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THE MICROBIAL ECOLOGY OF THE LARGE BOWEL OF BREAST-FED AND FORMULA-FED INFANTS DURING THE FIRST YEAR OF LIFE

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SUMMARY. The succession of bacterial populations in the large bowel of seven breast-fed and seven formula-fed infants was examined during the first year of life. The composition of the intestinal microflora varied according to the infant's diet. During the first week of life breast-fed and formula-fed infants were colonised by enterobacteria and enterococci followed by bifidobacteria, *Bacteroides* spp., clostridia and anaerobic streptococci. From week 4 until solid foods were given, breast-fed babies had a simple flora consisting of bifidobacteria and relatively few enterobacteria and enterococci. Formula-fed babies during the corresponding period were more often colonised by other anaerobes in addition to bifidobacteria and had higher counts of facultatively anaerobic bacteria. The introduction of solid food to the breast-fed infants caused a major disturbance in the microbial ecology of the large bowel as counts of enterobacteria and enterococci rose sharply and colonisation by *Bacteroides* spp., clostridia and anaerobic streptococci occurred. This was not observed when formula-fed infants began to take solids; instead, counts of facultative anaerobes remained high while colonisation by anaerobes other than bifidobacteria continued. At 12 months, the anaerobic bacterial populations of the large bowel of breast-fed and formula-fed infants were beginning to resemble those of adults in number and composition and there was a corresponding decrease in the number of facultative anaerobes. These changes are discussed in relation to changes in susceptibility to gastro-intestinal infection.

INTRODUCTION

The autochthonous bacteria inhabiting the gastro-intestinal tract of man influence the health of their host in various ways. For example, they contribute to resistance against colonisation by enteric pathogens (van der Waaij, 1979) and are thought to be involved in the aetiology of cancer of the large bowel (Hill, 1974). The normal balance between competing organisms is established during infancy and might be expected to be attained by successive stages, following a definite time sequence and under the influence of dietary change as has already been demonstrated in rodents (Lee *et al.*, 1971).

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Mata and Urrutia (1971) reported a prospective study of the succession of bacteria in the large bowel of breast-fed infants. The infants studied, however, lived in a rural area of Guatemala where standards of hygiene and nutrition are poor and breast feeding continues for much longer periods than is practised in industrialised societies. Other recent studies have been short term or have grouped together infants of widely differing ages and sometimes also differing feeding practices.

In the present investigation we have examined the succession of bacteria in the large bowel of healthy breast-fed and bottle-fed infants during the first year of life.

MATERIALS AND METHODS

Selection of subjects. Fourteen babies born at the Women's Hospital, Crown Street, Sydney were selected for the prospective study. They lived in Sydney suburban homes where high standards of nutrition and hygiene were practised. None of the children suffered from gastro-intestinal or any major illness or received antibiotic therapy during the course of the survey.

Seven babies were breast fed for periods ranging from 3 to 10 months. Seven were artificially fed from birth although three of these also received limited amounts of breast milk during the first 1–2 weeks of life. Solid foods, usually in the form of cereals in cow's milk, were first given between the 10th and 28th weeks (average 17 weeks).

In addition, single specimens of faeces were collected from 20 healthy 9-month-old infants. Ten had been formula fed since birth and 10 were still receiving breast milk in addition to some solid foods. The infants lived in Sydney homes and were contacted during routine visits to Baby Health Clinics. Single specimens of faeces were also collected from 20 adults from among the staff and postgraduate students at the University of New South Wales.

Collection of specimens. Faeces (*c.* 0·5 g) were collected with sterile wooden spatulas and transported in 5·5-ml screw-capped glass bottles containing 4·5 ml of salts solution (Holdeman and Moore, 1975), with glycerol 10%, cysteine HCl 0·5 g/L, and resazurin 1 mg/L, overlaid by a thin layer of paraffin oil. The transport devices were pre-reduced in an anaerobic chamber and discarded if at any time the resazurin turned pink. All samples were frozen at −60°C on the day of collection and held at that temperature until cultured (Crowther, 1971).

Four or five samples were collected from each baby in the prospective study during the first week of life, and single samples at 4 weeks and at approximately 6-weekly intervals thereafter.

Culture and identification of bacteria. A flexible vinyl anaerobic chamber, containing a gas mixture of 90% CO_2 and 10% H_2 circulated over a palladium catalyst, was used for the culture of anaerobes. Frozen samples were thawed inside the chamber and serial tenfold dilutions made in 4·5 ml of pre-reduced brain-heart infusion (BHI).

