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Bifidobacterium, *Bacteroides*, and *Clostridium* spp. in Fecal Samples from Breast-Fed and Bottle-Fed Infants with and without Iron Supplement

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***Bifidobacterium*, *Bacteroides*, and *Clostridium* spp. isolated from the feces of 23 neonates during the first 3 months of life were identified. Of the 23 neonates, 10 were breast fed, 6 received an infant formula with iron supplement (5 mg/liter), and 7 received the formula without iron supplement (iron concentration, <0.5 mg/liter). The *Bifidobacterium* spp. most frequently isolated from the three groups of infants were *B. longum*, *B. breve*, *B. adolescentis*, and *B. bifidum*. The *Bacteroides* spp. most frequently isolated were *B. fragilis* and *B. vulgatus*. The most common *Clostridium* sp. in the three groups of infants was *C. perfringens*. The type of milk did not select for species of *Bifidobacterium*, *Bacteroides*, or *Clostridium*, except for *Clostridium butyricum*, which was isolated significantly more often from bottle-fed infants with iron supplement than from the other groups, and *Clostridium tertium*, which was more often isolated from breast-fed infants. The species of the three anaerobic genera did not persist for a long period of time in the three groups of infants.**

Colonization of the large bowel of the neonate starts immediately after delivery. Gram-negative aerobic bacteria together with gram-positive and -negative anaerobic bacteria can be recovered from fecal specimens within 3 days after birth (24, 25). The vaginal and fecal flora of the mother, and also the environment (air, food, etc.), are important sources of bacteria. The fecal flora of infants fed with breast milk differs from that of bottle-fed infants (6). Several factors are responsible for this difference in the spectrum of colonization, including the high lactose content and poor buffering capacity of human milk together with the presence of iron-binding proteins such as lactoferrin (4–6).

Although it is known that the anaerobic flora of breast-fed infants differs from that of bottle-fed infants, few data are available on the species of anaerobes inhabiting the gastrointestinal tract of these infants. Therefore, we extended our previous work on the effect of iron on the neonatal gut flora (16, 17). Because *Bifidobacterium* and *Bacteroides* spp. are the major components of the normal neonatal flora (2, 24, 25) and *Clostridium* spp. are important as human intestinal pathogens, particularly in infants (7), these anaerobes received special attention.

MATERIALS AND METHODS

Infants. Twenty-three neonates delivered by a midwife in Utrecht, The Netherlands, were enrolled in the study. Of these, 10 were breast fed (iron concentration, < 0.5 mg/liter), 6 were given an infant formula with iron supplement (concentration, 5 mg/liter; Almiron-M_2; Nutricia, Zoetermeer, The Netherlands), and 7 received the formula without iron supplement (concentration, <0.5 mg/liter). None of the infants was fed solid foods.

Collection and transport of specimens. Stool samples (about 1 g) were collected once weekly by the mother and transferred to 19 ml of a prereduced transport medium (27). The medium was then kept at 4°C at home until the samples were collected by laboratory workers. In this way, all samples could be analyzed within 24 h for viable counts of *Bifidobacterium*, *Bacteroides*, and *Clostridium* spp. This procedure has been found to give reliable and reproducible results (26). In the laboratory, the preweighed bottle was weighed again for determination of the exact weight of the fecal specimen the mother had collected. In this way, the number of bacteria per gram of feces could be calculated.

Isolation of anaerobic bacteria. After homogenization, 20-fold dilutions of the specimens were prepared in cysteine-peptone broth; 0.03-ml samples of appropriate dilutions were inoculated on suitable media by the method of Miles et al. (19). Reinforced clostridial agar (Oxoid CM 151; Pharmachemie, Haarlem, The Netherlands) supplemented with 0.5% glucose, 7.5% horse blood, and 0.03% China blue (ferri-ferrocyanide; Schmid Co., Stuttgart, Federal Republic of Germany) (RBC) was used for isolation of *Bifidobacterium* and *Bacteroides* spp. This medium does not inhibit growth of the anaerobes. *Bifidobacterium* spp. produce dark brown colonies, and *Bacteroides* spp. produce blue, translucent colonies (26). The limit of detection with this medium is 10^4 CFU/g of feces. *Clostridium* spp. were isolated on sulfite-polymyxin-milk agar containing 15 g of tryptone (Difco Laboratories, Detroit, Mich.), 10 g of yeast extract (E. Merck AG, Darmstadt, Federal Republic of Germany), 0.5 g of iron(III) citrate (Merck), and 18 g of Bacto-Agar (Difco) per 930 ml of distilled water; after sterilization and cooling of the medium to 56°C, 5 ml of filter-sterilized 5% Na_2SO_3, 10 ml of 0.1% colistin sulfate (Laboratoire Roger Bellon, Paris, France), 4 ml of 1% neutral red (Difco), and 50 ml of sterile whole cow milk were added (8). This selective medium is suitable for the isolation of many *Clostridium* spp. and inhibits the growth of *Bifidobacterium* and *Bacteroides* spp. The medium permits the isolation of *Clostridium* spp. when they are present in amounts as low as 2×10^3 CFU/g of feces. The media were incubated at 37°C in anaerobic jars containing 90% H_2, 5% CO_2, and 5% N_2 and read after 72 h. All media were prepared weekly and kept under nitrogen at 4°C until use. After incubation, each different colony of the representative genera from RBC and sulfite-polymyxin-milk

