STUDIES OF INFANT FEEDING XVI

A BACTERIOLOGIC STUDY OF THE FECES AND THE FOOD OF NORMAL BABIES RECEIVING BREAST MILK *

ELSIE W. BROWN AND ALFRED W. BOSWORTH

BOSTON

INTRODUCTION

Much has been said concerning the intestinal flora of infants, both breast fed and bottle fed, but owing to the intangibility of certain aspects of the subject, the field seems to be open to further investigation.

Description of the bacteriology of breast fed babies' stools which have been published heretofore are based chiefly on small groups, on very young infants in maternity wards, or on diseased babies. In the Research Laboratories of The Boston Floating Hospital, during the past year, we have had an unusual opportunity in connection with certain metabolism investigations for studying a fairly large group of normal breast fed babies of different ages.

The work here described has been undertaken as an independent research subject although no claims are made as to its originality. Its chief aim has been to obtain complete bacteriologic data as a parallel to metabolism work done simultaneously on the same babies.

With the idea of obtaining as complete data as possible concerning the source of intestinal bacteria, samples of the breast milk which the babies were receiving were examined bacteriologically also, in an effort to determine, if possible, what relationship there might be between the bacteria in breast milk as fed to normal babies and the fecal flora of the same babies.

During our investigation a "bifidus-like" bacillus was isolated from the breast milk and from the skin around the nipple. It is a pleomorphic slender bacillus exhibiting on artificial mediums, under various conditions, forked forms, clubbed forms, V and Y forms, crossed forms, coccal forms and straight or slightly curved forms generally in parallel arrangement. In young cultures and in rapidly growing cultures the organisms are usually gram-positive.

On one occasion definite spores were seen when stained by the Ziehl-Neelsen method. After heating cultures to 80 C. for ten minutes a feeble growth could usually be obtained but a longer exposure prevented growth.

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* From The Boston Floating Hospital Laboratories.

The bacillus was isolated only under aerobic conditions but in the second generation on artificial mediums it grew almost as well anaerobically. Culturally, it ferments, without gas, dextrose, saccharose, lactose, maltose, raffinose and mannite but grows very poorly in mannite medium. Milk is clotted very slightly.

Because of its typical morphology and its similarity in other respects to the descriptions of *B. bifidus* by preceding investigators, we do not hesitate to call this organism *B. bifidus* also. It differs from *B. bifidus* as isolated from nurslings' feces in one respect only, namely, its ability to grow well initially under aerobic conditions. One would expect that an organism which had existed on the skin would thrive in the presence of oxygen.

As the result of our investigations we believe that the *B. bifidus* which is present in or on the mother's nipple is an important source of the bifidus organisms found in the nursling's intestine. The reason for their predominance in the nursling's feces has been explained adequately by Kendall and preceding investigators. In their passage through the intestinal tract the bacilli have had to adapt themselves to increasingly anaerobic conditions so that as a fecal organism *B. bifidus* is a voluntary, though not necessarily a strict anerobe. Because of its sensitiveness to environmental changes it is difficult to cultivate artificially.

One of our reasons for believing that this organism which we have isolated from breast milk is a predecessor of the fecal *B. bifidus* is that after anaerobic cultivation in milk digested by intestinal ferments a straight slender rod, morphologically typical of *B. bifidus* as it appears in direct smears from the feces, resulted.

After going over the available literature we find that several other investigators have been able to cultivate *B. bifidus* aerobically. Noguchi [1] believes that this organism has an aerobic phase, and Howe [2] has been able to obtain good aerobic cultures of his organisms isolated from carious teeth. Torrey,[3] by using a special method, has also succeeded in aerobic cultivation.

We can find no definite statement in the literature as to the isolation of *B. bifidus* from breast milk. The majority of writers [4] are interested only in pathogenic cocci, although several mention finding occasionally a slender bacillus to which no further allusion is made.

Escherich [5] made cultures from women's milk but was apparently more interested in the numbers than in the types of bacteria present.

1. Noguchi: J. Exper. M. **12**:182, 1910.
2. Howe: J. M. Research, N. S. **31**:481, 1917.
3. Torrey: J. Bacteriol. **2**:435, 1917.
4. Koestlin: Arch. f. Gynäk. **3**:201, 1897.
5. Escherich: Fortschr. f. Med. **3**:231, 1885.

The following year, however,[6] he offered the supposition that lactic acid bacilli may be among the adventitious types entering the intestines before the first nursing or that they may develop on the nipples or in the milk that remains in the baby's mouth.

