PAPERS AND ORIGINALS

Breast Milk Substitute: A Bacteriological Study

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Summary

The increased susceptibility of infants fed on cows' milk preparations has been attributed, at least in part, to differences in the nature of the large-bowel content—owing to the acidity of the faeces and their high content of *Lactobacillus bifidus*. In an attempt to mimic these features of the breast-fed infant in one who is fed artificially, a breast milk substitute was devised which resembles breast milk in several important ways. When this material was fed to newborn infants the faeces developed the characteristics of those of the breast-fed child.

Introduction

Earlier workers on the resistance of the breast-fed infant to gastroenteritis (Bullen and Willis, 1971; Bullen *et al.*, 1973) reported the results of in-vitro studies of breast milk and cows' milk preparations, and of the faeces from breast-fed and bottle-fed infants. Several factors seemed likely to influence the production and maintenance of a *Lactobacillus bifidus* flora and low pH in the faeces of newborn infants, and these were mainly attributable to the nature of the feed.

It was suggested that responsible factors in breast milk included its high lactose, low protein, low phosphate content together with its poor buffering capacity. Importance was also attached to the fact that breast milk seems to provide a fluid feed of small bulk and low residue. Cows' milk, on the other

hand, which has a low lactose, high protein, high phosphate content and a high buffering capacity is a relatively bulky, high-residue feed.

Since these observations the results of the important studies of iron-binding proteins in milk by Bullen *et al.* (1972) have been published. They showed that lactoferrin, which is present in substantial amounts in human milk but not in cow's milk, in combination with specific antibody to *Escherichia coli*, has a specific and powerful inhibitory effect on the growth of this organism. They suggested that the initial inhibition of *E. coli* in the gut of the breast-fed infant was likely to occur in the small intestine as a result of this mechanism. The importance of these observations and the cogency of the arguments put forward by Bullen *et al.* (1972) demand close attention. Nevertheless, we have not been able to look at the problem from this point of view. In the present study we describe the development of a breast milk substitute, whose composition is based on the results of our earlier findings, and the results obtained when newborn infants were fed with this material.

Methods

Modification of Infant Feed.—For our purpose breast-fed infants differ from bottle-fed infants in two outstanding ways: (1) the microbiological and physicochemical properties of the faeces, and (2) the composition of the feed. The results of our previous in-vitro studies suggested that the first is partly dependent on the second. So that the artificial feed should simulate breast milk when fed to newborn infants we concluded that the breast milk substitute (B.M.S.) should be not only broadly comparable nutritionally to breast milk but that it should also resemble human milk in its lactose, protein, and phosphate content, and in its buffering capacity. Table I compares some of the major constituents and properties of human and cows' milk, and shows how divergent they are from one another. Because it was virtually impossible for us to modify any available cows' milk preparation so that its properties matched those of breast milk, we devised an entirely artificial "milk" which, except for a lower pH (breast milk pH 6.8; B.M.S. pH 5.5), satisfied these requirements; we could fault it only on appearance and flavour. The general

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TABLE I—Some Properties and Major Constituents of Human and Cows' Milk (g/100 ml of Whole Milk)*

Constituent or Property	Breast Milk	Cows' Milk
Fat	4.6	3.5
Carbohydrate (lactose)	6.9	4.8
Total protein	1.3	3.2
Casein	0.4	2.5
Soluble	0.9	0.7
Total phosphorus	0.013	0.1
Casein	—	0.075
Soluble	—	0.025
pH (approximate)	6.8	6.9
Buffering capacity ratio	1	3

*Information compiled mainly from Oser (1965).

recipe of this feed is shown in the appendix. The composition of Aminosol (Paines and Byrne Ltd.), which is a tryptic digest of casein, is detailed in table II, and the average composition of the spray-dried whey powder (Unigate, Ltd.) is shown in table III. A dry mixture of the whey powder, Aminosol, and lactose was kindly prepared by Paines and Byrne, Ltd. Before use the dry powder mixture was examined bacteriologically to ensure that it was free of undesirable organisms. In addition, its buffering capacity and its performance in "continuous culture" with a mixture of *E. coli* and *L. bifidus* (Bullen and Willis, 1971) were checked to ensure that the mixture resembled breast milk in these respects.