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TABLE 1. Number and incidence of organisms in feces during the first 12 weeks of life

Genus	No. (%) of viable organisms (log_{10}; mean ± SD)/g of feces for infants:		
	Breast fed (91)[a]	Bottle fed without iron supplement (73)	Bottle fed with iron supplement (65)
Bifidobacterium	9.8 ± 0.7 (96)	9.6 ± 0.7 (90)	9.5 ± 0.6 (71)[b]
Bacteroides	9.0 ± 0.7 (38)	9.3 ± 0.6 (53)	9.0 ± 0.7 (77)[b]
Clostridium	6.4 ± 1.6 (25)[c]	7.3 ± 1.5 (79)	7.1 ± 1.5 (88)

[a] Number of specimens.
[b] Difference was statistically significant ($P < 0.005$) between infants bottle fed with iron-supplement and the other two groups.
[c] Difference was statistically significant ($P < 0.005$) between breast-fed infants and the two bottle-fed groups.

agars was subcultured on 5% sheep blood agar (to check for aerobic growth) and on reinforced clostridial or sulfite-polymyxin-milk agar (to check for purity and anaerobic growth).

Identification of isolates. Isolates confirmed to be obligate anaerobes were identified based on their appearance in a Gram-stained preparation and on the detection of the end products of glucose metabolism by gas-liquid chromatography. They were assigned to species on the basis of their biochemical reactions.

Gas-liquid chromatography. Cultures (7 day) were centrifuged at 3,000 × *g* for 15 min. The supernatants were used for quantitative determination of short-chain fatty acids and stored at −20°C if necessary. The volatile fatty acids (VFA), acetic acid, propionic acid, isobutyric acid, and butyric acid, and the nonvolatile lactic acid were determined, after deionization, by the method of Lindner and Marcelis (14). The nonvolatile fatty acids (non-VFA) lactic acid and succinic acid were determined after esterification with ethanol (modified esterification method of Holdeman et al. [11]). Analyses were performed on a dual glass-column Packard Becker gas chromatograph (type 417) with a flame ionization detector and a 1.8-m glass column packed with Chromosorb 101 (80/100 mesh; Johns-Manville Products Corp., Denver, Col.). The operating temperature was 210°C for the VFA and 210 to 250°C for the non-VFA. Nitrogen (40 ml/min) was used as the carrier gas. A 2-μl portion of the sample for analysis was injected into the column.

Biochemical tests. Fermentation reactions were performed in sterile tubes containing 5 ml of basal medium, based on the procedures outlined by Holdeman et al. (11). The basal medium was the Viande Levure medium of Beerens and Fievez (1) supplemented with hemin 2 mg/liter), with a final pH of 6.9. The cultures were incubated anaerobically at 37°C for 7 days. The final pH of the reaction mixtures was estimated by the addition of 2 drops of bromocresol purple indicator and measured with a combined Microelektrode (no. 40 104023316; Ingold GmBH, Udorf, Switzerland) together with a pH meter. The biochemical tests for production of indole, gelatin liquefaction, hydrolysis of esculin, and production of lecithinase were performed as described by Holdeman et al. (11). *Bifidobacterium* spp. were identified by their ability to ferment amygdalin, arabinose, cellobiose, glucose, inositol, lactose, mannitol, mannose, starch, sucrose, trehalose, and xylose and by the biochemical test for the hydrolysis of esculin and starch. *Bacteroides* spp. were identified by fermentation tests with arabinose, fructose, glucose, lactose, maltose, mannitol, mannose, rhamnose, sucrose, trehalose, and xylose and by the production of indole. *Clostridium* spp. were identified by their ability to ferment fructose, glucose, lactose, maltose, mannitol, mannose, starch, and sucrose, produce indole, gelatinase, and lecithinase, and hydrolyze esculin and starch.