Culture media and incubation conditions are summarised in table I. Plates for anaerobic incubation contained cysteine HCl 0·5 g/L and were stored inside the chamber for 24 h before inoculation with 0·1-ml quantities of the faecal dilutions. Willis and Hobbs (1959) agar was inoculated with dilutions which had been heat shocked at 75°C for 12 min.

After incubation, 10 colonies were picked at random from BHI-agar plates and subcultured for aerobic and anaerobic incubation. Isolates confirmed to be obligate anaerobes were inoculated into BHI broth containing yeast extract 0·5%, glucose 0·4%, cellobiose 0·1%, maltose 0·1% and starch 0·1% for gas-liquid chromatography. In addition, colonies of each distinctive morphology were picked from vancomycin-kanamycin, tomato-juice and Willis and Hobbs agar plates and subcultured as described previously. With the exception of heat shocking, all procedures described above were done inside the anaerobic chamber.

Lactobacilli were counted by the same technique except that incubation was in a candle jar. Because MRS medium (Oxoid CM361) also supports the growth of gram-positive cocci and some gram-negative rods, colonies from these plates were gram stained before subculture into broths.

Enterobacteria and enterococci were counted by a modified Miles and Misra technique (Miles, Misra and Irwin, 1938).

Bacterial counts were expressed as colony-forming units (cfu)/g of wet faeces.

Identification of anaerobic isolates was based on the gram-stain morphology and the gas-chromatographic identification of volatile fatty acids and nonvolatile acids produced as end products of carbohydrate fermentation. Sugar-fermentation reactions and spore tests were sometimes required for differentiation between members of the genera *Clostridium*, *Bifidobacterium* and *Eubacterium* (Holdeman and Moore, 1975).

RESULTS

Preliminary evaluation of results showed that bacterial populations were influenced by the diet rather than the age of the babies. For this reason data were analysed in terms of changing feeding regimens as shown in table II. When more than one sample was collected during a particular stage of feeding, the culture results of only one specimen are given because it was found that bacterial populations for each baby did not change appreciably within the feeding modes described.

Obligate anaerobes

Bifidobacteria. The culture results for the bifidobacteria are shown in table III. During the first week of life, six of the seven breast-fed babies were colonised by bifidobacteria. By week 4 all the breast-fed babies were colonised and counts remained in the range 10^9–10^{11} cfu/g of faeces throughout the first year.

Colonisation of two of the bottle-fed group was delayed, bifidobacteria being first isolated from baby no. 9 at week 27 and baby no. 12 at week 30. When colonisation had occurred, counts in the bottle-fed infants were in the same range as those in the breast-fed group.

TABLE I

Culture media and conditions of incubation

Culture medium	Dilutions cultured ($\log_{10}$)	Incubation			Bacteria
		time	temp.	atmosphere	
Brain heart infusion agar *(Holdeman and Moore, 1972)	6–9	3 days	37°C	Anaerobic	Total anaerobes
Vancomycin-kanamycin agar *(Finegold *et al.*, 1971)	2–5†	3 days	37°C	Anaerobic	*Bacteroides* spp. *Fusobacterium* spp. *Veillonella* spp.
Tomato-juice agar with kanamycin and nalidixic acid (Finegold *et al.*, 1971)	2–5†	3 days	37°C	Anaerobic	Bifidobacteria
Willis' and Hobbs' agar (Willis and Hobbs, 1959)	2–4	3 days	37°C	Anaerobic	Clostridia
MRS (Oxoid, CM361)	2–5	3 days	37°C	CO_2	Lactobacilli
5% sheep-blood agar	2–7	over-night	37°C	Aerobic	Total aerobes
MacConkey agar	2–7	over-night	37°C	Aerobic	Enterobacteria enterococci

* Supplemented with 10% horse blood, vitamin K, haemin and yeast extract.
† Higher dilutions were cultured when the baby was known to be already colonised by the organisms being selected.

TABLE II

Stages in the succession of bacterial populations in the infant large bowel

Stage (and average age) of babies that were		Description
breast fed	formula fed	
Week 1	Week 1	First bacterial inoculum
Week 4	Week 4	Early colonisation under the influence of either breast milk or formula
Pre-weaning (14 weeks)	Pre-solids (12 weeks)	Bacterial flora which stabilises under the influence of breast milk or formula
Early weaning (20 weeks)	After solids (23 weeks)	Immediate effect of introducing solid foods to the diet
Late weaning (37 weeks)	9 months (39 weeks)	Effect of a prolonged mixed diet of either breast milk plus solids or formula plus solids
12 months	12 months	Bacterial flora of fully weaned breast-fed infant compared with formula-fed infant of the same age