Mixing the Feed.—The feed was prepared as required by the nursing staff in the milk kitchen. The material was reconstituted by mixing 8.5 g of powder in 100 ml hot boiled water; sterile cows' cream 4.5 g was added to each 100 ml of feed. The strength (up to a maximum of double-strength) and volume of the feed were increased as required.

TABLE II—Amino-acid Composition of Aminosol

Substance	Aminosol Powder :
Essential Amino-acids:	(%)
Isoleucine	5.6
Leucine	7.8
Lysine	6.1
Aromatic:	
Phenylalanine	4.7
Tyrosine	5.1
Sulphur-containing:	
Methionine	2.2
Cystine-cystin	0.2
Threonine	4.5
Tryptophan	0.84
Valine	5.1
Non-essential amino-acids:	
Arginine	2.7
Alanine	2.4
Aspartic Acid	5.1
Glutamic Acid	15.0
Glycine	1.7
Histidine	2.6
Proline	8.8
Serine	7.0
Hydroxyproline	0.0
Dialyzable Peptides	24.0
Electrolytes:	(mg/100 g)
Na	90.0
K	62.5
Ca	20.0

TABLE III—Approximate Composition for Cows' Milk Whey Powder (Unigate)

Substance	Powder (%)
Lactose (as hydrate)	66.5
Total protein	16.6
Moisture	3.2
Fat	1.65
Ash	9.5

Selection of Infants.—In order that the efficacy of B.M.S. could be properly assessed, it was necessary that infants receiving the feed and control infants receiving standard cows' milk preparations or breast milk should remain in hospital for at least eight days. In all cases the nature of the feed and the object of the test feeding was explained to the parents, and per-

mission was obtained before feeding began. Before discharge from hospital all infants who had received B.M.S. were established on a standard cows' milk preparation. A total of 83 babies were studied of whom 38 received B.M.S., 23 received a standard cows' milk preparation, and 22 received breast milk.

Clinical Control.—All infants were nursed in hospital and received the customary paediatric surveillance and nursing care. Daily weighings were made, and note was taken of feeding habits and general progress. Test infants received B.M.S. only; the two control groups of infants received either a standard cows' milk preparation or breast milk only. After 6-14 days infants receiving B.M.S. were placed on a standard cows' milk preparation before discharge.

Bacteriological Control.—Bacteriological studies, which were performed daily on "24-hour" specimens of faeces, included a record of their macroscopic appearance, pH determinations on faecal emulsions in saline, assessment of the bacterial flora by direct microscopical examination of Gram-stained films, and viable counts of the aerobic and anaerobic bacterial flora with special reference to enterobacteriaceae, streptococci, staphylococci, yeasts, clostridia, bacteroides, and *L. bifidus*. Total viable counts were made by the method of Miles *et al.* (1938). Counts of *E. coli* were made on MacConkey agar, and those for other aerobic organisms were performed on horse blood agar or MacConkey agar or both. Counts for *L. bifidus* were performed on reinforced clostridium medium (Oxoid), solidified with 0.75% New Zealand agar, pH 5.0, and containing 0.1% cotton blue. This medium is inhibitory to the growth of *E. coli* owing to its low pH value, and its low agar concentration permits the growth of large colonies of *L. bifidus*. Counts for the clostridia and bacteroides were performed on Columbia blood agar containing neomycin sulphate as a selective agent.

Results

Various factors prevented us from following through all the babies in any one group for the full eight-day period. Thus, some infants were discharged home earlier than expected, others had their feed changed unexpectedly. In addition, many infants, and especially those receiving breast milk or B.M.S., did not pass faeces each day. Consequently, the recorded results showed no continuity from day to day as to the number of infants studied in a single group. For example, among the group of infants yielding *L. bifidus* who were fed on B.M.S. (fig. 1) specimens from only five babies were examined on day 1, specimens from 16 were examined on day 6, and only one specimen was available on day 14.