Statistical analysis. The frequency of isolation of the species of the different anaerobic genera and the percentage of specimens yielding one, two, or more species were determined. Statistical significance among the different groups of children was tested by the chi-square test (significance level, 1%).

RESULTS

The incidence of the various anaerobes isolated from fecal samples from the three groups of infants are presented in Table 1. The isolation frequency of *Bifidobacterium* and *Bacteroides* spp. from the breast-fed infants and infants bottle fed without iron supplement was higher and lower, respectively, than from infants bottle fed with iron supplement ($P < 0.005$). The isolation frequency of *Clostridium* spp. was lower from breast-fed infants than from either group of bottle-fed infants ($P < 0.005$).

***Bifidobacterium* spp.** A total of 550 strains of bifidobacteria were isolated from the feces of the infants: 241 were from breast-fed infants, 185 were from infants bottle fed without iron supplement, and 124 were from infants bottle fed with iron supplement. All strains could be assigned to species. The *Bifidobacterium* spp. identified from the fecal specimens and their frequency of isolation during the first 3 months of life are shown in Fig. 1. *B. longum*, *B. breve*, *B. adolescentis*, and *B. bifidum* were the species most frequently identified in fecal specimens from all three groups of infants, but the differences among the three groups of infants were not statistically significant. The other *Bifidobacterium* spp. included *B. pseudolongum*, *B. asteroides*, *B. cornutum*, and *B. magnum*; they were isolated infrequently from the three groups of infants. Persistence of a species during the entire 3-month period occurred in two breast-fed infants (*B. breve* in one infant and *B. adolescentis* in the other) and in one infant bottle fed without iron supplement (*B. longum*).

***Bacteroides* spp.** A total of 220 *Bacteroides* strains were isolated from the fecal samples of the infants: 63 were from breast-fed infants, 64 were from infants bottle fed without iron supplement, and 93 were from infants bottle fed with iron supplement. Five isolates (8%) from the breast-fed infants and two (2%) from the infants bottle fed with iron supplement could not be assigned to species. The *Bacteroides* spp. identified from the fecal specimens and their frequency of isolation during the first 3 months of life are shown in Fig. 2. *B. fragilis* and *B. vulgatus* were the species most frequently identified in fecal specimens from all three groups of infants. The differences in the frequency of isolation of the species among the three groups were not statistically significant. The other *Bacteroides* spp. included *B. eggerthii*, *B. thetaiotaomicron*, *B. hypermegas*, *B. disiens*, *B. capillosus*, *B. bivius*, and *B. distasonis*, but they were isolated so infrequently that no comment can be made as to their distribution. Persistence of a species occurred in two breast-fed infants (*B. vulgatus*), in two infants bottle fed without iron supplement (*B. fragilis* in one infant and *B. vulgatus* in the other), and in one infant bottle fed with iron supplement (*B. vulgatus*).

***Clostridium* spp.** A total of 274 *Clostridium* strains were isolated from the feces of the infants: 43 were from breast-fed infants, 106 were from infants bottle fed without iron supplement, and 125 were from infants bottle fed with iron

supplement. The *Clostridium* spp. identified from the fecal specimens and their frequency of isolation during the first 3 months of life are shown in Fig. 3. *C. perfringens* was the species most frequently isolated from the fecal specimens of all three groups of infants. *C. butyricum* was isolated significantly more often from infants bottle fed with iron supplement than from those bottle fed without iron supplement ($P < 0.005$). *C. tertium* was isolated significantly more often from breast-fed infants than from either group of bottle-fed infants ($P < 0.01$). The other *Clostridium* spp. included *C. nexile*, *C. tyrobutyricum*, *C. clostridiiforme*, *C. novyi*, *C. ramosum*, *C. barati*, *C. cadaveris*, *C. sphenoides*, *C. septicum*, *C. beijerinckii*, *C. felsineum*, and *C. glycolicum*; they were isolated mainly from both groups of bottle-fed infants. Persistence of a species occurred in six breast-fed infants (four with *C. perfringens*, one with *C. tertium*, and one with *C. butyricum*), in one infant bottle fed without iron supplement (*C. perfringens*), and in one infant bottle fed with iron supplement (*C. butyricum*).