TABLE III

Colonisation of the infant large bowel by bifidobacteria

Baby no.	Viable count (log_{10} cfu/g wet faeces) in breast-fed infants					
	Week 1*	Week 4	Pre-weaning	Early weaning	Late weaning	12 months
1	8·8–9·5	9·7	9·3	8·3	11·1	10·1
2	9·5–12·1	11·2	11·1	12·3	10·2	10·1
3	7·1–10·9	11·1	9·3	10·2	9·1	10·2
4	<4	10·2	10·2	9·6	NA	9·4
5	9·9–10·2	...	10·3	11·1	9·7	10·2
6	7·5–11·1	9·4	9·7	10·2	10·2	10·2
7	9·5–11·3	11·8	11·1	9·5	NA	9·2
Mean count in babies colonised	*	10·6	10·1	10·2	10·1	9·9

Baby no.	Viable count (log_{10} cfu/g wet faeces) in formula-fed infants					
	Week 1*	Week 4	Pre-solids	After solids	9 months	12 months
8	8·1	10·1	10·3	11·1	9·6	11·7
9	<4	<4	9·2	<4	10·2	11·5
10	7·2	10·1	9·1	9·1	8·7	9·1
11	<4	...	11·2	9·1	9·1	9·2
12	5·1	<4	...	<4	11·9	11·9
13	10·4	10·5	11·2	10·1	10·1	9·7
14	4·4–9·2	10·4	11·5	9·8	9·8	9·9
Mean count in babies colonised	*	10·3	10·4	9·8	9·9	10·4

* Because counts fluctuated widely during week 1, the range of values is given, and mean could not be calculated.

... = Specimen not obtained; NA = not applicable, babies weaned over a short period.

TABLE IV

Colonisation of the infant large bowel by Bacteroides spp.

Baby no.	Viable count (log_{10} cfu/g wet faeces) in breast-fed infants: Week 1*	Week 4	Pre-weaning	Early weaning	Late weaning	12 months
1	<3	<3	<3	<3	7·1	10·8
2	6·3–11·2	<3	<3	<3	9·6	11·1
3	<3	<3	<3	<3	8·1	10·3
4	10·7–11·2	<3	<3	10·1	NA	9·6
5	4·2–10·2	...	<3	8·2	9·3	9·3
6	7·2	<3	<3	<3	7·1	8·1
7	<3	7·2	7·1	9·7	NA	10·2
Mean count in babies colonised	*	...	...	9·3	8·2	9·9

Baby no.	Viable count (log_{10} cfu/g wet faeces) in formula-fed infants: Week 1*	Week 4	Pre-solids	After solids	9 months	12 months
8	<3	<3	<3	<3	10·1	11·9
9	<3	<3	<3	<3	<3	10·9
10	<3	<3	8·9	10·4	10·3	9·3
11	<3	...	8·1	8·1	9·2	8·9
12	<3	10·5	...	8·1	9·3	9·1
13	7·3	8·1	8·1	8·1	9·2	8·9
14	<3	<3	<3	<3	9·5	9·1
Mean count in babies colonised	*	9·3	8·4	8·7	9·6	9·7

Footnotes as in table III.

Bacteroides spp. The isolation of *Bacteroides* spp. is shown in table IV. These strict anaerobes were isolated from four breast-fed babies during the first week of life. Although many were present for several consecutive days, persistent colonisation was not achieved because bacteroides were not present in any of the four babies at the age of 1 month. All the breast-fed babies except one (no. 7) were colonised some time after the introduction of solid food. Stable colonisation by *Bacteroides* spp. often occurred at an earlier stage in the formula-fed group, four individuals being colonised before solid food was given. By 12 months of age all infants in both groups were colonised by *Bacteroides* spp. with numbers ranging from 10^8 to 10^{11} cfu/g.

Anaerobic cocci. Anaerobic gram-positive cocci of the genera *Peptostreptococcus* and *Peptococcus* were isolated from breast-fed and formula-fed babies as shown in table V. These bacteria were present as transient members of the faecal flora in one breast-fed baby during the first week of life and one formula-fed baby at week 4. With the exception of one baby (no. 5), colonisation in both groups occurred at some time after the introduction of solid food. Anaerobic gram-positive cocci had reached 10^8–10^{11} cfu/g in breast-fed and in formula-fed infants by 12 months of age.

Veillonellae were also isolated from four formula-fed infants before and shortly after solid foods were added to the diet. Concentrations ranged from 10^6 to 10^9 cfu/g.