Moro [7] states that it is easy to obtain *B. acidophilus* from breast milk on beerwort agar. At that time he considered *B. acidophilus* the predominant organism in nurslings' stools, but later [8] he corrected this statement, charging himself with the neglect of anaerobic methods whereby he failed to isolate *B. bifidus* as the predominant organism. While he corrects his work on the fecal flora he does not speak here about the organism which he isolated from breast milk as *B. acidophilus.* However, from his later description of *B. bifidus,* which he considers a very polymorphic, strictly anaerobic bacillus, one may assume that he did not isolate *B. bifidus* as such from breast milk.

Noguchi [1] considers the breast of the mother one source of *B. bifidus* in the stools of breast fed babies. He thinks that in its aerobic phase the organism may inhabit the breast and skin of the mother and that the anaerobic phase also is met with in the nursling's feces as *B. bifidus communis* (Tissier). This is somewhat parallel to our belief but while his aerobic organism resembles a simple spore bearer, ours has all the morphologic characteristics of the fecal *B. bifidus.*

A STUDY OF THE FECAL FLORA

The babies selected were nurslings whose physical examinations rated them as normal infants. The bacteriologic investigations were made on sixty-five specimens from thirty-eight babies; one specimen was examined after admission to the ward and another, in the majority of cases, at the end of the metabolism period.

The thirty-eight babies ranged in age from 2 days to 6 months. Of these, twenty-eight had received only breast milk since birth and the other ten had had some cow's milk. All babies who had received cow's milk were placed on a strict breast milk diet after admission.

In spite of our efforts to use only breast fed babies, six orphans from St. Mary's Infant Asylum and four breast fed babies who had previously received some cow's milk, were included by mistake. However, the mistake was a happy one in that it offered several points of comparison.

The routine procedure consisted of the examination of direct smears and of aerobic and anaerobic cultures from the feces. The specimens were obtained by rectal tube and sent directly to the laboratory, where smears and cultures were made immediately.

6. Escherich: Die Darmbakterien des Säuglings, 1886.
7. Moro: Jahrb. f. Kinderh. **2**:38, 1900.
8. Moro: Jahrb. f. Kinderh. **11**:687, 1905.

About 1 gram of feces was emulsified in 10 c.c. of sterile physiologic solution of sodium chlorid and from this suspension the smears and cultures were made. For the study of aerobic organisms Endo plates or lactose litmus agar plates were used and colonies finished for identification by cultural reactions in carbohydrate and other mediums. For the anaerobic work dextrose or lactose agar slants were inoculated and incubated in vacuum bottles after Stitt's method with slight alterations.

Direct Smears.—Of the strictly breast fed babies, all but three showed either a practically pure growth of *B. bifidus* in the direct smears or a large predominance of this organism with only a few cocci and gram-negative bacilli. A few spore bearers were observed in almost all cases. The three exceptions were:

No. 35, whose smear showed a large predominance of gram-negative bacilli, was having loose stools at the time her second specimen was taken. Since *B. alkaligenes* was isolated in unusually large numbers by aerobic culture she presumably was having an intestinal upset. Her first specimen seven days before was typical of the other breast fed babies.

No. 43 had an unusually large number of sporebearers. This organism was typical of *B. aerogenes capsulatus* and did not appear in the aerobic cultures.

No. 57 had many gram-negative bacilli. She had been having enemas for several days before the specimen was taken which presumably eliminated these organisms more quickly than normally.

Of the ten babies who had received some cow's milk:

No. 38, after nine days on a strict breast milk diet showed a direct smear typical of a breast fed baby. He had been entirely breast fed for two weeks after birth and had received cow's milk alone for three or four days.

Nos. 39 and 40, who had received supplementary feedings of cow's milk since birth because their mothers had an inadequate supply, showed a high percentage of gram-negative bacilli after nine and sixteen days, respectively, on a strict breast milk diet.

No. 41 had received supplementary feedings of cow's milk in addition to the breast but a direct smear from her stool showed a practically pure growth of *B. bifidus* after sixteen days of straight breast milk.

No. 44 had received cow's milk just one day, but was only eight days old when a specimen of feces was examined. Her direct smear showed a fairly high percentage of cocci and gram-negative bacilli. Probably she was still at the transitional stage described by Kendall[9] which bridges the period of early adventitious bacterial infection of the intestinal tract to the period of the dominant breast milk flora.

No. 45 had received cow's milk for several days before admission and three weeks later still showed a high percentage of gram-negative bacilli.

No. 54 had received cow's milk alone for three or four days before admission. There was no history of the previous feeding. At the beginning of the fourth week the direct smear from this case still showed a high percentage of cocci and gram-negative bacilli in spite of a continuous breast milk diet.