Number of species per specimen. Two or three *Bifidobacterium* spp. per specimen were more often found in specimens from breast-fed infants than in specimens from either group of bottle-fed infants. The mean number of *Bifidobacterium* spp. per specimen for breast-fed infants was 2.9, for infants bottle fed without iron supplement it was 1.4, and for infants bottle fed with iron supplement it was 2.4 (difference not significant). Two or more *Bacteroides* spp. per specimen were more often found in specimens from infants bottle fed with iron supplement than in specimens from either of the other groups. The mean number of *Bacteroides* spp. per specimen for breast-fed infants and infants bottle fed without iron supplement was 1.7, and for infants bottle fed with iron supplement it was 2.0 (difference not significant). One *Clostridium* sp. per specimen was more often found in specimens from breast-fed infants than in specimens from either group of bottle-fed infants. The mean number of *Clostridium* spp. per specimen for breast-fed infants was 1.6, and for infants bottle fed with or without iron supplement it was 1.9 (difference not significant).

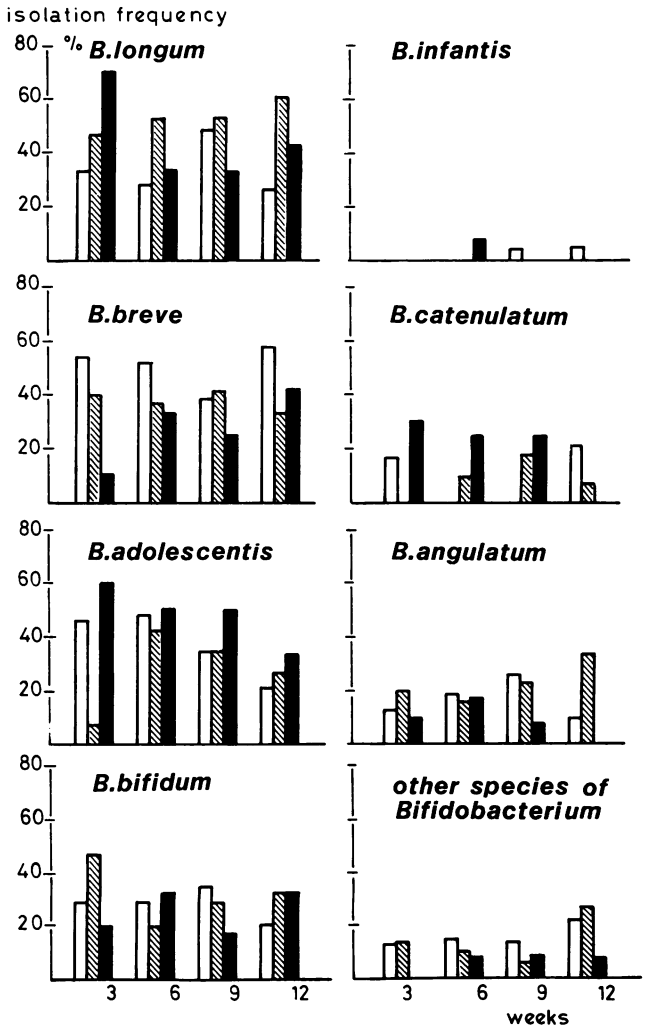

FIG. 1. Isolation frequency of *Bifidobacterium* spp. from fecal samples of breast-fed infants (□), infants bottle fed without iron supplement (▨), and infants bottle fed with iron supplement (■) during the first 12 weeks of life.

