

# The Journal of Medical Microbiology

Vol. 10 No. 4

## THE EFFECT OF "HUMANISED" MILKS AND SUPPLEMENTED BREAST FEEDING ON THE FAECAL FLORA OF INFANTS

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THE type of milk fed to newborn infants greatly influences the physicochemical and microbiological properties of their faeces. Breast-fed babies are relatively resistant to gastroenteritis (Alexander, 1948; Ross and Dawes, 1954; Hinton and MacGregor, 1958); various explanations suggested for this include passive transfer of antibodies to *Escherichia coli* in colostrum (Sussman, 1961; Michael, Ringenback and Hottenstein, 1971), contamination of artificial feeds during preparation (Neter, 1959) and the nature of the intestinal environment (Ross and Dawes, 1954). The continuing occurrence of enteropathogenic *E. coli* infections in infants, together with the introduction of "humanised" milks and the popularity of supplementing breast feeding with cows' milk preparations during the 1st week of life, has led us to re-examine this problem.

From in-vitro studies of breast-fed infants, a number of factors seem likely to influence the establishment and maintenance of the bifidobacterial flora and low pH characteristic of the faeces of young infants. There may be specific factors in human milk that either encourage the growth of bifidobacteria or suppress that of *E. coli*. Gyorgy (1953) showed that breast milk contained a factor that was essential for the growth of one strain of lactobacillus. Petuely and Kristen (1949), and MacGillivray, Finlay and Binns (1959) showed that addition of lactulose to modified cows' milk preparations induced a predominance of lactobacilli in the faeces of infants, but this was not accompanied by a consistently low pH. J. J. Bullen and his colleagues (Bullen, Rogers and Leigh, 1972; Bullen, Rogers and Griffiths, 1974) showed that the high content of iron-binding proteins, predominantly lactoferrin, in human milk can inhibit *E. coli*. They found that lactoferrin in combination with specific antibody to *E. coli* had a powerful bacteriostatic effect that was abolished when the lactoferrin was saturated with iron.

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Received 18 Jan. 1977; accepted 24 Feb. 1977.

The results of earlier studies (Bullen and Willis, 1971) are in general agreement with the observations and conclusions of Ross and Dawes (1954). Breast-fed infants produce an acidic environment in the lumen of the large intestine, and acetic acid is frequently present as an acetate buffer (Bullen, Tearle and Willis, 1976). In-vitro experiments (Bullen and Tearle, 1976) suggest that such a buffer exerts a bacteriostatic effect upon Gram-negative and putrefactive organisms. Although we do not exclude the possible role of other specific factors, our findings point to the importance of the ingredients and properties of breast milk, which seems to provide an intestinal content that is favourable both for the growth of bifidobacteria and for the production of an acid environment.

We decided therefore to investigate the microbiological and physicochemical properties of the faeces from infants fed (1) breast milk only, (2) "humanised" milks *i.e.*, cows' milk preparations with the protein and carbohydrate content adjusted to concentrations similar to those of breast milk and with buffering capacities only slightly greater than that of breast milk, and (3) breast milk supplemented with cows' milk preparations during the 1st week of life and thereafter breast milk only.

#### MATERIALS AND METHODS

*Test and control feeding groups.* The study was carried out with 47 infants; 13 were breast-fed and received no supplement, nine were fed Cow and Gate Premium milk, 10 were fed Scientific Milk Adaptation (S.M.A.) Gold Cap milk and 15 were breast-fed but received cows'-milk supplementary feeding once every 24 h during the 1st week of life. Weekly faecal samples were examined from birth to the end of the 6th week for their viable bacterial counts, pH, and the presence and identity of volatile products of bacterial metabolism; the samples were stored at 4°C before delivery to the laboratory. Various factors prevented us from collecting all the specimens from every infant. Moreover, some infants changed to a different milk preparation before the 6th week. Consequently the number of infants in the four groups changed from week to week.

