Archives of Disease in Childhood, 1989, **64**, 1672–1677

Diet and faecal flora in the newborn: breast milk and infant formula

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SUMMARY This study examined the faecal flora on days 4, 14, and 28 of 17 breast fed babies and 26 bottle fed babies receiving a modern infant formula based on demineralised whey. Generally among breast fed babies bifidobacteria and staphylococci were the predominant organisms, whereas in the formula fed babies the predominant organisms were enterococci, coliforms, and clostridia. Despite the extensive modification of cows' milk to make an infant formula resemble human breast milk, the results are very similar to those previously reported with unmodified cows' milk baby feeds. The exact dietary factor responsible for these microbiological differences is unclear and in succeeding papers we have looked at the effects of protein quality, in particular the content of whey proteins, casein, and lactoferrin.

For many years it was accepted that the faecal flora of a breast fed baby differed from that of a bottle fed one.[1] There are three reasons for re-examining this association between diet and faecal flora in the newborn. Firstly, many of these studies were performed using infant formulas that consisted of cows' milk with only minimal modification, whereas cows' milk is now extensively modified in the manufacture of an infant formula.[2] Secondly, breast milk differs from cows' milk and cows' milk based formula in so many ways it is impossible to know which dietary difference is responsible for the microbiological difference. Thirdly, some studies have not found these differences: the faecal flora of breast and bottle fed babies were similar.[3 4] It was suggested that the antiseptic environment of modern obstetrics may have over-ridden the dietary effects.

For these reasons we have re-examined the association between diet and faecal flora. This first paper determines whether the faecal microflora of the breast fed baby differs from that of a bottle fed baby receiving a well defined modern infant formula and succeeding studies explore the effects of specific nutrients.

Subjects and methods

DIET AND BABIES (TABLES 1 AND 2)

The infant formulas most commonly used in early life are based on demineralised whey plus skimmed milk, a blend of vegetable and animal fats, vitamins,

Table 1 *Composition of feeds compared with unmodified cows' milk (per l)*

	Breast milk	*Modern whey formula*	*Unmodified cows' milk*
Protein g (nitrogen×6·38)	11	17	33
Total 'true' protein (%)	9 (100)	16 (100)	31 (100)
Caseins	2·5 (28)	6·4 (40)	24·5 (79)
Whey proteins	6·5 (72)	9·6 (60)	6·5 (21)
Phosphorus (mg)	150	340	950
Iron (mg)	0·76	8·00	0·50

Table 2 *Data of babies taking part in the study*

	Breast fed (n=17)	*Formula fed (n=26)*
Sex:		
Male	8	12
Female	9	14
Race:		
White	15	19
Asian	0	6
Afro-Caribbean	2	1
Born by caesarean section	1	10
Mean (SD) birth weight (g)	3330 (390)	3290 (530)
Mean (SD) weight gain day 1–day 28 (g)	770 (340)	810 (330)

and minerals including iron. We therefore used such a formula (Nan supplied by Nestlé) for one group of babies, the other group was breast fed. Allocation of the babies to the two dietary groups depended solely on the mother's wish to breast or bottle feed her baby and so was not random. The babies in the two groups were similar in mode of delivery, size, etc. All were well and none received antibiotics. Mothers of the breast fed babies did not receive antibiotics postnatally. Breast fed babies were allowed feeds of water between breast feeds and the use of dummies was not prohibited.

PROCEDURES

Both groups of babies were fed on demand. The formula was reconstituted by the project nurses until the babies were 14 days old. From day 14 to day 28 mothers reconstituted the powdered formula. Anthropometric measurements were made at days 1 and 28. Faeces were collected on days 4, 14, and 28.