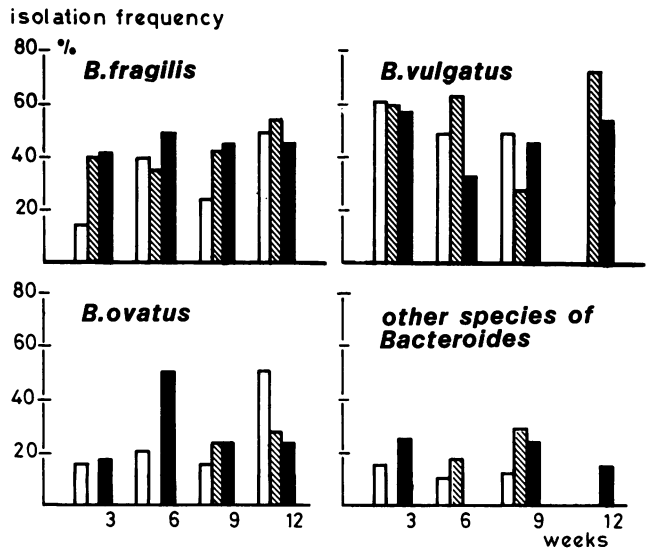

FIG. 2. Isolation frequency of *Bacteroides* spp. from fecal samples of breast-fed infants (□), infants bottle fed without iron supplement (▨), and infants bottle fed with iron supplement (■) during the first 12 weeks of life.

DISCUSSION

Anaerobic bacteriological studies were performed by conventional bench handling combined with carefully controlled anaerobic techniques, using fresh reduced media. Other workers have shown that these methods are adequate for quantitative recovery of anaerobic bacteria from human feces and give results comparable to those obtained with an anaerobic cabinet (28).

Previously we showed that colonization of the infant bowel by *Bifidobacterium* spp. was influenced by diet; the organisms were isolated from breast-fed infants and infants bottle fed without iron supplement more frequently than from infants bottle fed with iron supplement (17). However, in the present study we showed that the same *Bifidobacterium* spp. were isolated from breast-fed infants and infants bottle fed with or without iron supplement. The predominant *Bifidobacterium* spp. isolated from the neonates were *B. breve*, *B. adolescentis*, and *B. longum*. Our results are in agreement with those of Mitsuoka et al. (20) and Reuter (22). Some workers (10, 21) have shown that the predominant species in breast-fed infants is *B. infantis*. However, we

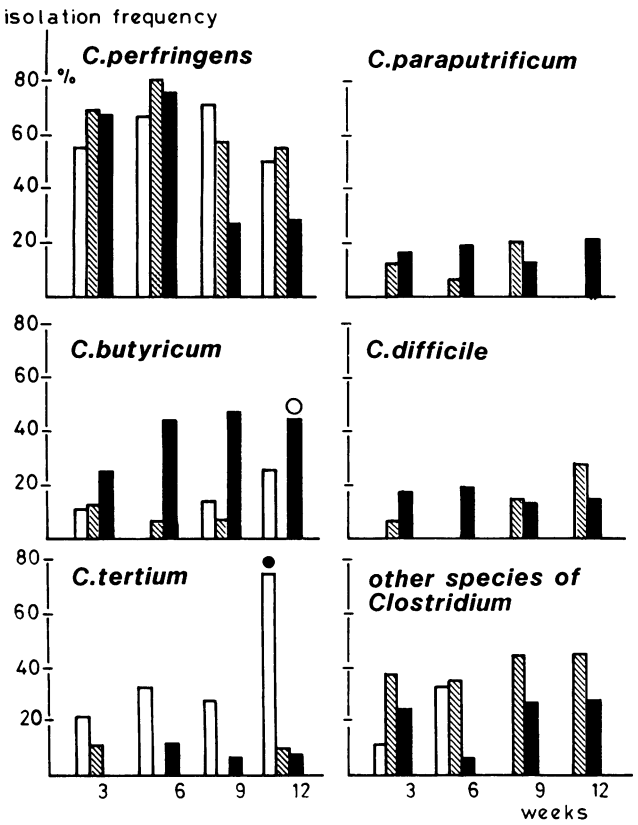

FIG. 3. Isolation frequency of *Clostridium* spp. from fecal samples of breast-fed infants (□), infants bottle fed without iron supplement (▨), and infants bottle fed with iron supplement (■) during the first 12 weeks of life. Symbols: ○, $P < 0.005$ between bottle-fed infants with and without iron supplement; ●, $P < 0.005$ between breast-fed infants and both groups of bottle-fed infants.

