/ml concentrations of untreated milk. Table 1 shows the decrease in the maximum bacteriostatic dilution after precipitation, against the milk-sensitive strain for one individual milk and a pool milk each diluted three fold. Removal of IgA caused a greater than six-fold reduction of titre.

The milks used in these experiments were not active enough against the milk-resistant strain to permit assay of loss of bacteriostatic activity by dilution.

https://doi.org/10.1017/S0022172400026048 Published online by Cambridge University Press

A comparison of the number of times the inoculum grew in undiluted milk before and after treatment is shown in Table 2. The growth of the resistant strain in a 1/2 dilution of milk is given for comparison. Transferrin was again added to

Table 2. *The dependence of bacteriostasis for a milk-resistant strain of* E. coli *on IgA*

	No. times inoculum increased in milk + Bic/TF*		
	V97, 1/1	V97, 1/2	Pool milk 29376, 1/1
Untreated milk	3	68	10
Milk after precipitation with rabbit anti-human IgA	16	110	14
'Milk 100°' diluent	100	110	65

* Sodium bicarbonate (Bic) was at 0·04% and transferrin (TF) at 2 mg/ml.

each tube to ensure that iron-binding protein was not the limiting factor. Bicarbonate was added to activate the milk for the resistant strain. The role of IgA is definite but less marked than for the milk-sensitive strain.

Evidence of the lack of strain specificity of the bacteriostatic activity of milk for E. coli *from milk activity and strain sensitivity tests*

We have reported previously (Dolby, Honour & Valman, 1977) that about 50 specimens of 1-week *post partum* milk collected locally were inhibitory to the growth of some strains, commensal and of enteropathogenic serotype and not to others but that the strains which were resistant to one milk were resistant to all. These milk-resistant strains became sensitive to the activity of milk in the presence of low concentrations of bicarbonate; the bicarbonate concentration could be reduced further when transferrin was added whereas transferrin alone was inactive (Dolby, Stephens & Honour, 1977). The determination of the activity of 150 or more locally collected milks from 1 week to 7 months *post partum* against six or so local indicator strains does not alter this conclusion.

We have now also had the opportunity to test 40–50 strains of *E. coli* isolated from the stools of mothers and babies in the Gambia and from food and water. These strains were first tested for growth in one of three bacteriostatic local milks and classified as sensitive or resistant. Two-thirds of the strains derived from stools of mothers and babies were then tested against the milk of the donor or donor's mother. Of these, one third were tested against milks from other Gambian mothers. The remainder of the strains isolated from food and water were tested against 1 or 2 Harrow and 1 or 2 Gambian milks. The Gambian milks were measured for bacteriostatic activity against two indicator strains isolated locally in Harrow.

All strains behaved consistently without evidence of strain specificity. Those sensitive to one milk, local or Gambian, were also sensitive to other local or Gambian milks which had already been shown to be active against the local

https://doi.org/10.1017/S0022172400026048 Published online by Cambridge University Press

milk-sensitive indicator strain, and against the local milk-resistant strain in the presence of bicarbonate and transferrin. All the milks behaved consistently, their activities being similar whether Gambian or Harrow to Gambian and Harrow strains already classified by a local milk. Inactive or less active milks also behaved consistently against a variety of strains.

An example of the repeated findings is shown in Table 3 in which the bacteriostatic activity of Gambian and Harrow milks is compared. The sensitivity of our two indicator strains V21/1 and VB71/1 is shown for the two milks.

Table 3. *Comparison of activity of milks and sensitivity of* E. coli *to bacteriostasis*

		No. times inoculum increased in milk	
Milk donor	Source of faecal *E. coli*	Alone	Bic/TF*
Gambian mother K 525	Gambian mother K 525	8	4
	K 525's baby	24	37
	Harrow mother V21	9	6
	Harrow baby VB71	35	8
Harrow mother V144	Gambian mother K 525	3	4
	K 525's baby	14	22
	Harrow mother V21	8	5
	Harrow baby VB71	50	5

* Sodium bicarbonate (Bic) was at 0·04% and transferrin (TF) at 2 mg/ml.