*Buffering capacity.* The buffering capacities of breast milk, Premium milk and Gold Cap milk, were measured over the pH range 6.8-4 with N/10 lactic acid and a Pye Unicam pH meter. The powdered milks were reconstituted according to the manufacturers' instructions.

*Chromatographic analysis.* Gas chromatographic analysis was performed as described by Bullen *et al.* (1976).

*Microbiological studies.* The pH of 10% faecal suspensions in 0.15 M NaCl was measured. Viable counts of the aerobic and anaerobic faecal flora were made, with special reference to the enterobacteria, streptococci, staphylococci, yeasts, clostridia, bacteroides and bifidobacteria. Total viable counts were made by the method of Miles, Misra and Irwin (1938). The media and methods were those of Willis *et al.* (1973).

#### RESULTS

##### *Studies on milk preparations*

*Composition of Premium, Gold Cap and breast milks.* Table I shows the concentrations of fat, protein, carbohydrate and phosphorus in breast milk, and in Premium and Gold Cap milk reconstituted as recommended by the manufacturer.

*Buffering capacity.* The ratios of the buffering capacities of breast, Premium and Gold Cap milks were 1.0 : 1.6 : 1.9.

TABLE I

*The composition of breast milk and reconstituted "humanised" milks*

Ingredients	Concentration (g per 100 ml) of the stated ingredient in		
	Cow and Gate Premium milk*	S.M.A. Gold Cap milk*	breast milk†
Fat	3.3	3.6	4.6
Total protein	1.8	1.5	1.3
Casein	0.57	0.6	0.4
Soluble protein	1.23	0.9	0.9
Carbohydrate	6.9	7.2	6.9
Phosphorus	0.04	0.044	0.013

\* Figures compiled mainly from manufacturers' current products information.

† Figures compiled from Oser (1965).

*Studies on faeces*

*Macroscopic appearance of faeces.* Stools from the breast-fed babies were of small bulk, with a watery curdled consistency, yellow-green colour, and "cheesy" odour. In the 1st week, the stools of the bottle-fed infants bore a resemblance to those of the breast-fed infants; they had a slightly "cheesy" odour and contained curds, but the number of motions passed was less and the stools were larger and firmer. After the 1st week, the stools of bottle-fed babies became putrid and were of a firm putty-like consistency. The stools from the infants fed supplemented breast milk appeared identical to those of the breast-fed infants but they did not develop a "cheesy" odour for several weeks.

*Acetate buffer in the faeces.* The faecal material from breast-fed infants produced a chromatographic pattern that showed a striking increase in the acetic acid content after acidification with sulphuric acid. This acetate buffer was present in the faeces of more than 60% of the breast-fed infants during the first 5 weeks of life, and in 50% in the 6th week. At no time was an acetate buffer demonstrated in the faeces of the Premium-fed infants. The infants fed Gold Cap milk produced no acetate buffer in their faeces until the 5th and 6th weeks, when a buffer was detected in 16% and 32% respectively. Of those infants fed breast milk plus supplement, 20% produced a buffer in the 1st week, 11.5% in the 2nd, and 60% in the 4th (fig. 1).

*Volatile fatty acids in the faeces.* All acidified faecal suspensions from breast-fed infants contained acetic acid; propionic acid appeared occasionally after the 1st week of life. The acidified faecal suspensions from the Premium-fed infants always contained acetic acid. Propionic acid appeared in >83% of the specimens, and isobutyric, butyric, isovaleric, valeric and isocaproic acids were also commonly present. The suspensions from infants fed Gold Cap milk contained acetic acid at all times except during the first 2 weeks of life when