isolated this species only infrequently. Similar results were found by other investigators (20, 22, 25). Also, the isolation rate for *B. infantis* from bottle-fed infants was much lower than in other investigations (20, 25). This may have been due to testing infants from a different geographical region. As did Mitsuoka et al. (20), we found that most specimens from breast-fed and bottle-fed infants had one or two *Bifidobacterium* spp. per specimen. Colonization of the large bowel by *Bacteroides* spp. was significantly higher in infants bottle fed with iron supplement than in breast-fed infants and infants bottle fed without iron supplement. However, the feeding pattern did not influence the species of *Bacteroides* found in the bowel. The predominant *Bacteroides* spp. in the neonates were *B. vulgatus* and *B. fragilis*. *B. fragilis* and *B. vulgatus* belong to the *B. fragilis* group that has been recognized generally as the predominant group of *Bacteroides* species in both human adult and neonatal fecal flora (9, 15, 23). The high incidence of *B. vulgatus* in the neonatal fecal flora is in agreement with the findings of Rotimi and Duerden (23). Most specimens from breast-fed infants and infants bottle fed without iron supplement had only one *Bacteroides* sp. per specimen, indicating a relatively simple flora. Colonization of the large intestine by *Clostridium* spp. was much higher in infants bottle fed with or without iron supplement than in breast-fed infants. The present study suggests that both the feeding pattern and iron content of the food influences the isolation rate of some *Clostridium* spp. from the large intestine of the infant. *C. tertium* was more often isolated from breast-fed infants than from either group of bottle-fed infants, and *C. butyricum* was more frequently isolated from infants bottle fed with iron supplement than from breast-fed infants or infants bottle fed without iron supplement. Enhancement of bacterial growth by iron has been recognized for some *Clostridium* spp. (3, 18). *C. difficile* and *C. paraputrificum* were not isolated from breast-fed infants but were isolated from the stools of healthy bottle-fed infants. *C. butyricum*, *C. paraputrificum*, *C. perfringens*, and the toxin of *C. difficile* have been implicated in the pathogenesis of necrotizing enteritis (13). It is unclear whether these organisms are primary pathogens or secondary invaders of an otherwise damaged intestinal mucosa. Since we found, as have other investigators (12), that *C. perfringens* was the most common species to colonize the healthy infant gut in the neonatal period, it is unlikely that *C. perfringens*, *C. butyricum*, *C. paraputrificum*, and *C. difficile* are primary pathogens in necrotizing enteritis, and thus they must be considered part of the normal neonatal gut flora. However, it can be postulated that bottle-fed infants, especially infants bottle fed with iron supplement, are at a greater risk for developing necrotizing enteritis caused by *C. butyricum*, *C. difficile*, and *C. paraputrificum* than are breast-fed infants in cases of a damaged intestinal mucosa. Although infant botulism can be a rather common disorder between 3 and 20 weeks of age, *C. botulinum* was not isolated from any infants in this study.

This investigation showed that bacterial species of different anaerobic genera did not persist in the intestine over a long period of time. This may have been due to continuous exposure of the species to food, whereby different anaerobic species gained entrance to the infant gastrointestinal tract. However, it is also possible that a number of different species may have been continuously present in the bowel, but their relative concentrations may have fluctuated.

LITERATURE CITED

1. **Beerens, H., and L. Fievez.** 1971. Isolation of Bacteroides fragilis and Sphaerophorus fusiformes groups. Ann. Inst. Pasteur **121**:109-113.
2. **Beerens, H., C. Romond, and C. Neut.** 1980. Influence of breast-feeding on the bifid flora of the newborn intestine. Am. J. Clin. Nutr. **33**:2434–2439.
3. **Bullen, J. J., G. H. Cushnie, and H. J. Rogers.** 1967. The abolition of the protective effect of *Clostridium welchii* type A antiserum by ferric iron. J. Immunol. **12**:303–312.
4. **Bullen, J. J., H. J. Rogers, and L. Leigh.** 1972. Iron-binding proteins in milk and resistance to *Escherichia coli* infection in infants. Br. Med. J. **1**:69–75.
5. **Bullen, C. L., and P. V. Tearle.** 1976. Bifidobacterium in the intestinal tract of infants: an in-vitro study. J. Med. Microbiol. **9**:335–344.
6. **Bullen, C. L., and A. T. Willis.** 1971. Resistance of the breast-fed infant to gastroenteritis. Br. Med. J. **3**:338–343.
7. **Cashore, W. J., G. Peter, M. Laverman, B. S. Stonestreet, and W. Oh.** 1981. Clostridium colonization and clostridial toxin in neonatal necrotizing enterocolitis. J. Pediatr. **98**:308–311.
8. **De Vos, N., E. Mevissen-Verhage, W. Harmsen-van Amerongen, and J. Marcelis.** 1982. A new selective medium for the culture of *Clostridium* from human feces. Eur. J. Clin. Microbiol. **1**:267–271.
9. **Duerden, B. I.** 1980. The isolation and identification of *Bacteroides* spp. from the normal human fecal flora. J. Med. Microbiol. **13**:69–78.
10. **Haenel, H., W. Müller-Beuthow, and F. K. Grütte.** 1970. Zur fäkalen Mikroökologie des Säuglings in Abhängigkeit von der Ernährung: Zusammensetzung der Mikroflora sowie Vorkommen der *Lactobacillus bifidus*-Typen. Zentralbl. Bakteriol.