9. Kendall: Bacteriology, p. 581.

No. 55 had received cow's milk alone for the first week after birth. After seven days of a strict breast milk diet his direct smear showed only gram-negative bacilli. Five days later *B. bifidus* began to predominate but the percentage of colon-like bacilli was still high.

No. 58 was an interesting case. She was only two days old when admitted and had received cow's milk for twenty-four hours. When the first specimen from this baby was examined the stool was still a meconium stool, and, as was to be expected, contained many cocci and gram-negative bacilli. Since she was started on a breast milk diet at the end of her second day this baby was practically on the same basis of feeding as a breast fed baby. The feces examined eight days later contained a flora typical of a breast fed baby, as indicated by the direct smear. Apparently the small amount of cow's milk ingested during a period of physiologic adjustment had no permanent effect.

No. 59 was two weeks old when admitted. There was no history of feeding prior to admission to St. Mary's Asylum but she received cow's milk alone for several days at that institution. The first specimen examined after four days on drawn breast milk showed a proportion of cocci and gram-negative bacilli larger than in the direct smears of the normal breast fed babies. A second and third specimen examined at intervals of eight days showed a continual reduction of these organisms, but it was not until the fourth week that a flora absolutely typical of a breast fed baby was established.

Anaerobic Cultures.—Unquestionably a large number of the bacteria present in the feces are dead but it is of interest to know whether the bifidus type survives the last period of intestinal existence in as high a percentage as the direct smears seem to indicate.

Therefore, the chief aim of this anaerobic study has been to find a culture method which would give an approximate estimate of the living bacterial types naturally present in the feces of normal nurslings.

Since *B. bifidus* is generally considered an obligate anaerobe and since the other types of bacteria ordinarily found in such feces, as *B. coli, B. lactis aerogenes, M. ovalis* and staphlyococci, are all facultative anaerobes and may grow well under anaerobic conditions it was logical to assume that proper anaerobic cultivation would offer an environment to all the above types as suitable as that part of the intestine where they had passed the last period of their metabolism.

After trying out various anaerobic methods, the most uniform results were obtained by altering slightly Stitt's vacuum bottle method. Dextrose and lactose agar slants were used instead of stab cultures except for isolation work. By omitting the layer of liquid petrolatum and leaving the plugs out of the tubes a high degree of anaerobiosis was insured.

This corroborates the work of Gates and Olitsky [10] and of Ivan Hall [11] who have concluded, after careful investigation, that the layer of oil is inefficient in procuring anaerobic conditions. We also agree

10. Gates and Olitsky: J. Exper. M. **33**:51 (Jan.) 1921.
11. Hall: J. Bacteriol. **6**:1 (Jan.) 1921.

TABLE 1.—STUDY OF THE FECAL FLORA OF INFANTS *

Baby	Admission Age	Feeding Before Admission	Feeding After Admission	Specimen	Days After Admission	Proportion of Bacterial Types in			Remarks
						Direct Smear	Anaerobic Culture	Aerobic Culture	
35	8 weeks	Breast	Breast	1	4	Bifidus Few colon-like	.....	B. coli Few staphylococci	
				2	11	Colon-like Few bifidus Rare coccus	.....	B. alkaligenes Few B. subtilis Rare diplococcus	Tendency to loose stools
36	2 months	Breast	Breast	1	9	Bifidus Few colon-like Rare coccus	.....	B. coli Few M. ovalis Few paratyphoid-like	
37	4 months	Breast	Breast	1	2	Bifidus Few cocci	.....	B. pyocyaneus Few B. coli	Specimen left in ward over night
				2	9	Bifidus Few colon-like Rare coccus	.....	B. coli Few M. ovalis	
38 St. M.	3 weeks	Breast; cow's milk 3-4 days	Drawn breast milk	1	9	Bifidus Few colon-like Few cocci	.....	B. coli Rare M. ovalis Rare B. pyocyaneus	Cow's milk formula made up with lactose
				2	16	Bifidus Few colon-like Few cocci	.....	B. coli Few pyocyaneus	
39	16 days	Breast; cow's milk supplementary	Breast; drawn breast milk	1	9	Bifidus Many colon-like	.....	B. coli Rare staphylococcus	Mother's milk inadequate
40	6½ weeks	Breast; cow's milk supplementary	Breast; drawn breast milk	1	16	Bifidus Many colon-like Few spore bearers Rare coccus	.....	B. coli	Mother's milk inadequate
41	6½ weeks	Breast; cow's milk supplementary	Breast; drawn breast milk	1	16	Bifidus	.....	B. coli	
42	5½ weeks	Breast	Breast	1	6	Bifidus	....	B. lactis aerogenes Many staphylococci	
43	3½ months	Breast	Breast	1	8	Bifidus Many spore bearers Few cocci Rare colon-like	.....	B. coli Few B. lactis aerogenes	No clinical evidence for prominent spore bearer
				2	8	Bifidus Spore bearer Few cocci	.....	B. coli Few M. ovalis Rare B. alkaligenes Rare B. megaltherium	