TABLE V

Colonisation of the infant large bowel by anaerobic gram-positive cocci

Baby no.	Viable count (log_{10} cfu/g wet faeces) in breast-fed infants					
	Week 1*	Week 4	Pre-weaning	Early weaning	Late weaning	12 months
1	<7	<7	<7	<7	<7	10·4
2	10·2–11·2	<7	<7	<7	7·1	10·2
3	<7	<7	<7	<7	<7	9·1
4	<7	<7	<7	9·2	NA	10·1
5	<7	...	10·1	<7	9·3	<7
6	<7	<7	<7	<7	8·1	10·5
7	<7	<7	<7	<7	NA	10·1
Mean count in babies colonised	*	...	...	...	8·2	10·1

Baby no.	Viable count (log_{10} cfu/g wet faeces) in formula-fed infants					
	Week 1*	Week 4	Pre-solids	After solids	9 months	12 months
8	<7	<7	<7	<7	10·1	11·5
9	<7	<7	<7	8·9	9·7	11·1
10	<7	<7	<7	<7	10·1	8·8
11	<7	...	<7	<7	<7	<7
12	<7	11·1	...	<7	<7	<7
13	<7	<7	<7	9·1	<7	9·1
14	<7	<7	<7	<7	10·1	9·2
Mean count in babies colonised	*	...	...	9·1	10·1	9·9

Footnotes as in table III.

These organisms did not seem able to persist as major components of the bacterial faecal flora when other anaerobic groups had become established.

Clostridia. Spore counts of clostridia, obtained by the heat-shock technique, are shown in table VI. Clostridia were isolated from six breast-fed infants during the first week of life but from none during the period lasting from week 4 until the introduction of solid food. All breast-fed infants were colonised during the weaning period. Clostridia were isolated from four formula-fed infants during week 1 and permanent colonisation occurred earlier in this group, six babies being colonised before solids were given. By 12 months all infants were colonised by clostridia in numbers ranging from 10^3 to 10^6 spores/g of faeces.

Although heat shocking is the best method available for the selective isolation of clostridia, it must be remembered that spore counts are not accurate estimates of total numbers of clostridia. They may be used, however, as a guide to the relative numbers of clostridia in breast-fed and formula-fed infants.

Facultative anaerobes

The results for the culture of the facultative anaerobic bacteria are shown in tables VII and VIII.

TABLE VI

Colonisation of the infant large bowel by clostridia

Baby no.	Spore count (log_{10}/g wet faeces) in breast-fed infants: Week 1*	Week 4	Pre-weaning	Early weaning	Late weaning	12 months
1	<3	<3	<3	<3	5·2	4·3
2	3·4–6·2	<3	<3	<3	5·5	5·2
3	8·5	<3	<3	<3	5·5	5·2
4	5·4	<3	<3	5·7	NA	5·8
5	4·7	...	<3	5·3	4·2	5·5
6	3·3–4·1	<3	<3	<3	5·2	4·6
7	3·4–6·8	<3	<3	4·7	NA	6·1
Mean count in babies colonised	*	...	...	5·2	5·1	5·2

Baby no.	Spore count (log_{10}/g wet faeces) in formula-fed infants: Week 1*	Week 4	Pre-solids	After solids	9 months	12 months
8	<3	7·1	<3	<3	5·5	4·3
9	<3	<3	5·3	5·1	4·5	3·5
10	<3	<3	5·1	4·1	5·7	5·3
11	5·1	...	4·3	4·1	<3	4·3
12	5·2	5·1	...	4·1	6·4	5·2
13	3·2–5·1	6·2	5·2	5·9	6·2	5·6
14	5·8	7·1	6·6	4·6	5·3	6·1
Mean count in babies colonised	*	6·4	5·3	4·7	5·6	4·9

Footnotes as in table III.

First week. Enterococci were isolated from all babies, and members of the family Enterobacteriaceae from all but three babies during the first week of life. These were the first bacterial groups to colonise the infant intestine and numbers fluctuated widely from 10^4 to 10^{11} cfu/g.

Week 4 to weaning. By week 4, counts of enterobacteria in breast-fed babies had fallen (mean $10^{6\cdot1}$ cfu/g) and they stayed at these levels until solid foods were given. By contrast, counts of enterobacteria in formula-fed infants remained higher throughout this period (mean $10^{9\cdot4}$ cfu/g).

The enterococci showed similar trends; in the breast-fed group counts fell (mean $10^{6\cdot3}$ cfu/g) while in formula-fed babies counts remained higher (mean $10^{9\cdot6}$ cfu/g).