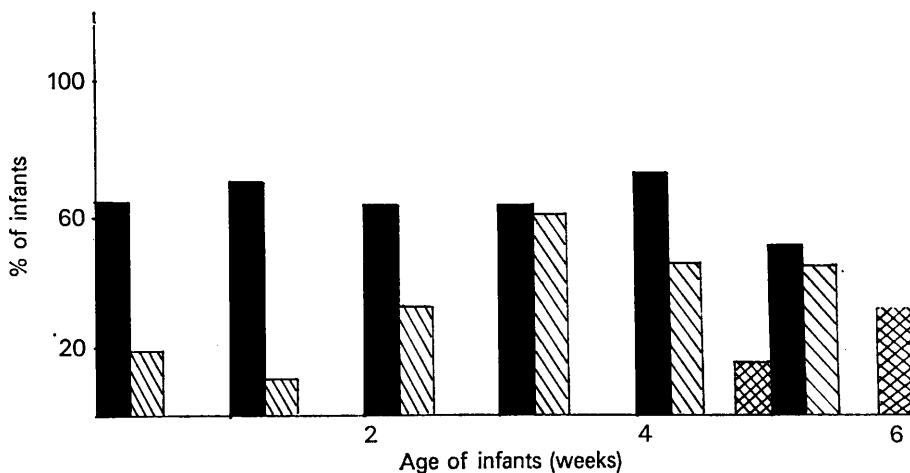


FIG. 1.—The presence of an acetate buffer in the faeces of infants in the first 6 weeks of life. ■ = Breast-fed infants; ▨ = infants fed breast milk plus supplement; ▩ = infants fed Gold Cap milk.

it appeared in >85% of the suspensions. Propionic acid was present in >62% of the suspensions, and the other volatile fatty acids were also frequently present. In the 1st week, 71.4% of the infants fed breast milk plus supplements had propionic and butyric acids in addition to acetic acid in their faeces. In the 3rd week 7.6% of the infants had *isobutyric* and *isovaleric* acids also, but by the 6th week all acids had disappeared except for acetic and propionic acids. In all infants the occurrence of volatile fatty acids in the faeces became more frequent during the first 3 weeks of life (table II).

*The pH of faeces.* Faecal specimens from breast-fed infants were of pH 5.1–5.4 throughout the 6 weeks. In the two bottle-fed groups of infants, the values were in the range pH 5.9–7.3 during the first 2 weeks of life; from the 2nd to the 5th week the pH was in the range 7–8.2, but after the 5th week the pH of faecal suspensions from infants fed Gold Cap milk fell to 6.4. In infants fed breast milk plus supplement the mean pH was 5.7–6.0 during the first 4 weeks, falling to 5.45 by the 6th week (fig. 2).

#### *Mean viable bacterial counts of faeces*

*Breast-fed infants.* From the 1st week of life there was a marked predominance of bifidobacteria over coliform bacilli and streptococci. Counts of bacteroides were <10<sup>8</sup> per g. Counts of clostridia (*Clostridium perfringens* and *Clostridium paraputrificum*) rarely exceeded 10<sup>3</sup> per g of faeces, and at times these organisms were not found (fig. 3a).

*Infants fed Premium milk.* In the 1st week of life, bifidobacteria gave a count of >10<sup>9</sup> per g of faeces and were predominant over all other bacteria. By the 2nd week, however, the counts of coliform bacilli, streptococci and bacteroides had risen to values of >10<sup>9</sup> per g, which were sustained during

TABLE II  
*Volatile fatty acids present in the faeces of infants fed "humanised" cows' milk preparations, breast-milk,  
 or breast-milk plus supplement*