FIG. 1—pH of samples of faeces from "bifid" subgroup of 20 infants fed on breast milk substitute during first 14 days of life, showing highest and lowest values for each day (●) and a plot of mean values.

FAECAL pH, COLI AND *L. BIFIDUS*

Several infants in each feeding group failed to yield *L. bifidus* at any time; seven of 22 breast-fed infants (32%), 18 of 38 B.M.S.-fed infants (47%), and 14 of 23 cows' milk-fed infants (61%) fell into this category. The results for the three groups of babies have, therefore, each been divided into two subgroups—those with, and those without *L. bifidus*.

Infants Receiving B.M.S.—The mean faecal pH values and the mean viable counts of *E. coli* and *L. bifidus* present in faecal samples for this group of infants, recorded daily from birth to the 14th day, are shown in figs. 1, 2, and 3. In the first subgroup of infants (figs. 1 and 2), all of whose faeces gave "bifid" counts at some stage during the first two weeks of life, there was no clear overall difference between the counts of *L. bifidus* and those of *E. coli*; in most of the faecal specimens both organisms commonly gave counts in excess of 10^5 organisms/g of faeces, and counts of 10^9 and 10^{10} were not uncommon. The corresponding mean faecal pH values showed a general decline from 6.1 to 5.6. In the second subgroup of infants fed on B.M.S., all of whom gave zero "bifid" counts, the *E. coli* counts and mean pH values were generally similar to those of the first subgroup. It is notable that despite the absence of *L. bifidus* there was a steady fall in the mean faecal pH from 6.2 to 5.4 (fig. 3).

FIG. 2—Counts of *E. coli* (●—●) and *L. bifidus* (○—○) per gramme of faeces in samples considered in fig. 1. Each plot represents mean of logarithms of daily counts.

FIG. 3—pH of samples of faeces from "non-bifid" subgroup of 18 infants fed on breast milk substitute during first 14 days of life, showing highest and lowest values obtained each day (●), and a plot of mean values.

Infants Receiving Breast Milk.—The mean faecal pH values and the mean viable counts of *E. coli* and *L. bifidus* present in faecal samples for this group of infants, recorded daily from birth to the 14th day, are shown in figs. 4, 5, and 6. In the first subgroup of infants (figs. 4 and 5), all of whose faeces gave "bifid" counts at some stage during the first 14 days of life, there was no clear overall difference between the counts of *L. bifidus* and those of *E. coli*; in most of the faecal specimens both organisms commonly gave counts in excess of 10^5 organisms/g of faeces, and counts of 10^9 and 10^{10} were not uncommon. The corresponding faecal pH values showed a general decline from 6.1 to 5.0. In the second subgroup of infants fed on breast milk, all of whom gave zero "bifid" counts, the *E. coli* counts and mean pH values were generally similar to those of the first subgroup. As in the corresponding group of infants fed on B.M.S., there was a steady fall in the faecal pH from 6.5 to 5.2, despite the absence of *L. bifidus* (fig. 6).

FIG. 4—pH of samples of faeces from the "bifid" subgroup of 15 infants fed on breast milk during first 14 days of life, showing highest and lowest values obtained each day (●) and a plot of the mean values.

FIG. 5—Counts of *E. coli* (●—●) and *L. bifidus* (○—○) per gramme of faeces in samples considered in fig. 4. Each plot represents mean of logarithms of daily counts.

FIG. 6—pH of samples of faeces from "non-bifid" subgroup of seven infants fed on breast milk during first 14 days of life, showing highest and lowest values obtained each day (●) and a plot of mean values.

FIG. 7—pH of samples of faeces from "bifid" subgroup of nine infants fed on cows' milk preparations during first 14 days of life, showing highest and lowest values obtained each day (●), and a plot of mean values.