Mikrobiol. Hyg. 1 Abt. Orig. A **215**:333–347.
11. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
12. **Kindley, A. D., P. J. Roberts, and W. H. Tullock.** 1977. Neonatal necrotising enterocolitis. Lancet **i**:649.
13. **Koshoske, A. M.** 1979. Necrotizing enterocolitis in the neonate. Surg. Gynecol. Obstet. **148**:259–269.
14. **Lindner, J. G. E. M., and J. H. Marcelis.** 1978. Quantitative gas chromatography of *Bacteroides* species under different growth conditions. Antonie van Leeuwenhoek J. Microbiol. Serol. **44**: 1–14.
15. **Long, S. S., and R. M. Swenson.** 1977. Development of anaerobic fecal flora in healthy newborn infants. J. Pediatr. **91**:298–301.
16. **Mevissen-Verhage, E. A. E., J. H. Marcelis, W. C. M. Harmsen-van Amerongen, N. M. De Vos, J. Berkel, and J. Verhoef.** 1985. Effect of iron on neonatal gut flora during the first week of life. Eur. J. Clin. Microbiol. **4**:14–18.
17. **Mevissen-Verhage, E. A. E., J. H. Marcelis, W. C. M. Harmsen-van Amerongen, N. M. De Vos, and J. Verhoef.** 1985. Effect of iron on neonatal gut flora during the first three months of life. Eur. J. Clin. Microbiol. **4**:273–278.
18. **Miles, A. A., P. L. Khimji, and J. Mastkell.** 1979. The variable response of bacteria to excess ferric iron in host tissues. J. Med. Microbiol. **12**:17–28.
19. **Miles, A. A., S. S. Misra, and J. O. Irwin.** 1938. The estimation of bactericidal power of the blood. J. Hyg. **38**:732–750.
20. **Mitsuoka, T., K. Hayakawa, and N. Kimura.** 1974. The fecal flora of man. II. Communication: the composition of bifidobacterium flora of different age groups. Zentralbl. Bakteriol. Mikrobiol. Hyg. 1 Abt. Orig. A **226**:469–478.
21. **Petuely, F., and G. Lindner.** 1960. Kritische Untersuchung über die Darmflora. III. Mitteilung: Bewertung quantitativer Züchtungsmethoden. Die Darmflora des Brustkindes. Zentralbl. Bakteriol. Mikrobiol. Hyg. 1 Abt. Orig. A **195**:347–484.
22. **Reuter, G.** 1963. Vergleichende Untersuchungen über die Bifidus-Flora im Säuglings- und Erwachsenensthul. Zentralbl. Bakteriol. Mikrobiol. Hyg. 1 Abt. Orig. **191**:486–507.
23. **Rotimi, V. O., and B. I. Duerden.** 1981. *Bacteroides* species in the normal neonatal fecal flora. J. Hyg. **87**:299–304.
24. **Rotimi, V. O., and B. I. Duerden.** 1981. The development of the bacterial flora in normal neonates. J. Med. Microbiol. **14**:51–62.
25. **Stark, P. L., and A. Lee.** 1982. The microbial ecology of the large bowel of breast-fed and formula-fed infants during the first year of life. J. Med. Microbiol. **15**:189–203.
26. **van der Wiel-Korstanje, J. A. A., and K. C. Winkler.** 1970. Medium for differential count of the anaerobic flora in human feces. Appl. Microbiol. **20**:168–169.
27. **van der Wiel-Korstanje, J. A. A., and K. C. Winkler.** 1975. The fecal flora in ulcerative colitis. J. Med. Microbiol. **8**:491–501.
28. **Watt, B., J. G. Collee, and R. Brown.** 1974. The isolation of strict anaerobes: the use of an anaerobic cabinet compared with a conventional procedure. J. Med. Microbiol. **7**:315–324.