MICROBIOLOGICAL METHODS

Faeces were collected in nappy liners, weighed and emulsified in a transport medium (BHI broth containing 10% glycerol and 0·03% sodium formaldehyde sulphoxalate). This was immediately frozen and stored at −20°C. All specimens were analysed within one month of collection. Eight 10 fold dilutions were made in transport medium, and 20 µl of each dilution were inoculated in triplicate on to MacConkey agar (for enterobacteriaceae, *Staphylococcus* spp, and *Enterococcus* spp), Rogosa agar (for *Lactobacillus* spp and *Bifidobacterium* spp), and Blood agar containing 0·01% neomycin (for *Clostridium, Bacteroides,* and *Bifidobacterium* spp). Bacteria were identified by standard methods outlined by Cowan.[5] API20E were used to identify enterobacteriaceae, API20A were used to identify anaerobes (API System SA). *Lactobacillus* spp were identified using API50CHL. *Bifidobacterium* spp were identified biochemically using API50CHL as media and comparing the profiles with those of reference species of bifidobacteria obtained from the National Institute of Research in Dairying, Shinfield and to the schemes drawn up by Holdeman, Cato, and Moore and Mitsuoka and Kaneuchi.[6 7]

FAECAL PH

The pH of each faeces sample was measured in a 10% suspension in normal saline. All measurements were taken in a single batch using a Bibby 3 in 1 stick meter with an SMP 1 electrode.

STATISTICAL ANALYSIS

The results were analysed statistically using Mann-Whitney, Wilcoxon, χ^2 (with Yates's correction), and McNemar (with Yates's correction) tests as appropriate.

ETHICAL APPROVAL

Approval for the study was obtained from the ethics committee of the South Birmingham Health Authority. The mothers of all babies taking part in the study gave written informed consent.

Results

MICROBIOLOGY

There is no accepted convention for presenting descriptions of faecal flora. We found difficulty in summarising the data (for example, as means and SDs, etc) without covering up details of the distribution. We have therefore chosen to present the results in two ways: (1) counts of individual *organisms* in each baby shown diagrammatically; these were analysed statistically by Mann-Whitney, Wilcoxon, and χ^2 tests. (2) patterns of dominance in individual *babies* (that is, a particular genus accounting for the highest count in the bacterial population examined); these were analysed by χ^2 and McNemar tests.

(1) Counts of individual organisms (figs 1 and 2)

(a) Changes with time—The faecal flora changed with time but more changes were seen in the formula fed babies. Significant changes in the number of babies colonised are shown in columns marked y:

(i) breast fed babies had an increase in coliforms other than *Escherichia coli* but a decrease in enterococci;

(ii) formula fed babies had an increase in *E coli*, enterococci, and lactobacilli but a decrease in staphylococci.

Significant changes in the counts of organisms are shown in columns marked x:

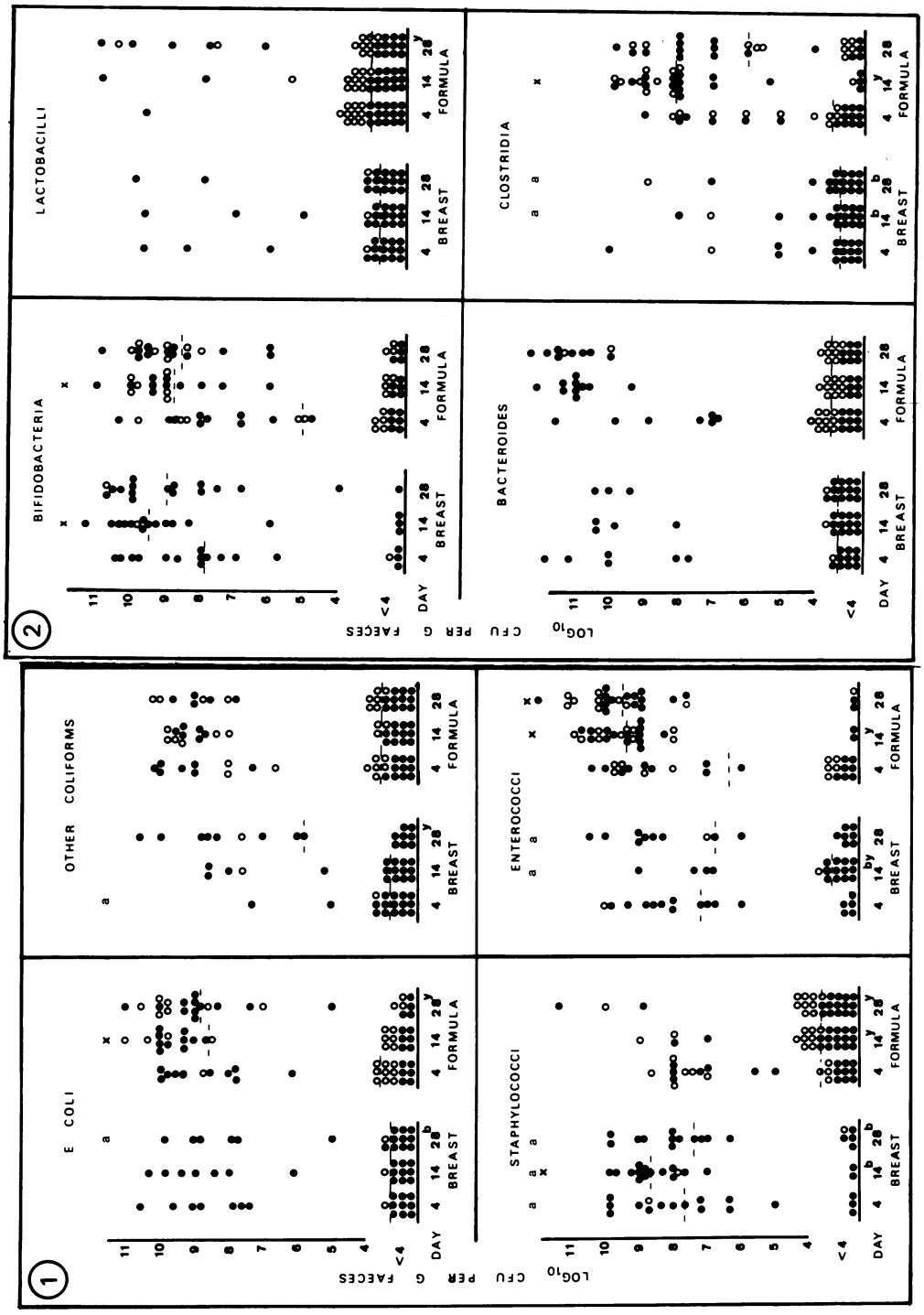
(i) breast fed babies had an increase in staphylococci and bifidobacteria;

(ii) formula fed babies had increase in *E coli*, enterococci, bifidobacteria, and clostridia.

(b) Dietary differences—More breast fed babies were colonised with staphylococci but fewer with clostridia (columns marked b).

Breast fed babies had lower counts *E coli* coliforms, enterococci, and clostridia but higher counts of staphylococci (columns marked a).

(c) Mode of delivery—Only one breast fed baby was delivered by caesarean section but 10 formula fed babies were delivered by section. None of the 10 babies were colonised by bacteroides on either day 4



Figs 1 and 2 *Counts of individual organisms (log_{10} colony forming units (CFU) per g of faeces) in the two dieury groups on days 4, 14, and 28. Median count shown by horizontal broken line; a, distribution of counts significantly different ($p<0·05$) from counts in formula fed babies of the same age (Mann-Whitney test); b, proportion of breast fed babies in whom an organism was not detected, is significantly different ($p<0·05$) from formula fed babies of the same age (χ^2 with Yates's correction); x, distribution of counts is significantly different ($p<0·05$) from counts at day 4 (Wilcoxon); and y, proportion of babies in whom an organism was not detected is significantly different ($p<0·05$) from that proportion at day 4 (McNemar with Yates's correction). ●= Babies delivered vaginally and ○= babies delivered by caesarean section.*