Infants Receiving a Standard Cows' Milk Feed.—The mean faecal pH values and the mean viable counts of *E. coli* and *L. bifidus* present in faecal samples for this group of infants, recorded daily from birth to the 14th day, are shown in figs. 7, 8, and 9. In the first subgroup of infants (figs. 7 and 8), all of whose faeces gave "bifid" counts at some stage during the first 14 days of life, the counts for *E. coli* were, in general, of a higher order than those for *L. bifidus*. Moreover, the cor-

responding mean faecal pH values showed a general rise from 6.0 to 6.8, despite the presence of lactobacilli. In the second subgroup of infants fed on cows' milk, all of whose faeces gave zero 'bifid' counts, the *E. coli* counts and mean pH values were generally similar to those of the first subgroup. The mean pH values rose from 6.4 to 7.0 (fig. 9).

FIG. 8—Counts of *E. coli* (●—●) and *L. bifidus* (○—○) per gramme of faeces in samples considered in fig. 7. Each plot represents mean of logarithms of daily counts.

FIG. 9—pH of samples of faeces from "non-bifid" subgroup of 14 infants fed on cows' milk preparations during first 14 days of life, showing highest and lowest values obtained each day (●) and a plot of mean values.

OTHER ORGANISMS

Staphylococci were rarely isolated. *Staphylococcus aureus* was never encountered, but *Staph. albus* was identified in small numbers in a few faecal samples during the first two days of life. Streptococci were commonly present in the faeces from all groups of infants. *Streptococcus faecium* was the usual species isolated, and was present in numbers similar to those of *E. coli*. *Pseudomonas aeruginosa* or *Proteus* species were isolated from the faeces of 11 infants. On no occasion were both organisms present. These non-lactose-fermenting bacilli were uncommon in infants fed on breast milk and on B.M.S. (one out of 22, and two out of 38 babies respectively), but were present in five out of 23 infants fed on cows' milk preparations. Three of 28 infants fed on B.M.S. yielded the organisms after their feed had been changed to a cows' milk preparation.

Yeasts were present in small numbers in the faeces of about 10% of babies in each feeding group. These organisms disappeared between the fifth and eighth days.

Bacteroides species were present in faecal specimens from all groups of babies in substantially similar numbers. Though breast-fed infants appeared to carry these organisms more commonly than others this was not sustained, and all feeding groups gave zero counts after the 10th day. Clostridia, usually *Clostridium welchii* but occasionally *Cl. parapatrificum*, were most commonly encountered in the faeces of infants receiving cows' milk preparations. The three feeding groups of babies were colonized by clostridia in the first days (fig. 10). There-

FIG. 10—Percentage of samples of faeces containing clostridia during the first 14 days of life, from infants receiving cows' milk preparations (○—○), breast milk (●—●), and breast milk substitute (X—X).

after, the organisms persisted in a high proportion of faecal specimens from cows' milk-fed infants. In infants fed on breast milk and on B.M.S., however, there was a sudden decline in the number of positive samples. A toxigenic strain of *Cl. tetani* was isolated on one occasion from the faeces of an infant who had been fed on B.M.S.; the organism appeared after the child's feed had been changed to a cows' milk preparation.

EFFECT ON FAECAL FLORA AND pH OF CHANGE IN FEED

Of the 38 infants fed on B.M.S., all of whom were established on a cows' milk feed before discharge from hospital, 28 were followed bacteriologically for three days after the change-over; 16 of these babies fell into a "bifid" subgroup and 12 into a "non-bifid" subgroup. Figs. 11 and 12 show the mean faecal pH values and the mean *E. coli* and *L. bifidus* counts over a period of six days (three before and three after the change-over) in the "bifid" subgroup. Clearly (see graphs) there was no significant change in the bacterial counts during this short period of observation. However, the mean faecal pH changed dramatically. During the three days before the changeover the mean pH remained stable at around pH 5.0; within 24 hours of the changeover the pH had risen to 6.0, and by the third day of cows' milk feeding it had risen above pH 7.0. This result is entirely in keeping with the behaviour of all control infants fed only on cows' milk preparations (figs. 7 and 9).