* Explanation of Table 1: Predominant organism stands first without modification. "Colon-like" by cultural tests are *B, coli* in most cases, *B. lactis aerogenes* in a few, and *B. alkaligenes* in one. Since the chief aim was to determine the proportion of types the group name rather than the name of the individual organisms has been noted more especially.

44	8 days	Breast; cow s milk	Drawn breast milk	1	Same day	Bifidus Moderate number of colon-like cocci	.....	B. coli B. lactis aerogenes Few staphylococci Few M. ovalis Few yeast	
				2	17	.....	.....	B. coli Few M. ovalis	
45	5 days	Breast; cow's milk	Drawn breast milk	1	21	Bifidus Many colon-like Few cocci	.....	B. coli	
48	4 weeks	Breast	Breast	1	2	Bifidus Few cocci Rare colon-like	.....	B. coli Rare B. subtilis Rare staphylococci	
				2	7	.....	.....	B. coli Few B. lactis aerogenes	
49	1 month	Breast	Breast; drawn breast milk	1	4	Bifidus	.....	B. coli	
				2	9	Bifidus Few colon-like Rare coccus	.....	Staphylococcus Many M. ovalis Few B. cloacae	Frequent loose stools for 5 days previous
50	6 months	Breast	Breast	1	5	Bifidus	.....	B. coli Few staphylococci	
				2	14	Bifidus Few spore bearers Few cocci Rare colon-like	.....	B. coli Rare staphylococci	
52	1 month	Breast	Breast	1	7	Bifidus	.....	B. coli Rare staphylococci	
54	1 week	Breast	Drawn	1	7	Bifidus Moderate number of colon-like cocci	.....	B. coli Few staphylococci	
				2	12	Bifidus Many colon-like Many cocci	.....	B. coli Many B. lactis aerogenes	
55	1 week	Cow's milk	Drawn breast milk	1	7	Colon-like	.....	B. lactis aerogenes Many yeast Many B. coli	No history of any breast feeding
				2	12	Bifidus Many colon-like	Bifidus Small number of M. ovalis	B. coli B. lactis aerogenes	

TABLE 1.—STUDY OF THE FECAL FLORA OF INFANTS.—*(Continued)*

Baby	Admission Age	Feeding Before Admission	Feeding After Admission	Specimen	Days After Admission	Proportion of Bacterial Types in			Remarks
						Direct Smear	Anaerobic Culture	Aerobic Culture	
57	6 weeks	Breast	Breast; drawn breast milk	1	8	Bifidus Many colon-like Rare coccus	.....	B. coli	Enemas for several days before taking specimen
58	2 days	Cow's milk (24 hours)	Drawn breast milk	1	2	Bifidus Many cocci Many colon-like	Colon-like Many staphylococci Occasional bifidus	B. coli	Still a meconium stool
				2	10	Bifidus Few colon-like	Colon-like Many bifidus	B. coli Few M. ovalis Few diplococci	
59 St. M.	2 weeks	? Cow's milk (2 days)	Drawn breast milk	1	4	Bifidus Many colon-like Many cocci	Bifidus Many colon-like Many cocci	B. coli Many diplococci	No history of feeding before admission to St. Mary's
				2	22	Bifidus Moderate number of colon-like Occasional coccus	Bifidus Colon-like M. ovalis	B. lactis aerogenes Few B. coli	
				3	30	Bifidus Small number of colon-like Few cocci	Colon-like Many M. ovalis Few bifidus	B. coli Few M. ovalis Few staphylococci	
				4	38	Bifidus Few colon-like	Bifidus Many colon-like Few yeasts Few cocci		
60	8 weeks	Breast	Breast	1	2	Bifidus Few colon-like Few cocci	Bifidus Few colon-like	B. coli	
61	1 month	Breast	Breast; drawn breast milk	1	1	Bifidus Small number colon-like Few cocci	.....	B. coli Many diplococci Moderate number B. aerogenes M. ovalis	Mother's milk inadequate
				2	9	Bifidus Few colon-like Few cocci	Bifidus Many colon-like	B. coli Rare staphylococci	
62	1 month	Breast	Breast; drawn breast milk	1	1	Bifidus	.....	Staphylococci only	Mother had follicular tonsillitis and caked breast
				2	19	Bifidus Rare colon-like Rare coccus	Bifidus Many colon-like	B. coli Rare staphylococci	Mother recovered