Table 4. *The absorption of bacteriostatic activity for a milk-sensitive strain* V21/1 *from milk with bacterial suspensions*

	No. times inoculum V21/1 increased in milk						
Absorption of milk with:	56 °C	56 °C, 1/4 in 100 °C	100 °C	56 °C abs.	100 °C abs.	56 °C, 1/4 in 100 °C abs.	56 °C abs, 1/4 in 100 °C
Milk-sensitive strain V21/1 × 63 °C	1	6	90	233	300	262	64
Milk-resistant strain VB71/1 × 63 °C	1	6	90	184	200	500	40
Milk-sensitive strain V21/1 × 100 °C	4	6	340	6	700	6	9

Absorption of activity from milk by suspensions of E. coli

Table 4 shows that the bacteriostatic activity for the milk-sensitive strain V21/1 was removed by 2–3 absorptions with strain V21/1, live or killed, by heating the culture or a saline suspension to 63 °C for 30 min, but that heating the absorbing organisms to 100 °C destroyed the ability to absorb bacteriostatic activity. Other milk-sensitive strains were similarly capable of absorbing activity for V21/1. The bacteriostatic activity for the milk-sensitive strain was also

https://doi.org/10.1017/S0022172400026048 Published online by Cambridge University Press

removed by absorbing with a milk-resistant strain (Tables 4 and 5). The results were not significantly affected by the addition of extra iron-binding protein as transferrin.

Table 5. *The absorption of bacteriostatic activity for a milk-sensitive and a milk-resistant strain after absorption of milk pool* V162, 5 *day* post partum *with both strains*

		No. times inoculum increased in milk	
Milk absorbed with:	Milk tested against:	Alone	With Bic/TF*
V21/1 Sensitive	V21/1	140	53
	VB71/1	61	75
VB71/1 Resistant	V21/1	51	50
	VB71/1	42	69
Unabsorbed	V21/1	5	10
	VB71/1	47	7

* Sodium bicarbonate (Bic) was at 0·04 % concentration and transferrin (TF) at 2 mg/ml.

Testing these absorbed milks with a milk-sensitive strain of different antigenic composition, serotype O128 compared with the commensal strain V21/1 which was not of enteropathogenic serotype, produced similar results to those given in Table 4. These results suggest that for a milk-sensitive strain, activity can be removed by absorption with any strain of *E. coli* live or heated at 63 °C but not heated at 100 °C.

In order to test for removal of activity against a milk-resistant strain it was necessary to add bicarbonate and additional iron-binding protein, as transferrin, to the assays. The results in Table 5 show that for milk-resistant VB71/1, activity can be removed by V21/1 and VB71/1.

As seen in Table 4 absorption of either the 56 °C-heated milk (active) or 100 °C-heated milk (inactive) used as diluent was able to reduce the activity of a 1/4 dilution. Presumably enough antigen involved was still present, unprecipitated, either washed off and cell-free or in unremoved organisms and able to block the activity when the dilution was made.

The absorption of activity for E. coli *from milk by suspensions of bacteria other than* E. coli

The bacteriostatic activity for V21/1 was not removed by suspensions of the unrelated bacteria *Staph. albus*, *Strept. viridans*, *N. pharyngis*. The milk of several mothers was however as active against a milk-sensitive strain of *S. newport* in the absence of any known infection as against *E. coli*. Many human milks have also been active against other members of the Enterobacteriaceae, e.g. *Proteus* sp., *Enterobacter* sp.