Type of food	Age of infants (weeks)	Number of infants that provided the faecal sample	Percentage of faecal samples in which the stated volatile fatty acids were found							
			Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	Isocaproic	
Cow and Gate Premium milk	1	8	100	100	25	25	25	25	0	12.5
	2	9	100	100	44.4	77.7	66.6	22.2	22.2	22.2
	3	9	100	100	66.6	88.8	77.7	44.4	16.6	0
	4	6	100	83.3	66.6	100	100	77.7	11.1	0
	5	9	100	100	55.5	88.8	77.7	11.1	28.5	42.8
	6	7	100	85.7	71.4	100	100	100	100	100
S.M.A. Gold Cap milk	1	8	87.5	62.5	50	62.5	37.5	12.5	12.5	12.5
	2	7	85.7	71.4	71.4	71.4	57.1	28.6	14.3	14.3
	3	5	100	80	80	100	100	20	20	20
	4	5	100	80	100	100	80	20	20	20
	5	6	100	100	83.3	100	83.3	16.6	16.6	16.6
	6	6	100	83.3	50	53.3	33.3	16.6	16.6	16.6
Breast milk	1	10	100	0	0	0	0	0	0	0
	2	10	100	20	0	0	0	0	0	0
	3	8	100	62.5	0	12.5	0	0	0	0
	4	9	100	22.2	0	0	0	0	12.5	0
	5	8	100	37.5	0	0	0	0	0	0
	6	5	100	40	0	0	0	0	0	0
Breast milk plus supplement	1	7	100	71.4	0	71.4	14.2	0	0	0
	2	11	100	54.5	0	45.4	11	0	0	0
	3	13	100	53.8	7.6	30.7	7.6	0	0	0
	4	10	100	40	10	40	0	0	0	0
	5	9	100	55	11.1	11.1	0	0	0	0
	6	7	100	57.1	0	0	0	0	0	0

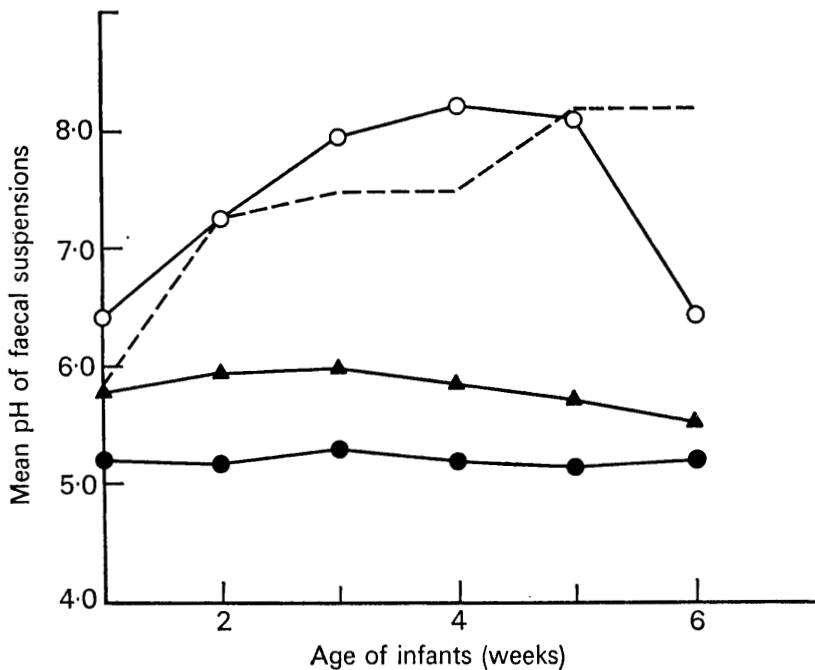


FIG. 2.—Mean pH of faecal suspensions from breast-fed infants (●—●), infants fed breast milk plus supplement (▲—▲), infants fed Cow and Gate Premium milk (---), and infants fed S.M.A. Gold Cap milk (○—○).

the 6-week period; no organism predominated. Viable counts of *C. perfringens* and *C. paraputrificum* rose sharply from  $<10^5$  per g in the 1st week of life to  $>10^7$  per g in the 2nd and they remained at this level (fig. 3b).

*Infants fed Gold Cap milk.* For the first 3 weeks of life the counts of bifidobacteria, coliform bacilli and streptococci were  $10^8$ – $10^9$  per g of faeces. In the 4th week the coliform bacilli predominated reaching counts slightly above  $10^9$  per g, whereas the counts of bifidobacteria dropped to  $10^7$ – $10^8$  per g. From the 4th week to the 6th week the coliform counts remained above those of all other bacteria. At no time did the counts of bacteroides fall below  $10^6$  per g. The counts of *C. perfringens* and *C. paraputrificum* rose steadily from  $10^5$ – $10^6$  per g in the 1st week of life to  $>10^7$  per g in the 5th week (fig. 3c).