FIG. 11—pH of samples of faeces from a "bifid" subgroup of 16 infants over a period of six days. Infants received breast milk substitute on days 1-3, and a cows' milk preparation on days 4-6. Highest and lowest values obtained each day are shown (●) together with a plot of mean values.

FIG. 12—Counts of *E. coli* (●—●) and *L. bifidus* (○—○) per gramme of faeces in samples considered in fig. 11. Each plot represents mean of logarithms of daily counts.

The bacterial counts in the small “non-bifid” subgroup of infants, like those in the “bifid” subgroup, showed no significant change during the three days after the change of feed. The mean faecal pH values, however, showed the same rapid rise after the changeover (fig. 13), rising from 5.4 to 7.6.

FIG. 13—pH of samples of faeces from a “non-bifid” sub-group of 12 infants over a period of six days. Infants received breast milk substitute on days 1-3, and a cows’ milk preparation on days 4-6. Highest and lowest values obtained each day are shown (●) together with a plot of mean values.

CLINICAL OBSERVATIONS

Faeces.—Though there was some variation from child to child the following generalizations were true for the groups of infants studied. Infants receiving cows’ milk preparations produced relatively frequent motions that were bulky, firm, and of a pale chalky colour, and had an offensive odour. Infants fed on breast milk and on B.M.S., however, produced motions much less often. They were of small bulk, of a watery “curdled” loose consistency, yellow to greenish in colour, and commonly had a “cheesy” but never an offensive odour.

Tolerance to B.M.S.—In general, B.M.S. was accepted no less readily than other types of milk. During the initial stages of feeding some regurgitation was to be expected, but this occurred no more often than with other types of milk. Only one infant refused B.M.S., but this child was also reluctant to accept any other type of feed.

Progress.—In general, infants’ appetites were satisfied by B.M.S. When this was not the case the problem was resolved by increasing the volume or the concentration of the feed or both factors. The mean birth weights and mean weights on the eighth day of infants in the three feeding groups are shown in table IV. In all cases the pattern of an initial weight loss followed by a steady weight increase occurred. Infants receiving B.M.S. were marginally slower to gain weight than were those in the other two groups.

Conversion of Feed from B.M.S. to a Cows’ Milk Preparation.—In all cases this was accomplished without difficulty at a single feed. Within 24 hours of conversion the characteristics of the faeces changed radically from those of the breast-fed infant to those of the infant fed on cows’ milk.

TABLE IV—Average Weights of Infants in Three Feeding Groups

No. of Infants in Feeding Group	Mean Weight (g) at	
	Birth	Eighth Day
Breast milk substitute:		
Full term (33)	3,320	3,129
Premature (5)	2,213	2,150
Cows’ milk preparations:		
Full term (19)	3,304	3,270
Premature (4)	2,083	2,125
Breast milk:		
Full term (22)	3,373	3,325

Discussion

The most striking result that emerged from this investigation was the influence which the feed had on the pH of the faeces. Thus, the faecal pH of those infants fed on breast milk and on B.M.S. showed a steady decline during the first 14 days of life from pH 6.1 to pH 5.0-5.5. In contrast, the faecal pH of infants fed on cows’ milk preparations rose steadily over the same period to 6.8-7.0. Moreover, in those infants whose feed was changed from B.M.S. to a standard cows’ milk preparation the mean faecal pH rose abruptly from 5.0 to above 7.0 in three days.