Absorption of milk by six members of the Enterobacteriaceae, all gram-negative bacteria commonly found in the gut and milk-sensitive (the *Proteus* sp. was only marginally so) was carried out and the resultant absorbed milk tested against

https://doi.org/10.1017/S0022172400026048 Published online by Cambridge University Press

homologous and heterologous strains. The results of one set of absorptions, the average of three assays, is shown in Table 6 and the cross relationships are shown in Table 7. The deduced, resultant make-up of these strains with respect

Table 6. *Cross-absorption of bacteriostatic antibody for milk-sensitive strains from milk by Enterobacteriaceae*

	No. of times inoculum increased in milk						
		Absorbed with					
Indicator strain	Un-absorbed	*Klebs.*	*Salm.*	*Entero.*	*Pseud.*	*Proteus*	*E. coli*
Klebsiella sp.	4	**50**	8	13	*22*	3	6
Salmonella newport	4	3	**46**	8	4	6	**40**
Enterobacter sp.	6	3	20	**250**	**63**	**80**	**40**
Pseudomonas sp.	—	—	—	—	—	—	—
Proteus sp.	20	14	*34*	**49**	*38*	*38*	**50**
Escherichia coli	8	3	**40**	8	4	6	**55**

—, Not done.
The results are the average of three experiments.

Table 7. *Relationship in the Enterobacteriaceae of the antigen eliciting bacteriostatic antibody*

Indicator strain	Bacteriostatic activity removed by absorption with:	Will absorb out
Klebsiella sp. (K)	K, (Ps)	K
Salmonella newport (S)	S, C	S, C
Enterobacter sp. (E)	E, Ps, P, C	E (P)
Pseudomonas sp. (Ps)	not done	K, E, P
Proteus sp. (P)	E, C, (Ps, P, S)	E, P
Escherichia coli (C)	C, S	C, S, E, P

to the antigen eliciting bacteriostatic antibody is the same as that given in the last column of Table 7. The cross-reaction between *S. newport* and *E. coli* was complete, but *E. coli* also cross-reacted with *Proteus* sp. and *Enterobacter* sp. but not with *Pseudomonas* sp. (only half of these tests could be done as *Pseudomonas* grew poorly in milk). *Klebsiella* sp. was almost completely without cross reactions.

Absorption of milk by bentonite, and the role of lysozyme in bacteriostasis

As a control to the absorption with bacterial suspensions, milk was treated with bentonite which removes many milk components including lysozyme. The bentonite absorption dramatically reduced the lysozyme content of milk but had only a slight effect on the bacteriostatic activity of undiluted milk as shown in Table 8. The milk-sensitive strain was less inhibited but activity was restored by adding bicarbonate and transferrin, suggesting absorption of lactoferrin. The absorptions varied a little from batch to batch; in one experiment there was a six-fold loss of IgA from 600 to 100 μg/ml, but this was above the limiting concentration of IgA (Spik *et al.* 1978).

https://doi.org/10.1017/S0022172400026048 Published online by Cambridge University Press

Table 8. *Bacteriostatic activity of milk absorbed by bentonite*

Milk	Lysozyme (units/ml)	No. times inoculum increased: V21/1 Alone	V21/1 +Bic/TF*	VB71/1 Alone	VB71/1 +Bic/TF*
Unabsorbed V144	12500	4	2	66	20
Absorbed V144	< 100	17	6	56	6
Unabsorbed V162	nd	5	10	47	7
Absorbed V162	nd	35	4†	31	4

nd, Not done.

* Sodium bicarbonate (Bic) was at 0·04 % and transferrin (TF) at 2 mg/ml.

† Effect achieved with TF only.

Table 9. *Restoration of loss of bacteriostatic activity for* V21/1 *of bentonite absorbed milk*

Milk	1/1	1/4 in 100 °C	1/4 in 100 °C absorbed
Unabsorbed	3	4	4
Absorbed	5	38	—
Absorbed + L*	4	30	—
Absorbed + TF*	2	5	—

* Lysozyme (L) was at 0·4 mg/ml and transferrin (TF) at 2 mg/ml. In 100 °C milk lysozyme had no bacteriostatic effect, transferrin reduced the inoculum-increase from 50 to 20.

From Table 9 it again appears that the loss of activity from milk absorbed by bentonite was due to removal of lactoferrin and not to removal of lysozyme. Unlike V144 and V162 (Table 8) this milk was not sufficiently depleted to cause loss of activity when tested undiluted, but loss was obvious at a 1/4 dilution.