Weaning. The introduction of solid food heralded a sudden increase in numbers of facultative anaerobic bacteria in the faeces of all breast-fed infants. At early weaning, mean counts of enterobacteria and enterococci rose to $10^{8\cdot1}$ cfu/g and $10^{8\cdot5}$ cfu/g, respectively. These counts remained high throughout the weaning period.

Numbers of facultative anaerobic bacteria in the faeces of formula-fed infants altered little with the introduction of solid foods. Mean counts for enterobacteria and enterococci were $10^{8\cdot5}$ cfu/g and $10^{9\cdot5}$ cfu/g, respectively.

These results were confirmed by examination of single specimens of faeces from

TABLE VII

Colonisation of the infant large bowel by enterobacteria

Baby no.	Viable count (log_{10} cfu/g wet faeces) in breast-fed infants					
	Week 1*	Week 4	Pre-weaning	Early weaning	Late weaning	12 months
1	5·2–9·2	3·3	4·3	7·3	9·3	6·1
2	4·8–7·2	7·1	6·2	8·6	7·9	6·2
3	< 3	6·7	6·1	7·2	8·5	8·9
4	6·2–9·2	4·3	4·1	8·7	NA	8·1
5	5·1–9·3	...	6·4	8·5	9·3	7·8
6	3·4–7·2	8·3	5·1	9·1	8·2	8·1
7	7·7–10·2	6·5	6·7	7·4	NA	3·5
Mean count of babies colonised	*	6·1	5·5	8·1	8·6	6·9

Baby no.	Viable count (log_{10} cfu/g wet faeces) in formula-fed infants					
	Week 1*	Week 4	Pre-solids	After solids	9 months	12 months
8	10·3–10·5	10·2	7·4	8·7	8·1	9·2
9	5·2–6·7	9·1	7·5	7·9	6·8	6·2
10	10·5–11·1	10·2	9·2	9·3	8·5	8·4
11	7·1–10·3	...	8·1	9·4	9·1	6·5
12	< 3	8·6	...	7·1	7·2	7·1
13	8·5–10·5	9·1	8·6	8·6	9·1	7·8
14	8·3	9·2	9·2	8·3	9·1	8·3
Mean count of babies colonised	*	9·4	8·3	8·5	8·3	7·6

Footnotes as in table III.

another 20 nine-month-old infants, 10 of whom were receiving a mixed diet of breast milk and solid food, and 10 who had been formula-fed since birth. The results are summarised in table IX.

Counts of enterobacteria and enterococci in breast-fed and in formula-fed 9-month-old infants were significantly higher than corresponding counts in adults (table IX). Furthermore, there was no significant difference in counts of enterobacteria in breast-fed compared with formula-fed infants, while counts of enterococci were significantly higher in the breast-fed group than in the formula-fed group (in all tests of significance, by Student's t test, $p < 0{\cdot}05$).

Twelve months. Counts of faecal enterobacteria had fallen in four breast-fed and two formula-fed infants by 12 months of age. In addition, another three breast-fed and two formula-fed infants who were followed up beyond 12 months also showed decreases in faecal enterobacteria before reaching 18 months of age. Counts of enterococci in both groups of infants also fell to within the adult range as the babies entered the second year of life.

Other genera

Lactobacilli were isolated from four breast-fed and three bottle-fed infants in

TABLE VIII

Colonisation of the infant large bowel by enterococci

Baby no.	Viable count (log_{10} cfu/g wet faeces) in breast-fed infants					
	Week 1*	Week 4	Pre-weaning	Early weaning	Late weaning	12 months
1	6·2–7·2	5·4	5·3	8·3	9·3	7·2
2	7·7–10·3	5·5	4·2	7·2	7·2	7·1
3	6·7–10·5	8·3	8·2	8·6	10·1	8·1
4	8·8–10·1	6·2	6·5	9·1	NA	8·1
5	5·4	...	9·1	9·5	8·1	7·3
6	4·4–5·2	6·1	6·2	7·6	8·5	8·1
7	7·2	6·1	6·3	9·2	NA	5·7
Mean count of babies colonised	*	6·3	6·5	8·5	8·6	7·4

Baby no.	Viable count (log_{10} cfu/g wet faeces) in formula-fed infants					
	Week 1*	Week 4	Pre-solids	After solids	9 months	12 months
8	<4	9·4	10·1	10·1	8·1	9·4
9	3·6–8·3	9·2	9·2	9·4	6·1	6·8
10	7·1–9·1	10·2	9·4	10·1	8·5	8·1
11	8·2–9·5	...	8·1	11·1	7·2	6·3
12	5·3–9·1	10·1	...	9·6	8·9	7·3
13	7·9–9·8	9·1	8·7	8·1	8·6	7·1
14	7·9	9·5	9·4	8·2	8·4	7·2
Mean count of babies colonised	*	9·6	9·2	9·5	7·9	7·5

Footnotes as in table III.

counts ranging from 10^5 to 10^{10} cfu/g. Isolations were not continuous for any one baby, however, lactobacilli being present for 2–4 months and the disappearing. This suggests that lactobacilli are unable to form stable populations in the infant.