Though it has been customary and reasonable to attribute the acidic faeces of breast-fed infants to the characteristic preponderance of *L. bifidus*, the results presented here suggest that the converse is more likely. Undoubtedly breast milk and B.M.S. initiate a low faecal pH in the absence of significant colonization of the gut by *L. bifidus*. Thus among infants fed on breast milk and on B.M.S. two distinct subgroups of children emerged—those with substantial numbers of lactobacilli in their faeces, and those without these organisms. This overt bacteriological difference had no effect on the downward trend of the faecal pH. Conversely, in the group of children fed on cows’ milk preparations this same bacteriological difference did not influence the upward trend of the faecal pH. We thus favour the view that the low buffering capacity of the feed is the primary factor which enables a low pH to develop in the infant’s large intestine; and that it is this falling pH which provides the favourable environment for subsequent colonization of the large gut by *L. bifidus*. Thus, in infants fed on cows’ milk preparations, which have a high buffering capacity and a low lactose content, free acid production would be negligible. In infants fed on breast milk and on B.M.S., however, substantial amounts of free acid would accumulate in the poorly buffered environment, and the pH would tend to fall. Such a falling pH would directly favour the growth of any lactobacilli present, which in turn would lead to a further fall in pH (Bullen and Willis, 1971). Though *L. bifidus* is not absent from the faeces of infants fed on cows’ milk preparations it is present in lower numbers than in those of breast-fed and B.M.S.-fed infants. It is noteworthy that among the three feeding groups of infants—breast-fed, B.M.S.-fed, and cows’ milk-fed—the number of babies without *L. bifidus* at any time were in the ratio of about 1:1.5:2.

While we are thus convinced that the primary factor required to ensure an acidic faeces is a feed of low buffering capacity, production of acid is clearly dependent on the presence of lactose-fermenting organisms. The three bacterial species most commonly encountered in this investigation, *E. coli*, *Str. faecium*, and *L. bifidus*, are all lactose-fermenting organisms, and are normally present and widely distributed in the vagina and on the maternal skin (Haelen *et al.*, 1958; Bullen *et al.*, 1973). Though it seems reasonable to assume that both breast-fed and bottle-fed infants are exposed to the same bacterial contaminants of the intestinal tract undoubtedly the exposure of the breast-fed infant is more consistent and continuous. This constant exposure of the breast-fed baby during suckling to relatively many *L. bifidus* on the maternal skin would ensure continuous reinoculation of the organism into the infant’s large bowel. This would partly

explain why, in the present study, colonization of the large gut by *L. bifidus* was more common in breast-fed infants than in those fed on B.M.S., since the latter were bottle-fed.

Probably preliminary colonization of the gut by lactose-fermenting organisms such as *E. coli* and *Str. faecium* is an essential precursor for the subsequent rapid outgrowth of *L. bifidus*. In breast-fed and B.M.S.-fed infants these facultative organisms would produce the conditions of a falling pH and Eh which are so favourable to the growth of *L. bifidus*. In the bottle-fed infant receiving cows' milk, on the other hand, this "starter effect" would be nullified by the high buffering capacity of the feed. From in-vitro experiments we have some evidence to suggest that as the pH of the infant's large gut contents falls significant amounts of acetic acid/acetate buffer accumulate. This not only favours the continued growth of *L. bifidus* but produces conditions that are unfavourable to *E. coli*.

The presence of organisms other than *L. bifidus*, *E. coli*, and *Str. faecium* in the faeces of infants is characteristic of babies fed on cows' milk preparations, and their presence has been regarded as the cause of the putrefactive faeces of cows' milk-fed, as opposed to the fermentative faeces of breast-fed children (Haelen, 1961). Our findings not only accord with these observations but also indicate that infants fed in B.M.S. behave like breast-fed babies in this respect. Not only were organisms such as *Proteus* species, *Ps. aeruginosa*, and clostridia rarely present in the faeces of B.M.S.-fed babies but their faeces were also of the fermentative type. Moreover, in infants who were followed bacteriologically after conversion from B.M.S. to a cows' milk preparation these organisms started to appear in the faeces as they changed from the fermentative to the putrefactive type.