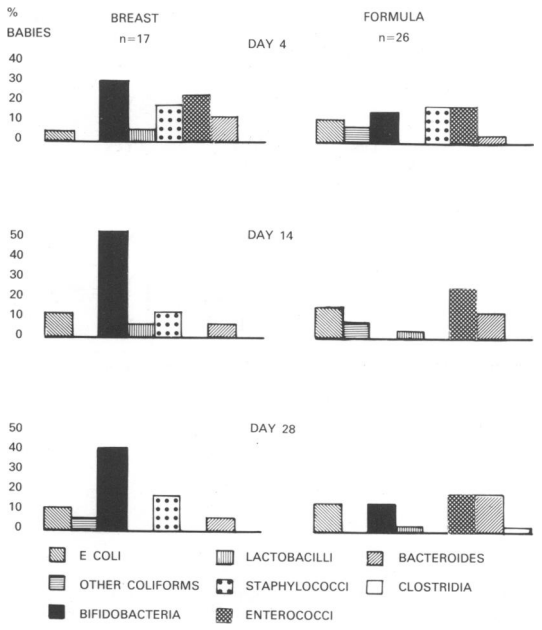


Fig 3 *Percentage of babies in whose faeces organisms were dominant.*

or day 14. Of the 16 vaginally born formula fed babies, however, seven were colonised with bacteriodes on day 4 ($p<0.02$) and 10 by day 14 ($p<0.001$).

(2) Patterns of dominance (fig 3)
(a) Changes with time—Enterococci were not dominant in any breast fed baby at days 14 or 28 and staphylococci were not dominant in any formula fed baby at days 14 and 28.

(b) Dietary differences—At day 4 no difference was observed. Later at day 14, when compared with breast fed babies, none of the formula fed babies had bifidobacteria as the dominant organism ($p<0.001$) and more had enterococci dominant, although this was not significant. This trend was continued at day 28 with an increased number of breast fed babies with bifidobacteria as the dominant organism, but it was no longer significant.

Table 3 *Mean (SD) pH of faeces*

	Breast fed	*Formula fed*
Day 4	5·56 (0·35)	5·90 (0·79)
Day 14	5·49 (0·40)*	6·91 (0·80)*†
Day 28	5·74 (1·03)*	7·07 (0·94)*†

*Difference between breast and formula fed babies $p<0.001$.
†Significant difference between value on day 4.

FAECAL PH (TABLE 3)
The pH of the breast fed babies was always more acidic than the formula fed babies and this was highly significant at days 14 and 28. The pH of the breast fed babies did not change significantly during the study but that of the formula fed babies became less acidic after day 4.

Discussion

Generally among breast fed babies bifidobacteria and staphylococci were the predominating organisms (for example, higher counts or more babies colonised or more babies in which the organisms were dominant), whereas in formula fed babies the predominant organisms were enterococci. It does seem therefore that modern infant formulas, despite extensive modification during manufacture, result in a faecal flora substantially different from that of the breast fed baby.

Table 4 summarises the results of this and some previous studies. The nature of the diets used is often not stated. However, like many other investigators we found a predominance of bifidobacteria in the breast fed babies. We are unclear why this was not observed in the babies born at St Bartholomew's Hospital, London.[3] The suggestion by the authors of that study that the antiseptic environment of modern obstetrics being responsible is an interesting one. In this hospital we use 1·5% chlorhexidine gluconate and 15% cetrimide diluted 1:50 (Savlon) for vulval washing and 1% chlorhexidine gluconate (Hibitane Obstetric cream) for vaginal examinations during labour, but antiseptic nipple sprays are not encouraged.