Eubacteria colonised four breast-fed and two bottle-fed infants between 9 and 12 months of age, reaching counts of 10^9–10^{10} cfu/g.

Staphylococcus aureus was cultured from baby no. 2 during weeks 4–11, and a coagulase-negative staphylococcus from baby no. 12 in the first week and from baby 6 for the first 11 weeks of life.

TABLE IX

Viable counts of facultative anaerobes in the large bowel of adults and 9-month-old infants

Group of organisms	Mean viable count (log_{10} cfu/g wet faeces ± SD) in		
	20 adults	10 formula-fed infants	10 breast-fed infants
Enterobacteria	6·5 ± 1·2*	7·7 ± 0·9	8·1 ± 0·9
Enterococci	6·3 ± 1·3*	7·6 ± 1·0	8·8 ± 0·9
All facultative anaerobes	6·9 ± 0·5*	8·4 ± 0·9	8·7 ± 0·9

* Significantly different from infant values ($p < 0·05$).

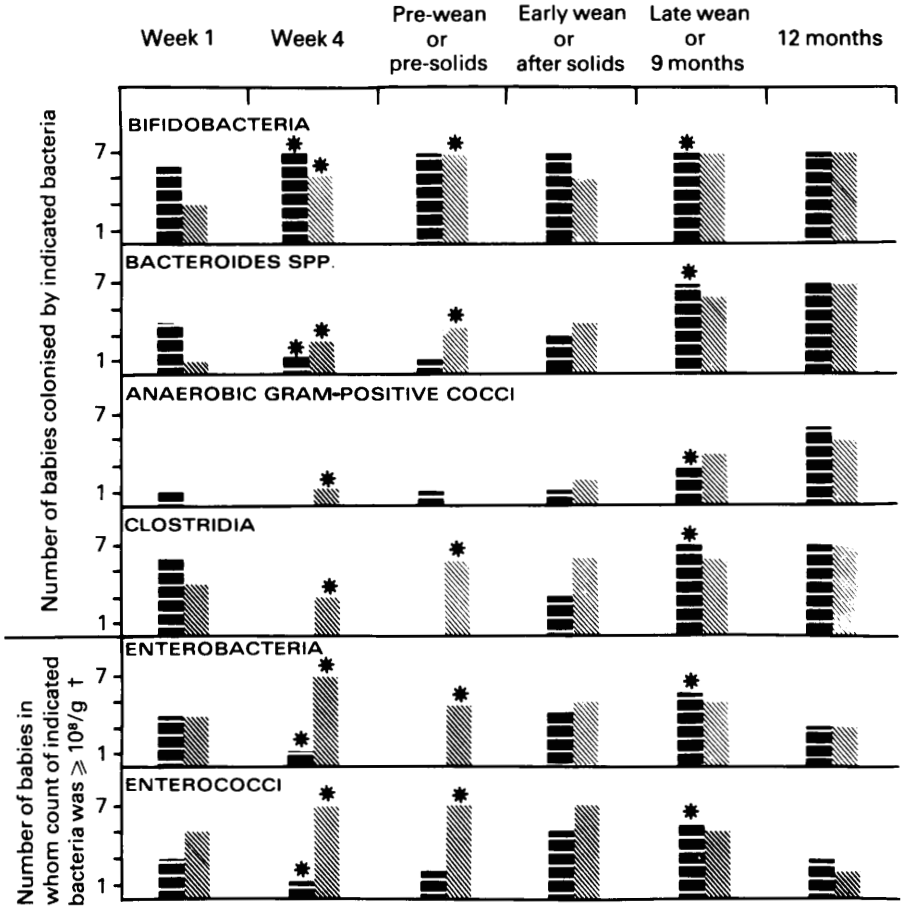

Figure 1—The succession of bacterial populations in the large bowel of breast-fed (■) and formula-fed (▨) infants.
* When fewer than seven babies were examined the results have been adjusted to a fraction of seven.
† Counts of facultative anaerobic bacteria $\geqslant 10^8$/g faeces are raised in comparison to counts in normal adults.