63	1 month	Breast	Breast	1	2	Bifidus	Bifidus Many colon-like Rare coccus	B. coli Occasional M. ovalis	
65	5 weeks	Breast	Breast	1	1	Bifidus Few cocci Rare colon-like	Bifidus Many colon-like Few cocci	B. coli Many M. ovalis	Mother had acute respiratory infection
				2	14	Bifidus	Bifidus Few staphylococci Few M. ovalis Rare colon-like	B. coli Many B. subtilis Few staphylococci	
66	16 days	Breast	Breast	1	5	Bifidus Many colon-like Few spore bearers Rare coccus	Bifidus Many colon-like Few cocci	B. coli Rare staphylococci	Five to seven loose stools daily
				2	12	Bifidus	Bifidus Small numbers colon-like cocci	B. coli Rare staphylococci	
67	5 weeks	Breast	Breast	1	2	Bifidus	Bifidus Moderate number colon-like	B. coli Many yeast Few dewdrop colonies not growing on Endo after fishing, perhaps diphtheria	
						Bifidus	Bifidus Many colon-like	B. coli Few staphylococci	Eight days after admission mother and baby had positive diphtheria cultures
68	2 months	Breast	Breast	1	Same day	Bifidus	.....	B. coli	
				2	7	Bifidus	Bifidus Many colon-like Few yeasts Few M. ovalis	B. coli Many M. ovalis Many M. ovalis	
69	2 weeks	Breast	Breast	1	Same day	Bifidus	.....	B. coli Few B. alkaligenes Few staphylococci	
				2	8	Bifidus	Bifidus Many colon-like Many cocci	B. coli Many staphylococci	
70	6 months	Breast	Breast	1	2	Bifidus	Bifidus	B. coli Many M. ovalis	
				2	12	Bifidus Rare colon-like			

TABLE 1.—Study of the Fecal Flora of Infants.—*(Continued)*

Baby	Admission Age	Feeding Before Admission	Feeding After Admission	Specimen	Days After Admission	Proportion of Bacterial Types in			Remarks
						Direct Smear	Anaerobic Culture	Aerobic Culture	
71	1 month	Breast	Breast	1	1	Bifidus	.....	B. coli Few M. ovalis	
				2	25	Bifidus	.....	B. coli Few M. ovalis	
72	6 weeks	Breast	Breast	1	Same day	Bifidus	Bifidus Many colon-like	B. coli Few M. ovalis Few staphylococci	
				2	8	Bifidus Few cocci	Bifidus Many cocci Few colon-like	B. coli Many staphylococci Many M. ovalis	
74	2 weeks	Breast	Breast	1	1	Bifidus Rare colon-like Rare cocci	Bifidus Many colon-like Few M. ovalis	B. coli Moderate number M. ovalis and staphylococci	
				2	8	Bifidus Rare colon-like			
75	18 days	Breast	Breast	1	1	Bifidus	Bifidus cocci Many colon-like	B. coli Many staphylococci Many yeast	
				2	12	Bifidus	Bifidus	B. coli Many yeast Few M. ovalis	
77	19 days	Breast	Breast	1	1	Bifidus	Bifidus Many colon-like	B. coli Few M. ovalis	
				2	8	Bifidus	Bifidus Few cocci		
79	14 days	Breast	Breast	1	1	Bifidus			
80	.....	Breast	Breast	1	1	Bifidus			
81	.....	Breast	Breast	1	1	Bifidus			

with Hall that the old Buchner method is more successful if the inoculated tubes are left unplugged.

The above investigators have had illuminating results with methylene blue as an indicator of the degree of anaerobiosis. The use of this is dependent on so many factors that it is rather complex for ordinary work. However, it is advantageous to have some way of determining the degree of anaerobiosis so in this work a culture of *B. pyocyaneus* was used which produced a large amount of pigment in the presence of free oxygen but did not produce any pigment under anaerobic conditions.

In this study the use of solid medium had an advantage over liquid mediums since the colonies could be seen growing and therefore definitely represented living bacteria.