What are the exact dietary constituents responsible for the observed differences in the faecal flora in the formula and breast fed infant? The substances and mechanisms implicated (casein, phosphate, buffering capacity, lactoferrin, secretory IgA) and the reviews of the subject are legion,[16–18] but as breast milk differs from an infant formula in so many ways it is difficult to know which is the main factor. In the succeeding papers we have looked at the microbiological effects of specific nutrients: casein and whey proteins, phosphate, lactoferrin, and iron.

This project was partially supported by Nestlé (Nestec Ltd, Research Centre), CH 1800 Vevey, Switzerland. We are grateful to Mrs C Boyle and Mrs N Burton for the preparation of feeds and the collection of specimens.

Table 4 *Comparison of studies examining faecal microflora in breast and formula fed babies. (Results refer to breast fed babies)*

Country	*No of babies*	*Age of babies*	*Type of milk*	*Less coliforms*	*More bifidobacteria*	*Less enterococci*	*More staphylococci*	*Less bactoides and clostridia*
United Kingdom (this study)	17	4 days	Breast	No	Yes	Yes	Yes	No
	26		Nan					
	17	14 days	Breast	Yes	Yes	Yes	Yes	Yes
	26		Nan					
	17	28 days	Breast	Yes	Yes	Yes	Yes	Yes
	26		Nan					
United Kingdom[1]	7	1 week	Breast	Yes	Yes	Yes	NT	Yes
	10		Cows' milk					
	5	2 weeks	Breast	No	Yes	Yes	NT	Yes
	4		Cows' milk					
	7	3 weeks	Breast	Yes	Yes	Yes	NT	Yes
	4		Cows' milk					
	4	5 weeks	Breast	Yes	Yes	Yes	NT	Yes
	3		Cows' milk					
	2	7 weeks	Breast	No	Yes	Yes	NT	Yes
	2		Cows' milk					
United Kingdom[8]	24	7–10 days	Breast	No	Yes	NT	NT	NT
	20		SMA					
	20		Cow and Gate special					
	10		Cow and Gate ½ cream					
	4		Golden Ostermilk					
United Kingdom[9]	11	5–7 days	Breast	No	Yes	NT	NT	NT
	21		SMA Gold Cap					
United Kingdom[10]	9	1–5 days	Breast	No	Yes	NT	NT	No
	6		Formula*					
	9	3 weeks	Breast	No	No	NT	NT	No
	6		Formula*					
	9	6 weeks	Breast	No	No	NT	NT	No
	6		Formula*					
United Kingdom[3]	15	2 weeks	Breast	No	No	Yes	Yes	Yes
	11		Formula*					
	16	4 weeks	Breast	No	No	Yes	Yes	Yes
	12		Formula*					
	15	6 weeks	Breast	No	No	Yes	Yes	Yes
	12		Formula*					
Nigeria	12		Breast	Yes	Yes	No	No	Yes
Sweden[4]	15	5 days	Breast	No	Yes	Yes	Yes	Yes
	7		Whey based formula					
	15	3 weeks	Breast	Yes	No	Yes	Yes	No
	7		Whey based formula					
	15	8 weeks	Breast	No	No	No	Yes	No
	7		Whey based formula					
Australia[11]	7	1 week	Breast	Yes	Yes	Yes	NT	Yes
	7	4 weeks	Formula*					
Japan[12]	70	28–46 days	Breast	Yes	No	Yes	No	Yes
	35		Formula*					
Japan[13]	6	1–6 days	Breast	Yes	Yes	Yes	No	Yes
	7		Cows' milk					
	6	4 weeks	Breast	No	Yes	Yes	No	Yes
	7		Cows' milk					
France[14]	22	1–8 days	Breast	No	Yes	No	NT	No
	11		Gallia					
Holland[15]	10	1–3 months	Breast	Yes	Yes	Yes	NT	No
	6		Almiron					
	7		Almiron—no added iron	Yes	No	Yes	NT	No

*Formula not specified; NT=not tested.

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Accepted 17 April 1989