The succession of bacterial populations

The succession of bacteria in the large bowel of breast-fed and formula-fed infants is summarised in figure 1.

Discussion

This is the first long-term prospective study of the microbial ecology of the large bowel of healthy breast-fed and formula-fed infants living in an industrialised community. Apart from one report from rural Guatemala (Mata and Urrutia, 1971), other investigations have concerned infants of different ages, and in some cases receiving different diets (Mitsuoka and Hayakawa, 1973; Ellis-Pegler, Crabtree and Lambert, 1975; Bullen and Tearle, 1976; Hewitt and Rigby, 1976; Albert *et al.*, 1978; Leclerc and Moriamez, 1980).

As in most studies of the human intestinal microflora, faecal bacterial populations were assumed to be representative of the flora of the lumen of the colon. This assumption has been verified by other authors (Hill and Drasar, 1975; Moore, Cato and Holdeman, 1978).

The number of microbial species in the human intestine is estimated to be more than 400 (Moore *et al.*, 1978). Lack of suitable selective culture media makes it impossible to count many of these organisms; Therefore studies on the development of the intestinal flora can feasibly quantitate only the dominant groups of bacteria or those than can be readily selected. The time and expenditure required for the culture and identification of strict anaerobes also limits the number of isolated colonies that can be identified. Nevertheless, the numerically dominant bacteria probably contribute most to homeostasis in the intestine.

The microbial ecology of the infant large bowel

Microbial colonisation of the infant large bowel is a complex process lasting throughout the first year of life. Distinct patterns of colonisation can be described although it must be remembered that some degree of individual variation occurs, e.g., staphylococci were isolated consistently from some babies but not from others; one breast-fed baby was colonised by a species of *Bacteroides* at 4 weeks of age while other infants in the group were not colonised by this organism until weaning was initiated.

In the first week the gastro-intestinal tract of the neonate is seeded with a wide variety of organisms from the birth canal and the baby's surroundings. Organisms best suited to the intestinal environment become established by a process of natural selection. A diet of breast milk creates an environment favouring the development of a simple flora of bifidobacteria and few other anaerobes and small numbers of facultatively anaerobic bacteria. A formula diet also allows bifidobacteria to reach high population densities, equal to those occurring in the climax community in the large bowel of adults, while permitting *Bacteroides* spp., clostridia and anaerobic streptococci to colonise more frequently, and facultative anaerobic bacteria to reach higher levels.

The introduction of solid food to the breast-fed infant causes a major perturbation in the gut ecosystem, with a rapid rise in the number of enterobacteria and enterococci, followed by progressive colonisation by *Bacteroides* spp., clostridia and anaerobic streptococci. The addition of solid food to the diet of the formula-fed infant does not have such an impact on the gastro-intestinal flora. Facultative anaerobes remain numerous while colonisation with anaerobes other than bifidobacteria continues. As the amount of solid food in the diet increases, the faecal bacterial flora of breast-fed and of formula-fed infants approaches that of adults.

Other authors consider the ratio of anaerobes to aerobes to be a useful index of intestinal populations (Ellis-Pegler *et al.*, 1975; Albert *et al.*, 1978 and Ellis-Pegler, Higgs and Lambert, 1979). Analysis of our data showed that the ratio of anaerobes to aerobes in breast-fed babies exceeded that in formula-fed babies during the period before solid foods were given (approximately 10:1 and 1000:1 respectively). However, after solids were introduced, ratios in both groups were similar, averaging about 50:1 at 9 months. This change in the breast-fed group was due to the increase in counts of aerobes. By 12 months, ratios in both groups had risen to over 100:1.

The differences between the faecal bacterial flora of infants who are exclusively breast-fed and those receiving formula has long been a subject of great interest. It was originally considered that the "bifidus flora" of breast-fed infants directly inhibited the growth of coliforms in the large bowel. The present study, in agreement with those of other authors (Hewitt and Rigby, 1976; Mitsuoka and Kaneuchi, 1977), shows that this is not the case because high counts of bifidobacteria were found to co-exist for many months with high coliform counts in the large bowel of formula-fed infants. A more plausible mechanism of coliform suppression has been elucidated by Bullen and colleagues (Bullen and Tearle, 1976; Bullen, Tearle and Willis, 1976; Bullen, Tearle and Stewart, 1977) who demonstrated an acetate buffer *p*H 5–6 in stools of breast-fed infants that inhibits the growth of gram-negative facultative anaerobes *in vitro*. In addition, breast-milk factors such as lactoferrin, an iron-binding protein, and secretory IgA may also act to suppress the growth of coliforms in the infant gut (Bullen, Rogers and Leigh, 1972; Bullen, Rogers and Griffiths, 1974).

