On the Intestinal Flora of Bottle-Fed Infant

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In order to obtain current information about the intestinal flora of bottle-fed infants, feces from 30 breast-fed infants and 43 bottle-fed infants, including 3 infants of low birth weights, were examined by the method of Mitsuoka. The frequency of occurrence and total number of bifidobacteria in bottle-fed infants were nearly equal to those of breast-fed infants. The number of enterobacteriaceae, streptococci, and anaerobes other than bifidobacteria were significantly higher in bottle-fed infants than in breast-fed infants. As for *Bifidobacterium* species, there was little difference between bottle-fed and breast-fed infants in average counts of all the species detected, but the frequency of occurrence of *Bifidobacterium bifidum* in bottle-fed infants was less than in breast-fed infants. The appearance of bifidobacteria in low birth weight bottle-fed infants was more delayed than that in normal bottle-fed infants, and their species composition was also simple, consisting of two species of bifidobacteria: *B. breve* plus *B. bifidum*, or *B. breve* plus *B. adolescentis*.

Key words: *Bifidobacterium*; bottle-fed infant; breast-fed infant; infant nutrition; intestinal flora

The comparison of intestinal flora of bottle-fed infants with that of breast-fed infants was first carried out by Tissier (12) in 1900, and has been repeated by various investigators (1-5, 7, 10, 11). Most of them reported coliform bacilli, enterococci, and lactobacilli as the predominant organisms of the intestinal flora in bottle-fed infants, whereas bifidobacteria were prevalent in breast-fed infants. The results, however, cannot be applied to the current bottle-fed infants that are fed with improved formulas. The results also had been obtained with study methods which are inadequate by the current standards. Thus, it is desirable to re-examine the intestinal flora of current bottle-fed infants by the advanced study methods. There are only few reports regarding the intestinal flora of low birth weight infants (6, 8), and their flora also

needs further investigations.

In this study, we examined the intestinal floras of bottle-fed and breast-fed infants, and the transition of intestinal flora along with the growth of bottle-fed infants of low birth weights.

1. Materials and Methods

Infants: Table 1 shows the life history of infants that were used in this study. Two of the infants, A₁ and A₂, were dizygotic twins of 38 weeks gestation. Their birth weights (2,560 and 2,680 g) were much less than the normal weight (2,800-3,000 g) for a gestation period of 38 weeks. We, therefore, used the infants, A₁ and A₂, to see the effects of the low birth weight.

Methods: Intestinal organisms were isolated from the feces of infants by the method

Table 1. Life history of infants

	Bottle-fed infants	Low birth weight infants			Breast-fed infants
		A ₁	A ₂	B	
Age of infants (days)	3-134	3-	3-	3-	33-135
Number of specimens	40	1	1	1	30
Gestation period (weeks)	—	38	38	38	—
Birth weight (g)	3,188±361	2,560	2,680	2,360	3,208±348
Initial feeding	3-Bottle	3-Bottle	3-Bottle	3-Bottle	Breast
	Clinic	Clinic	Clinic	Clinic	
Place of infants	Infant home	↓	↓	↓	
	Home	Home	Home	Home	Home
Period in incubator (days)	—	1	1	16	—
Staying period in clinic (days)	—	7	7	26	—
Weaning age (days)	/	153	153	144	/

Table 2. Comparison of fecal flora between bottle-fed infants and breast-fed infants

Organisms	Bottle-fed infants	Breast-fed infants
Enterobacteriaceae	9.5±0.5 ^a (100) ^b	8.6±1.3** (100)
Streptococci	9.8±0.6 (100)	7.9±1.5** (100)
Staphylococci	5.5±1.7 (100)	5.8±1.9 (97)
Lactobacilli	7.2±1.5 (78)	6.8±1.7 (80)
Bifidobacteria	10.0±2.2 (97)	10.7±0.9 (100)
Eubacteria	7.3±4.1 (78)	3.1±4.6** (33)**
Bacteroidaceae	9.9±1.1 (100)	6.1±4.3** (67)**
Peptococcaceae	7.9±4.0 (80)	2.4±4.1** (27)**
<i>Clostridium perfringens</i>	6.4±2.5 (50)	1.0±2.3** (17)*
Clostridia-other	0.9±2.6 (10)	1.3±3.2 (13)
Veillonellae	5.9±4.6 (63)	5.8±3.4 (83)