Everyone who has tried to cultivate these fecal organisms has spoken of the fact that the bifidus type is quickly crowded out by the other bacteria, *M. ovalis* and a yeast seeming to be the chief offenders in this respect. While it is undoubtedly true that artificial mediums offer a chemical environment more favorable to these organisms, a careful study of the colonies in these fecal cultures seemed to indicate that the antagonism was due to physical as well as chemical factors. The bifidus colonies are so much smaller and slower growing than those of the other types that the latter soon use all the available space on the surface of the medium, incidentally choking out the former. In diluted cultures the bifidus organisms multiply very well so long as there is space in which to proliferate. They also grow better under these conditions in symbiosis with the other bacteria than when isolated.

As may be seen in Table 1, all except five of the twenty-eight specimens examined by anaerobic cultures showed a large predominance of the bifidus group, as did the direct smears from these specimens. The five exceptions were as follows:

No. 58, in which gram-negative bacilli were predominant, staphylococci prominent and *B. bifidus* scantily represented. The direct smear from the same specimen showed a slight predominance of *B. bifidus* but since this was a meconium stool presumably the bifidus type had not established itself strongly.

No. 58.2 was the same case eight days later, but here the bifidus organisms were present in large numbers even though the colon type was still predominant; showing the gradual transition to the dominance of *B. bifidus* after a continuous breast milk diet.

Nos. 59.2 and 59.3 showed a large number of cocci and gram-negative bacilli as compared with the bifidus type. This baby had been on a cow's milk diet and its direct smears did not present the picture typical of a normal breast milk stool.

No. 75.1. The direct smear from this specimen showed practically a pure growth of the bifidus type. In the anaerobic culture cocci rivalled the bifidus organisms strongly. This was a strictly breast fed baby but the mother had been having nipple trouble which may have been the cause of a coccus infection. There were also many staphylococci found in the aerobic cultures.

Aerobic Cultures.—Of fifty specimens examined by aerobic culture all but four showed a predominance of the *B. coli-B. aerogenes* types. The four exceptions were:

No. 35.2, which showed a predominance of *B. alkaligenes.* At the time this specimen was examined the child was having loose stools. The direct smear also showed a predominantly gram-negative flora.

No. 37.1. This specimen was left in the ward over night and when plated showed a predominance of *B. pyocyaneus.*

No. 49.2. This baby, whose culture showed a predominance of staphylococci, had been having from five to seven loose stools for five days previous to the examination of this specimen. The increased peristalsis probably let free a larger number of living staphylococci than normally.

No. 62.1. In this specimen staphylococci only were found on the aerobic plates; the indication that there must have been an influx of staphylococci into the intestinal tract great enough to antagonize the prominent *B. coli-B. aerogenes* types. This was unquestionably true, for the baby had an infection of the nasal pharynx and the mother a definite follicular tonsillitis and an acute mastitis at the time the fecal specimen was examined.

A BACTERIOLOGIC STUDY OF BREAST MILK

The investigation here reported is divided into two parts.

Do the So-Called Adventitious Organisms Found in the Breast Milk Appear in the Feces?—The investigation first started in to discover how many of the so-called adventitious organisms found in the milk passed through the intestinal tract and appeared in the feces. This work was all done on samples of drawn breast milk which had been collected under the supervision of Miss Martha H. Stark of the On Shore Department of The Boston Floating Hospital and was to be used for supplementary bottle feedings. It was brought to the hospital in sterile bottles, kept on ice, and used in the order of arrival. This type of milk was used for this part of our investigation since it might be expected to contain more adventitious bacteria than milk which had been directly drawn from the breast and which had not been handled.

Fifty-four samples of drawn breast milk used to feed the babies enumerated in Table 1 were examined bacteriologically in this part of the investigation, forty by aerobic cultivation only and the other fourteen by anaerobic cultures in addition. The samples were examined before and during the metabolism period of the babies using the milk.

The supplementary feedings for the babies whose mothers had an inadequate amount of breast milk and the entire feedings for the orphans from St. Mary's were made up by mixing an adequate amount of the oldest milk of the supply on hand. When taken in the feedings, 75 per cent. of this milk was forty-eight hours old, 5 per cent. was seventy-two hours old, and the other 20 per cent. was from twelve to forty-eight hours old. Plated on ordinary agar, the different milks gave counts ranging from 10 to 150,000 bacteria in one cubic centimeter.