*Infants fed supplemented breast milk.* Although the bifidobacterial counts remained at  $>10^9$  per g, the difference between their values and those of the coliform bacilli was small. In the 4th week the counts were identical. Thereafter the counts diverged so that by the 6th week the bifidobacteria predominated and the coliform population continued to decrease. The streptococcal counts remained at  $10^8$ – $10^9$  per g whereas the counts of bacteroides ( $>10^9$  per g in the 1st week) fell sharply to  $10^5$ – $10^6$  per g by the 3rd week; thereafter the counts remained at  $10^6$ – $10^7$  per g. The counts of *C. perfringens* and *C. paraputrificum* rose from  $<10^5$  per g to  $>10^6$  per g by the 2nd week and remained at  $10^5$ – $10^7$  per g during the following 4 weeks (fig. 3d).

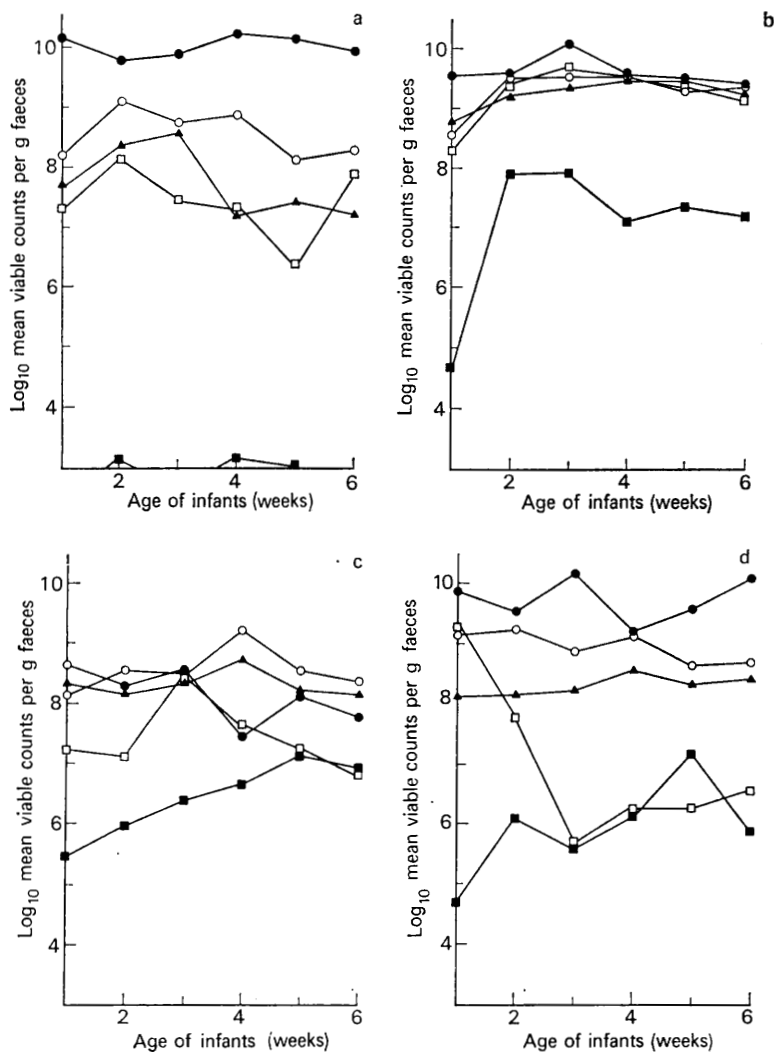


FIG. 3a.

FIG. 3b.