DISCUSSION

The IgA precipitation experiments described here provide other proof of the involvement of IgA in the bacteriostatic system of milk active against milk-sensitive and milk-resistant strains. These results confirm those of Nagy, Mackenzie & Bharucha (1976) for porcine milk. We found it more difficult by this method to demonstrate IgA activity against milk-resistant strains than milk-sensitive strains. Rogers & Synge (1978) however have demonstrated the involvement of IgA against a strain of O111 which is of intermediate resistance, using purified antibody and lactoferrin. The bacteriostatic activity remaining after precipitation of the IgA could presumably be accounted for by iron-binding protein acting alone or with antibody other than IgA.

From our results it seemed to us probable that the antigen stimulating the bacteriostatic antibody for *E. coli* is of widespread occurrence in the Enterobacteriaceae. Many of the milks which we tested and found to be active against

https://doi.org/10.1017/S0022172400026048 Published online by Cambridge University Press

one strain of *E. coli* were also active against other milk-sensitive strains of *E. coli* and other similar genera.

We could for instance demonstrate a bacteriostatic action for *S. newport* in the milk of mothers who had never knowingly been infected and who were not carriers, provided that the milk was active against *E. coli*. Allardyce *et al.* (1974) failed to demonstrate bacteriostatic activity for *Salmonella typhimurium* in the milk of mothers who were infected during pregnancy even although agglutinins were present in the milk. Their results may have been due to the milk insensitivity of the strain of *S. typhimurium* which was isolated or because the bacteriostatic activity was reversed by excess iron in the diluent used in the test.

Working with bovine and human milk, Griffiths & Humphreys (1978) have found what they consider evidence of strain specificity of bacteriostatic antibody: one strain of *E. coli* was inhibited by bovine but not human milk and another strain inhibited by the same bovine but not human milk. We have sometimes found that bovine milk was more active than human milk against certain strains but have never found strains in which the results could be reversed depending on the milk used.

Hanson (1976) raised the question of the specificity of protection but until we know on what protection depends this is difficult to answer. The elegant experiments of Goldblum *et al.* (1975) demonstrated anti-lipopolysaccharide antibody (i.e. antibody to the O antigen identified by serotyping) to a strain of *E. coli* ingested by pregnant mothers, and Lodinova & Jouja (1977) have demonstrated haemagglutinating antibodies in the milk of mothers nursing babies colonized with the same strain. Hanson (1976) however, quotes an experiment by Andersen and colleagues in which, during an epidemic of *E. coli* O111, breast-fed babies were protected whereas bottle-fed babies were not in spite of the fact that O-agglutinins to the infecting strain could not be demonstrated in the mothers' milk. On the other hand the Swedish workers had previously demonstrated that mice could be protected from fatal *E. coli* infection introduced intraperitoneally, by anti-O antibody (Kaijser, Holmgren & Hanson, 1972). Protection against this kind of infection may however be quite different from the protection of the small intestine.

Our cross-absorption experiments indicate that the bacteriostatic antibody at least can be elicited by antigen present in a number of members of the Enterobacteriaceae group and therefore not O, H or K antigens. Our results confirm and extend the results obtained by Nagy *et al.* (1976) and also demonstrate the heat lability of the antigen. It is interesting to note the complete cross-absorption which we obtained between *S. newport* and *E. coli*. Milk-resistant *E. coli* can absorb out the activity for milk-sensitive strains and *vice versa*. The likelihood of all human milk containing the antibody is therefore high and is indeed what we have found.

The lack of cross reaction between *E. coli* and *Klebsiella* sp. suggests that our absorption experiments cannot be explained by postulating that enterochelin is washed off the absorbing suspension enabling the indicator strain to grow since Miles & Khimji (1975) demonstrated that many members of the Enterobacteriaceae,

https://doi.org/10.1017/S0022172400026048 Published online by Cambridge University Press

including *E. coli* and *Klebsiella* sp. had interchangeable enterochelin. The bacterial absorption experiments do therefore indicate that an antibody on which bacteriostasis depends is being removed.