TABLE 2.—STUDY OF INTESTINAL FLORA OF STRICTLY BREAST FED BABIES

Baby	Type of Feeding Before Admission	Admission Age	Period of Receiving Supplementary Breast Milk*	Organisms Isolated from This Milk During This Period	Organisms Found in Feces at End of This Period	Remarks
38	Breast fed 2½ weeks; cow's milk 3-4 days	3 weeks	16 days	B. coli Staphylococci B. pyocyaneus (1 sample)	B. bifidus B. coli M. ovalis B. pyocaneus	It is hard to say whether the B. pyocyaneus in the feces had any relationship to that found in the breast milk 3 days before since this baby had had cow's milk before admission and B. pyocyaneus was isolated from a previous stool
39	Breast fed; cow's milk (supplementary)	16 days	9 days	B. coli Staphylococci B. pyocyaneus (1 sample)	B. bifidus B. coli Staphylococci	
40	Breast fed; cow's milk (supplementary)	6½ weeks	16 days	B. coli Staphylococci B. pyocyaneus (1 sample) B. subtilis (1 sample)	B. bifidus B. coli Staphylococci Spore bearer	A few sporogenous bacilli were seen in the direct fecal smear which did not appear in aerobic culture. It is a question whether these resulted from the ingestion of breast milk containing B. subtilis 6 days before or whether they came from the previous cow's milk formula or perhaps from a source outside the food
41	Breast fed; cow's milk (supplementary)	6½ weeks	6 days	B. coli Staphylococci B. pyocyaneus (1 sample) B. subtilis (1 sample)	B. bifidus B. coli	
54	Breast fed originally; cow's milk entirely for several days	1 week	12 days	B. coli Staphylococci B. subtilis	B. bifidus B. coli B. lactis aerogenes	
55	No history of breast feeding; cow's milk entirely	1 week	12 days	B. coli Staphylococci B. subtilis	B. bifidus B. coli B. lactis aerogenes	
57	.....	6 weeks	.....	B. coli Staphylococci B. subtilis	B. bifidus B. coli Cocci	

* All samples of breast milk contained staphylococci.

Since *B. Coli* and staphylococci are universally considered obligate inhabitants of the digestive tract it is of little value at this point to discuss the part which the *B. Coli* and the staphylococci found in the milk may have played in determining the fecal flora in these cases. As to the so-called adventitious *B. subtilis* and *B. pyocyaneus* isolated in small numbers from these samples of milk the relationship is little

clearer since the parallel specimens of feces containing these organisms were from babies who had previously received a cow's milk formula. However, Cases 40 and 41 were absolutely parallel in age, in type of feeding previous to admission, and in the feedings received in this hospital. If the bacteria of the food alone determined the bacteria of the feces one might expect both babies to have exactly the same fecal flora. During the course of these examinations several lots of breast milk were examined from mothers in the hospital who were contributing occasionally to the general supply since they had more milk than was needed for their own babies. It was noticed that the bacterial count of these samples was low and that the only organisms isolated were staphylococci. These specimens were collected with aseptic precautions. Comparing them with the milk obtained from outside sources brought out the fact that even a slight amount of handling may introduce adventitious bacteria into the feedings of infants receiving breast milk from a bottle.

What Bacteria May Enter the Baby's Mouth During Nursing.—The second part of the investigation dealt specifically with the kind of bacteria which may enter a baby's mouth directly from the breast. Six mothers in the hospital contributed entirely to this work.

In order to find out what types of organisms may naturally enter a baby's mouth directly from the breast it was decided to work only with mothers here in the hospital where the greatest aseptic precautions could be taken. The chief point of interest was to see if *B. bifidus* could be isolated from breast milk since it occupies such a prominent place in the fecal flora of normal breast fed babies. An attempt was made to obtain specimens of breast milk with as little handling as possible. Since it was realized that the skin around the area of the nipple must be a factor in contaminating the milk as it enters the baby's mouth, swabs from this area were made in all cases. The method of obtaining both milk and swab specimens was as follows:

Immediately before nursing time the area of the nipple was swabbed with a sterile swab soaked in sterile salt solution. The swab was then returned to its sterile test tube. Next the breast was wiped off with boric acid solution and about 10 c.c. milk was withdrawn by means of a sterile breast pump. The milk was evacuated very carefully into a sterile tube. After nursing a second specimen was obtained in similar fashion. Specimens from six mothers were obtained in this way (Table 3).

Lactose litmus agar plates were used for the aerobic cultivation of the first three specimens and dextrose agar slants for the anaerobic cultures. For the second three specimens dextrose agar slants for aerobic cultivation were used in addition. As soon as the specimens

reached the laboratory they were plated and smeared. They were kept for two weeks after this at room temperature, carefully protected from dust, and cultured and smeared at regular intervals. These later cultures failed to add any further information.