FIG. 3.—Mean viable counts of faecal organisms isolated from (a) 13 breast-fed infants, (b) nine infants fed Cow and Gate Premium milk, (c) 10 infants fed S.M.A. Gold Cap milk, and (d) 15 infants fed breast milk plus supplement. ●—●=Bifidobacteria; ○—○=coliform bacilli; ▲—▲=*Streptococcus faecium*; □—□=bacteroides; and ■—■=clostridia.

#### *The appearance of other bacterial species in the faeces*

*Breast-fed infants.* During the first 2 weeks, eight of the infants acquired *Staphylococcus epidermidis* in their faeces. By the 6th week, three infants had acquired a yeast. An aerobic lactobacillus was found in the faeces of one infant in the 2nd week.

*Infants fed Premium milk.* By the 3rd week of life, one-third of the infants in this group had acquired strains of *Proteus* that remained in their faeces thereafter. *Staph. epidermidis* was isolated from three infants on four occasions.

*Infants fed Gold Cap milk.* *Proteus* organisms were isolated from the faeces of four infants during the first 3 weeks of life. *Staph. epidermidis* was isolated from one infant on two occasions. Two infants acquired aerobic lactobacilli that were still present in the 6th week. One infant acquired a yeast in the 6th week.

*Infants fed supplemented breast milk.* *Staph. epidermidis* was isolated from the faeces of three infants at some time during the 6 weeks. One infant acquired a yeast in the 3rd week. Two infants had acquired an aerobic lactobacillus by the 2nd week. Strains of *Proteus* were isolated from three infants by the 4th week, and a strain of *Pseudomonas* was recovered from one infant in the 3rd week.

#### DISCUSSION

The results obtained from this survey support the conclusions reached in earlier feeding trials (Willis *et al.*, 1973; Bullen *et al.*, 1976) that an important factor that restricts the growth of the Enterobacteriaceae, streptococci, clostridia and bacteroides in the gut of breast-fed infants is the accumulation of acetic acid in the form of an acetate buffer. The primary factor required to ensure acidic faeces is food of poor buffering capacity. The amounts of insoluble protein and phosphorus in the two cows'-milk preparations studied in this trial are greater than those present in breast milk; the greater buffering capacities of "humanised" milks reflect their higher content of phospho-protein complex. The faeces of infants fed Premium and Gold Cap milks quickly acquire a *pH* of more than 6.2, and at such a *pH* an acetate buffer is unlikely to operate. It is interesting that the *pH* of the faeces continued to rise in these infants, whereas the *pH* values of faeces from the breast-fed group deviated by no more than 0.2 *pH* unit throughout the first 6 weeks of life.

The chromatographic patterns of acidified faecal suspensions from many of the infants fed the two artificial milk preparations showed the presence of a variety of volatile fatty acids. This is in sharp contrast to the patterns produced by the faeces from breast-fed infants in which fatty acids other than acetic acid were rarely present. An acetate buffer was demonstrated in >60% of wholly breast-fed infants in the first 4 weeks of life, whereas infants fed Premium and Gold Cap milks produced no buffer over this period. The presence or absence of an acetate buffer, and the patterns of volatile fatty acids, are reflections of the mean viable counts of the organisms isolated from faeces. Thus breast milk produced a fermentative flora, whereas Premium milk, which has a greater buffering capacity than that of breast milk, produced a putrefactive flora, although the counts of bifidobacteria were high. This suggests that the buffering capacity of the food was sufficiently low to encourage the growth of bifidobacteria, but too high to allow an accumulation of acetic acid in the faeces. Gold Cap milk, however, which has a buffering capacity almost twice that of breast milk, produced a typical putrefactive faecal flora similar to that of a bottle-fed group of infants examined by Bullen *et al.* (1976). Hewitt and Rigby (1976) could demonstrate no consistent relationship between high counts of bifidobacteria and low counts of *E. coli* in the stools of newborn infants fed breast

milk or artificial milk. This is not surprising as none of the infants they studied were wholly breast-fed and only a single observation (at about the 7th day) was made from each individual. Our studies show that breast-fed infants who receive supplements behave as bottle-fed babies. Moreover, the establishment and subsequent development of the faecal bacterial flora is a dynamic and continuing process that cannot be assessed from a single specimen, especially when such a specimen is taken as early as the 7th day.