^a Mean±SD of log organism counts. ^b Frequency of occurrence (%).Significant difference: ***p*<0.01; **p*<0.05.Table 3. Comparison of *Bifidobacterium* species between bottle-fed infants and breast-fed infants

<i>Bifidobacterium</i> species	Bottle-fed infants	Breast-fed infants
<i>B. bifidum</i>	9.3±1.0 ^a (23) ^b	9.9±0.6 (70)
<i>B. infantis</i>	10.2±0.2 (8)	10.6±0.4 (7)
<i>B. breve</i>	10.2±0.7 (93)	10.7±0.7 (90)
<i>B. adolescentis</i>	9.8±0.9 (53)	9.6±0.6 (40)
<i>B. longum</i>	9.5±0.9 (45)	9.6±0.8 (67)

^a Mean±SD of log bacterial counts. ^b Frequency of occurrence (%).

of Mitsuoka (9). Identification of genus of bacteria, and species of bifidobacteria were also done according to Mitsuoka (9).

Statistical analyses on the number and isolation frequency of the microflora were made for the purpose of comparing the flora between experimental groups: The mean difference of bacterial numbers was analyzed by the Student's *t* test; The frequencies of occurrence were compared by the χ^2 test.

2. Results and Discussion

Difference in intestinal flora between bottle-fed and breast-fed infants: The number and isolation frequency of the intestinal floral microorganisms of bottle-fed and breast-fed infants are shown in Table 2. The difference in the frequency of occurrence and total number of bifidobacteria between bottle-fed and

Fig. 1. Changes of fecal flora according to age of bottle-fed infants.
 —○—: Enterobacteriaceae, —●—: bifidobacteria, —△—: staphylococci, ---●---: lactobacilli, ---×---: bacteroidaceae, ---□---: *C. perfringens*.

Fig. 2. Changes of fecal flora according to age of low birth weight bottle-fed infant A₁.
 —○—: Enterobacteriaceae, —●—: bifidobacteria, ---●---: streptococci, —△—: staphylococci, ---●---: lactobacilli, ---×---: bacteroidaceae, —□---: *C. perfringens*.

breast-fed infants were not significant. The principal difference between bottle-fed and breast-fed infants was that the numbers of enterobacteriaceae, streptococci, and anaerobes other than bifidobacteria in bottle-fed infants were significantly greater than those in breast-fed infants. These results agree with those reported by Haenel et al. (5). Table 3 shows the difference in *Bifidobacterium* species

composition of intestinal flora between bottle-fed and breast-fed infants. The difference in number of each species between bottle-fed and breast-fed infants was not significant, but the frequency of occurrence of *B. bifidum* for bottle-fed infants were less than that for breast-fed infants. Such a high number of bifidobacteria in the recent bottle-fed infants may reflect the improvement of formula

Fig. 3. Changes of fecal flora according to age of low birth weight bottle-fed infant A₂.
 —○—: Enterobacteriaceae, —●—: bifidobacteria, ---●---: streptococci, —△—: staphylococci,
 ---●---: lactobacilli, ---×---: bacteroidaceae, —□---: *C. perfringens*.

Fig. 4. Changes of fecal flora according to age of low birth weight bottle-fed infant B.
 —○—: Enterobacteriaceae, —●—: bifidobacteria, ---●---: streptococci, —△—: staphylococci,
 ---×---: bacteroidaceae, —□---: *C. perfringens*.

component, i.e., the enhancement of protein digestability, decrease in protein and salt contents, and increase in lactose content.

Intestinal flora of low birth weight bottle-fed infants: In Fig. 1, the counts of fecal organisms of 40 bottle-fed infants are plotted against their ages. The time-courses of the changes of fecal organisms of bottle fed low birth weight infants, A₁, A₂, and B, are shown

in Figs. 2, 3, and 4, respectively. Bifidobacteria in normal bottle-fed infants gradually increased and they outnumbered Enterobacteriaceae in 10 days. On the other hand, it took a further 10–20 days for bifidobacteria in the low birth weight bottle-fed infants to dominate.