The organisms described as "bifidus-like" were morphologically typical of *B. bifidus* in subcultures isolated from stools.

TABLE 3.—STUDY OF INTESTINAL FLORA BEFORE AND AFTER NURSING

Specimen	Aerobic Cultivation			Anaerobic Cultivation		
	Before Nursing Milk 1	Nipple Swab	After Nursing Milk 2	Before Nursing Milk 1	Nipple Swab	After Nursing Milk 2
1	Cocci only good growth	Staphylococci only	Cocci only scanty growth	Cocci only fair growth	Staphylococci only	Cocci only scanty growth
2	Cocci only good growth	Staphylococci good growth B. subtilis rare colony	Cocci only scanty growth	Cocci only fair growth	Staphylococci only	Cocci only scanty growth
3	Cocci only good growth	Staphylococci Bifidus-like organism*	Cocci only scanty growth	Cocci only fair growth	Staphylococci only	Cocci only scanty growth
4	Cocci fair growth Bifidus-like organism† rare colony	Cocci only	Cocci only scanty growth	Cocci only fair growth	Cocci only	Cocci only scanty growth
5	Cocci good growth Bifidus-like organism‡ good growth	Cocci only	Omitted	No organisms of definite morphology	No growth	Omitted
6	Cocci Bifidus-like organism§ good growth	Cocci Bifidus-like organism§	Omitted	No organisms of definite morphology	Cocci only	Omitted

* Isolated from aerobic lactose litmus agar plates. Subcultures grew poorly and soon died out but finding this organism gave impetus to the work and the last three specimens were examined even more carefully.

† Isolated from aerobic dextrose agar slant, after 24 hours' incubation. Rather attenuated and died out after several generations.

‡ Isolated from aerobic dextrose agar slant after 48 hours' incubation. Subcultures showed good growth and were kept for future study.

§ Isolated from aerobic lactose litmus agar plates after three days' incubation. Also from aerobic dextrose agar slant after four days' incubation. Good subcultures kept for future study.

CONCLUSIONS

Bacteriology of Normal Breast Milk Stools.—1. Direct smears from normal breast fed babies present a practically constant picture which is characterized by an almost complete dominance of the bifidus group. In this picture cocci and gram-negative organisms are indistinguishable or are present in very small numbers. This proportion of bacterial types may be changed by abnormal physiologic conditions.

2. A baby who has been on cow's milk formula for several days, although originally breast fed, may show, bacteriologically, the effects of this diet for as long as four weeks even after the continuous ingestion of breast milk. This is indicated in the direct smears from the

feces by a larger number of cocci and gram-negative bacilli than is typically characteristic of a normal breast milk stool.

3. If a cow's milk formula is used before the third day after birth and breast milk used thereafter the establishment of fecal types of bacteria follows the course of a normal nursling very closely.

4. From the study of anaerobic cultures it has been found that *B. bifidus* is also the dominant living type of organism in the feces of normal breast fed babies.

5. The proportion of types represented in the direct smears is closely paralleled by the proportion of types growing on anaerobic cultures.

6. Aerobic cultures from the feces of normal nurslings typically show a predominance of the colon-aerogenes groups. This may be lessened by abnormal physiologic conditions.

7. While the study of the fecal flora of infants by anaerobic culture seems to be of great importance, aerobic cultures should always be used as a check to determine the presence of aerobic pathogens or adventitious bacteria.

Bacteriology of Breast Milk.—1. The results obtained from the study of drawn breast milk used for supplementary bottle feedings are inconclusive since no definite relationship could be established between the types of fecal bacteria and the bacteria isolated from the milk. This was made more difficult because the babies had not had a monotonous diet which could be used as a check and because the majority of the organisms isolated from the milk were staphylococci and *B. coli* which may be isolated from any normal stool. This study did emphasize the fact that even a slight amount of handling may introduce certain types of bacteria into a bottle fed baby's feedings which a breast fed baby would not ordinarily ingest.

2. That a typical monotonous fecal flora follows the continuous ingestion of breast milk has been shown in the results obtained from the study of the stools of normal breast fed babies.

3. From the examination of the breast milk from the mothers here in the hospital it would seem that staphylococci may be ingested in all cases and that a "lactic acid" bacillus typical of *B. bifidus* may be frequently present in the milk as it comes from the breast.

4. An important question arises as to the identity of the "bifidus-like" bacillus which has been isolated from the breast milk and from the skin around the nipple. We do not hesitate to call this organism *B. bifidus.*

5. It is our belief that the bifidus organisms which are present in or on the mother's nipple are an important source of the bifidus organisms found in the nursling's intestines.