In a feeding trial designed to create a preponderance of *L. bifidus* in the intestine of bottle-fed infants, MacGillivray *et al.* (1959) fed babies on a modified milk mixture containing 1% lactulose, which is regarded as a "bifidogenic" factor. Though they succeeded in obtaining a response to *L. bifidus* in 66% of their test infants the pH of the faeces was only slightly and inconsistently lowered. We fully agree with their conclusion that by itself *L. bifidus* does not make a major contribution towards producing the acidity of the stool of the breast-fed infant. On the other hand, probably *L. bifidus* plays an important part in maintaining the low pH once it has been established.

Since we first began studying this problem (Bullen and Willis, 1971) Harrison and Peate (1972) reported their observations on the significance of milk pH in newborn infants. They claimed that the addition of small amounts of alkali in the form of sodium bicarbonate or trometamol to cows' milk preparations produced a bacteriostatic effect on the growth of specific *E. coli* in vitro, and in infants produced a stool with a preponderance of lactobacilli over *E. coli* organisms. Though we have not fed infants on alkalized cows' milk we are in complete disagreement with their in-vitro findings. We conducted experiments similar to those described by Cox *et al.*, (1973) in which viable counts of *E. coli* growing in cows' milk preparations, with and without bicarbonate buffer, were compared. Like Cox *et al.*, we found that counts in the buffered and unbuffered milks were virtually identical; there was no evidence that alkalization exerted any suppressive effect on the growth of *E. coli*.

The overall results of our investigations with, admittedly, few babies indicate that B.M.S. produces essentially similar reactions in the infant's gut to those produced by breast milk during the first 14 days of life. The notable differences between the two groups of infants fed on the two types of milk were a slightly slower weight gain and somewhat lower *L. bifidus* colonization rate in the B.M.S. group. Extension of the feeding trial for a longer period might well have reduced these differences.

Whether or not the resistance to gastroenteritis of infants fed on B.M.S. would be comparable to that of breast-fed

babies is a matter for speculation. It would be unrealistic to suggest that the mechanisms we have studied are the only ones that determine the physicochemical and microbiological nature of the large bowel content of the infant. The studies of Bullen *et al.* (1972) lay emphasis on suppression of *E. coli* rather than on enhancement of growth of *L. bifidus*, due to specific *E. coli* antibody acting in the presence of iron-binding proteins. This, and other possible mechanisms in the defence against infection in the newborn, has been briefly reviewed by Hanson and Winberg (1972).

There is no easy way of determining whether the feeding of B.M.S. to infants provides any protection against gastrointestinal infections. This would probably require the setting up of large prospective feeding trial studies. Alternatively, since breast milk is known to speed the recovery of infants with gastroenteritis, some evidence about the protective effect of B.M.S. might be obtained by substituting it for breast milk in the treatment of the infected child.

We recognize that the breast milk substitute feed we have described is likely to be deficient in minerals and vitamins, and that it is therefore unsuitable for long-term use unless appropriately supplemented. The nine premature infants included in the present study received daily supplements of Abidec (Parke, Davis), Ferromyn (Calmic), and folic acid. There would seem to be no contraindication, however, to the addition of some supplements to the feed at the time of its manufacture.

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APPENDIX—Formulation and Preparation of Breast Milk Substitute (B.M.S.)

- (1) Dry powder mixture—contents/kg (prepared by Paines and Byrne, Ltd)

Aminosol (Paines and Byrne)	129g
Spray-dried whey powder (Unigate, Ltd.)	153g
Lactose	718g
  - (2) Sterile cows' cream . . . . . distributed in aliquots of 4.5g
- Immediately before required the feed was prepared as follows:
 8.5g dry powder mixture was made up to 100 ml with hot boiled water; sterile cows' cream 4.5g was added, and after thorough mixing the breast milk substitute was cooled to feeding temperature.
 Buffering capacity ratio . . . . . Breast milk : B.M.S. : : 1:1
 pH (approximate) 5.5
 As required, the concentration of the B.M.S. feed was varied up to a maximum of double strength.