Figure 5 shows the time-course of the changes in *Bifidobacterium* species of normal

Fig. 5. Changes of *Bifidobacterium* species according to age of bottle-fed infants.
 —●—: *B. breve*, —○—: *B. bifidum*, —×—: *B. adolescentis*, ---△---: *B. longum*.

Fig. 6. Changes of *Bifidobacterium* species according to age of low birth weight bottle-fed infant A₁.

—●—: *B. breve*, —○—: *B. bifidum*.

bottle-fed infants. Those for low birth weight bottle-fed infants, A₁, A₂, and B, are also shown in Figs. 6, 7, and 8.

As is evident from Figs. 6, 7, and 8, the species composition of bifidobacteria of low birth weight bottle-fed infants was simpler than that of normal bottle-fed infants, consisting of only two species. *Bifidobacterium breve* was detected at first, followed by an appearance of either *B. bifidum* or *B. adole-*

scensis. The delayed appearance of bifidobacteria might reflect the immaturity of the digestive organs of low birth weight infants.

References

- (1) Adam, A. 1921. Über Darmbakterien. III Über den Einfluß der H-Ionenkonzentration des Nährbodens auf die Entwicklung des *Bacillus bifidus*. Zeit. Kinderheilk. 29: 306-320.

Fig. 7. Changes of *Bifidobacterium* species according to age of low birth weight bottle-fed infant A₂.

—●—: *B. breve*, —○—: *B. bifidum*.

Fig. 8. Changes of *Bifidobacterium* species according to age of low birth weight bottle-fed infant B.

—●—: *B. breve*, —x—: *B. adolescentis*.

- (2) Asami, K. 1960. Studies of artificial mothermilk. *Acta Paed. Jpn.* **64**: 562-566.
- (3) Boverter, K. 1938. Untersuchungen über das *Bacterium bifidum*. *Zbl. Bakt. I. Abt. Orig.* **142**: 419-430.
- (4) Distaso, A. 1911. Sur les microbes acido-tolérants de la flore intestinale. *Zbl. Bakt. I. Abt. Orig.* **59**: 48-63.
- (5) Haenel, H., W. Müller-Beuthow, und F.K.

- Grütte. 1970. Zur fäkalen Mikroökologie des Säuglings in Abhängigkeit von der Ernährung: Zusammensetzung der Mikroflora sowie Vorkommen der *Lactobacillus bifidus*-Typen. *Zbl. Bakt. I. Abt. Orig.* **215**: 333-347.
- (6) Imura, S. 1981. Fecal nature and intestinal flora in the low birth weight bottle-fed infant. *Jpn. J. Paed.* **34**: 2295-2303.
- (7) Isoda, S. 1966. Studies on the intestinal flora of

- healthy infant. *Acta Paed. Jpn.* **70**: 110-116.
- (8) Leuterer, W. 1963. Das Verhalten der Bifidumflora und des Stuhl-pH von Frühgeborenen bei Ernährung mit Frauenmilch, adaptierter Milch und Verdünnter Kuhmilch. *Ann. Paediatr.* **200**: 7-19.
 - (9) Mitsuoka, T., K. Ohno, Y. Benno, K. Suzuki, und K. Namba. 1976. Die Faekalflora bei Menschen. IV. Mitteilung: Vergleich des neu entwickelten Verfahrens mit dem bisherigen üblichen Verfahren zur Darmfloraanalyse. *Zbl. Bakt. Hyg., I. Orig. A* **234**: 219-233.
 - (10) Negishi, A. 1958. The study on the intestinal flora of healthy infants. *Acta Paed. Jpn.* **63**: 187-195.
 - (11) Olsen, E. 1949. Studies on the intestinal flora of infants. Munksgaard, Copenhagen.
 - (12) Tissier, H. 1900. *Recherches sur la flore intestinale des nourrisson*. Paris.