We thank Dr M. G. M. Rowland of the Dunn Nutritional Laboratory, Cambridge for making available to us specimens of milk and strains of *E. coli* collected by the staff of the Medical Research Council Laboratory, Fajara, The Gambia during a field study in Keneba. The activity of Gambian milks and the sensitivity of strains of Gambian *E. coli* will be described more fully elsewhere. We thank the staff at Northwick Park Hospital for the collection of milk from local mothers and for strains of *E. coli*. (Approval for this study was given by the Northwick Park Hospital Ethical Committee.) We are grateful to Dr T. A. E. Platts-Mills of this Institute for precipitating IgA from milk and for the estimations of IgA.

REFERENCES

ALLARDYCE, R. A., SHEARMAN, D. J. C., MCCLELLAND, D. B. L., MARWICK, K., SIMPSON, A. J. & LAIDLAW, R. B. (1974). Appearance of specific colostral antibodies after clinical infection with *Salmonella typhimurium*. *British Medical Journal* **3**, 307.

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* **1**, 69.

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. & 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.

GOLDBLUM, R. M., AHLSTEDT, S., CARLSSON, B., HANSON, L. A., JODAL, U., LIDIN-HANSON, G. & SOHL-AKERLUND, A. (1975). Antibody-forming cells in human colostrum after oral immunisation. *Nature, London* **257**, 797.

GRIFFITHS, E. & HUMPHREYS, J. (1978). Alterations in tRNAs containing 2-Methylthio-N^6-(Δ^2 isopentenyl)-adenosine during growth of enteropathogenic *Escherichia coli* in the presence of iron-binding proteins. *European Journal of Biochemistry* **82**, 508.

HANSON, L. A. (1976). *Escherichia coli* infections in childhood. Significance of bacterial virulence and immune defense. *Archives of Disease in Childhood* **51**, 737.

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

KAIJSER, B., HOLMGREN, J. & HANSON, L. A. (1972). The protective effect against *E. coli* of O and K antibodies of different immunoglobulin classes. *Scandinavian Journal of Immunology* **1**, 27.

LODINOVA, R. & JOUJA, V. (1977). Antibody production by the mammary gland in mothers after artificial oral colonisation of their infants with a non-pathogenic strain *E. coli* O83. *Acta Paediatrica Scandinavica* **66**, 705.

MILES, A. A. & KHIMJI, P. L. (1975). Enterobacterial chelators of iron: their occurrence, detection, and relation to pathogenicity. *Journal of Medical Microbiology* **8**, 477.

NAGY, L. K., MACKENZIE, T. & BHARUCHA, Z. (1976). *In vitro* studies on the antimicrobial effects of colostrum and milk from vaccinated and unvaccinated pigs on *Escherichia coli*. *Research in Veterinary Science* **21**, 132.

ORAM, J. D. & REITER, B. (1968). Inhibition of bacteria by lactoferrin and other chelating agents. *Biochimica et Biophysica Acta* **170**, 351.

OSSERMAN, E. F. & LAWLER, D. P. (1966). Serum and urinary lysozyme in monocytic and monomyelocytic leukaemia. *Journal of Experimental Medicine* **124**, 921.

https://doi.org/10.1017/S0022172400026048 Published online by Cambridge University Press

Platts-Mills, T. A. E. & Ishizaka, K. (1975). IgG diphtheria antitoxin responses from human tonsil lymphocytes induced by anti-γ-chain antibodies. *Journal of Immunology* **114**, 1605.

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

Spik, G., Cheron, A., Montreuil, J. & Dolby, J. M. (1978). Bacteriostasis of a milk-sensitive strain of *Escherichia coli* by immunoglobulins and iron-binding proteins in association. *Immunology* **35**, 663.

https://doi.org/10.1017/S0022172400026048 Published online by Cambridge University Press