The results of the present survey endorsed earlier findings (Bullen and Willis, 1971; Bullen *et al.*, 1976), that the supplementation of breast feeding with artificial feeds of high buffering capacities interferes with the establishment of a fermentative bacterial flora in the gut of the newborn infant, and consequently with the development of acidic faeces. The mean *pH* of the faeces from the supplement-fed infants was within the acid range, but even after 6 weeks of life the *pH* was not as low as that of the faeces from the breast-fed infants. This was reflected in the slow appearance of an acetate buffer in the supplement-fed infants; it was not until the 4th week that 60% of these babies had an acetate buffer in their faeces, whereas >60% of the breast-fed group produced a buffer within the 1st week of life.

The chromatographic patterns of volatile fatty acids produced in the faeces of supplement-fed infants indicated an initial putrefactive flora that gradually changed to a fermentative one. When the feeding sequence was changed from a supplemented regimen to breast-feeding alone the subsequent faecal changes took several weeks to develop. The mean viable bacterial counts in the faeces of infants fed supplement reflected the physicochemical changes that occurred. After 4 weeks of life when, except for acetic acid, the volatile fatty acids were disappearing from the faeces, the bifidobacteria were increasing and gradually came to predominate.

In infants fed breast milk alone a strongly acidic environment is produced in the large intestine within the first few days. At the same time, protection is afforded by the bacteriostatic effect of lactoferrin in combination with specific antibody to *E. coli* (Bullen *et al.*, 1972 and 1974). As suckling continues, this protection is reinforced and ultimately replaced by that of the acidic environment in the large intestine. It is important to emphasise that when supplements were fed during the first 7 days of life the production of a strongly acidic environment was delayed and its full potential was never reached.

#### SUMMARY

Faecal specimens from 47 babies were examined each week during the first 6 weeks of life. Thirteen infants received breast milk alone, nine received Cow and Gate Premium milk, 10 received Scientific Milk Adaptation Gold Cap milk, and 15 received breast milk and in the first week of life a supplement of modified cows' milk. An acetate buffer was demonstrated in the faeces of >60% of breast-fed infants during the first 4 weeks of life. No buffer was demonstrated in the faeces of either bottle-fed group over the same period. Only 20% of breast-fed infants receiving a supplement produced an acetate buffer in the 1st

week, but this figure increased to 60% by the 4th week. Breast-fed babies produced faeces with a low pH (5.1–5.4), high counts of bifidobacteria and low counts of coliforms, bacteroides and clostridia; with the exception of acetic acid there was a notable absence of most volatile fatty acids from these faeces. Infants fed Premium milk produced faeces with a high pH (5.9–8.0) and a relatively high bifidobacterial count in the 1st week; thereafter no bacterial species predominated. A wide variety of volatile fatty acids was present in these infants' faeces. Infants fed Gold Cap milk produced faeces with a high pH (6.4–8.2) and a wide variety of volatile fatty acids; the counts of coliform bacilli and putrefactive bacteria were high, whilst counts of bifidobacteria were low. Breast-fed infants who received a supplement in the 1st week produced acid faeces, but the pH was higher than that of infants fed breast milk alone; the bacterial flora was putrefactive for the first 2 weeks but gradually became fermentative, and whilst several volatile fatty acids were detected in the faeces in the 1st week they gradually decreased during the following 5 weeks.

We are indebted to Dr A. T. Willis for his continuing encouragement and advice and to Dr C. G. Fagg for allowing specimens to be collected from his patients. We thank the nursing staff of the North Hertfordshire Hospital, Hitchin, for their conscientious co-operation in selecting mothers and babies, and for collection of the early specimens. We are grateful to Mr J. Harrison and Miss G. Frankland for the photographic reproduction of the figures, and to Mrs J. Holt for secretarial assistance.

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