Interviews with mothers taking part in this survey revealed that exclusively breast-fed infants frequently suffered from constipation whereas formula-fed infants did not. This suggests that transit times may be shorter in the latter group and this may contribute to higher coliform levels. Albert *et al.* (1978) found, in infants suffering from diarrhoea, that a more rapid passage of intestinal contents is associated with increased numbers of faecal enterobacteria.

Colonisation by anaerobes other than bifidobacteria occurred only in the presence of large numbers of facultative anaerobes and one might postulate that these facilitate colonisation by the strict anaerobes by lowering the redox potential of the intestinal lumen. Certainly this phenomenon has been demonstrated experimentally in mice, in which the redox potential of gut contents is considerably higher in germ-free animals than in gnotobiotes colonised by a facultative anaerobe (Celesk, Asano and Wagner, 1976). A highly reduced environment is not so critical for the growth of bifidobacteria (Mitsuoka and Kaneuchi, 1977), isolates from the babies often showing limited growth in aerobic conditions.

The results obtained for the breast-fed infants in this survey are in broad agreement with those reported by Mata and Urrutia (1971). The Guatemalan study, however, described a more gradual increase in enterobacteria throughout the first year, with no decrease around 12 months. This difference is probably due to the practice of prolonged breast-feeding and the insanitary environment in that society.

The low isolation rates for lactobacilli in the Sydney survey are similar to those reported for infants in Guatemala (Mata and Urrutia, 1971), and Britain (Ellis-Pegler *et al.*, 1975), but in contrast to a 75% isolation rate reported in Japan (Mitsuoka, Hayakawa and Kimura, 1975). This variation could be due to dietary differences between countries.

The influence of changes in the gastrointestinal flora on susceptibility to infection

Because the normal flora of the gastrointestinal tract is an integral part of the body's nonspecific defence mechanisms, changes in this ecosystem have important implications for the health of the infant. The observation that coliform counts are higher in young formula-fed infants than in exclusively breast-fed infants suggests that the large bowel of formula-fed babies would be more amenable to colonisation by

gram-negative enteric pathogens than that of their breast-fed counterparts. Several recent studies have shown that the incidence of gastro-intestinal infection is indeed lower in exclusively breast-fed infants. This difference, while more important in underdeveloped countries (James, 1972; Kanaaneh, 1972; Plank and Milanesi, 1973), has also been documented in industrialised societies (Wheatley, 1968; Larsen and Homer, 1978; France, Marmer and Steele, 1980).

Small numbers of coliforms were maintained in the large bowel of breast-fed infants only as long as breast milk was the sole source of nutrition. At weaning, the intestinal milieu is greatly disturbed with rapid changes in bacterial populations. This is in contrast to the homeostasis of the intestinal environment of the formula-fed infants, in whom aerobic populations have been established in large numbers for several months and would be better adapted to their ecological niche and therefore better able to compete against enteric pathogens than the more recently established aerobic organisms in the breast-fed babies. Thus it appears that when solid foods are added to the diet the breast-fed infant could be more susceptible to gastro-intestinal infection than the formula-fed infant. The perturbations occurring in the gut ecosystem of the breast-fed infant at weaning may be an important factor in the pathogenesis of weanling diarrhoea, a common disease of underdeveloped countries where a prolonged and inefficient weaning process is accompanied by malnutrition and intestinal infection (Gordon, 1971). It would be interesting to compare the incidence of intestinal infection occurring soon after solid foods are added to the diet in breast-fed infants with that in formula-fed infants in a westernised society.

An adult type of intestinal bacterial flora is not established in breast-fed or in formula-fed infants until at least 12 months of age. This has been confirmed in another study (Stark and Lee, 1982) on the volatile fatty-acid patterns of human faecal specimens. The acquisition of a range of anaerobic bacteria similar in type and number to those found in the adult presumably increases the infant's resistance to intestinal infection because the human gut anaerobes are considered to have a major role in colonisation resistance against enteric pathogens (van der Waaij, 1979).

The study of the gastrointestinal microflora of infants in pathological states

This investigation has demonstrated that the composition of the normal intestinal bacterial flora of infants depends on the diet at the time of sampling. Therefore it is very important when studying pathological states that are thought to be related to changes in the intestinal flora, such as neonatal necrotising enterocolitis and infantile diarrhoea, to compare results of faecal culture from sick infants with those from healthy infants receiving the same diet. We hope that the baseline data provided in this paper will be useful in such studies.

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