Host resistance to infection[1, 2]

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Pediatricians hold a common opinion that breast-fed infants experience less infectious disease than those artificially fed. The greater resistance of the breast-fed child is especially evident in gastrointestinal disorders, but it extends to a wide variety of other acute and chronic infections.

A classical British publication on the incidence of illnesses and mortality of infants according to the type of feeding (1) attests the beneficial effect of breast milk, as opposed to cow's milk. Wholly bottle-fed infants had greater morbidity rates for gastrointestinal disorders, respiratory infections, and otitis media. Infections tended to be of longer duration, and mortality and case fatality were notably greater. No differences were observed according to social class as determined by father's occupation.

In Sweden, Sydow and Faxén (2) studied infants from birth to 9 months; bouts of fever were fewer in breast-fed than artificially fed infants. Similarly, Mellander et al., in a more extensive investigation (3), found the incidence of acute infections, otitis media, febrile upper respiratory infections, and acute diarrhea lower in breast-fed than in bottle-fed infants aged 3 months to 1 year. These studies were in populations with adequate environmental sanitation. Differences would be expectably more marked in preindustrial societies exposed to highly deficient sanitary conditions and a greater burden of infection.

No similar controlled studies have been conducted in countries with unfavorable environmental conditions. However, it is well-known that children have a low incidence of diarrheal disease during the early months of exclusive breast feeding; the attack rates increase as weaning progresses, to reach a maximum nearing the period of definitive separation from the breast (4). It is also known that there is greater resistance to *Shigella* and other infections during the period of intensive breast feeding (5, 6). The remarkable association of diarrheal disease with weaning led to characterization of the syndrome "weanling diarrhea," referring to the combined action of a highly contaminated environment and deterioration of the nutritional status (7). The high point on frequency by geographical region occurs at different ages, governed by the weaning practices prevailing in the particular region: early in life with early weaning, much delayed with late weaning. Infant mortality follows the same trend with high rates in areas where children were weaned early (8), and a continued high mortality through preschool ages in the areas where weaning is late (8, 9).

These field observations support an assumption that human milk acts beneficially through induced host resistance to infection. The mechanisms have not been soundly established, but several factors appear to be involved, such as specific antibodies to infectious agents, influences stimulating or inhibiting certain intestinal microorganisms, or nonspecific antimicrobial factors.

Immunoglobulins in human milk

Schlossman and Moro (10) pioneered the demonstration of a relationship between proteins in serum and milk. A more critical study of the proteins responsible for humoral immunity had to await the development of electrophoretic, chromatographic, and immunologic techniques, particularly within the past 10 years. For a review on immuno-

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globulins, see Gitlin (11), Bellanti (12), Tomasi (13), and Putnam (14).

Immunoelectrophoresis revealed more than 30 components in human colostrum than in cow's milk. Some 18 milk proteins are related to those in serum, whereas 13 are peculiar to breast milk (15, 16). The first group includes the better known serum proteins, indicating a structural identity between these and milk proteins, although there may be differences in the determinants of antibodies, as with the immunoglobulins.

IgA, IgG, IgM, and IgD are all present in human milk. Of these, IgA is the most important in terms of relative concentration and biological characteristics. The immunological properties and high concentration of colostrum IgA suggested to some workers an identity with serum IgA. However, the strong variability in immunodiffusion tests with antibodies to serum IgA indicated to Hanson (15) a different structure than for serum IgA. Evidence of its different nature derived from the studies of Tomasi et al. (17) and South et al. (18). Using autoradiography and immunofluorescence, the first authors demonstrated that most of the IgA molecule is synthetized in lymphoid cells that underly the duct of the human salivary gland, whereas another component of the molecule, the "transport piece," is localized and synthetized in epithelial cells lining the glandular duct. Similar observations held for IgA secreted by the intestine, bronchi, and mammary glands of rabbits (19). South et al. (18) detected IgA, but no IgG or IgM, in the saliva of agammaglobulinemic patients after administration of large doses of normal blood plasma. IgA apparently is selectively transported from blood and lymphatic tissues to secretions of certain glands and systems. Colostrum IgA rather assuredly is synthetized in the gland from two molecules of serum IgA linked by disulfide bonds, related to each other by two "transport piece" molecules. The result is a larger structure with a molecular weight of approximately 375,000 (19).

Ammann and Stiehm (20) showed that IgA is present in initial colostrum to the extent of 17 mg/ml, and in 4-day colostrum in concentrations of 1 mg/ml; these amounts exceed those of IgG and IgM. The concentrations of IgA in bovine colostrum is less than in serum (21); human colostrum is rich in this immunoglobulin.

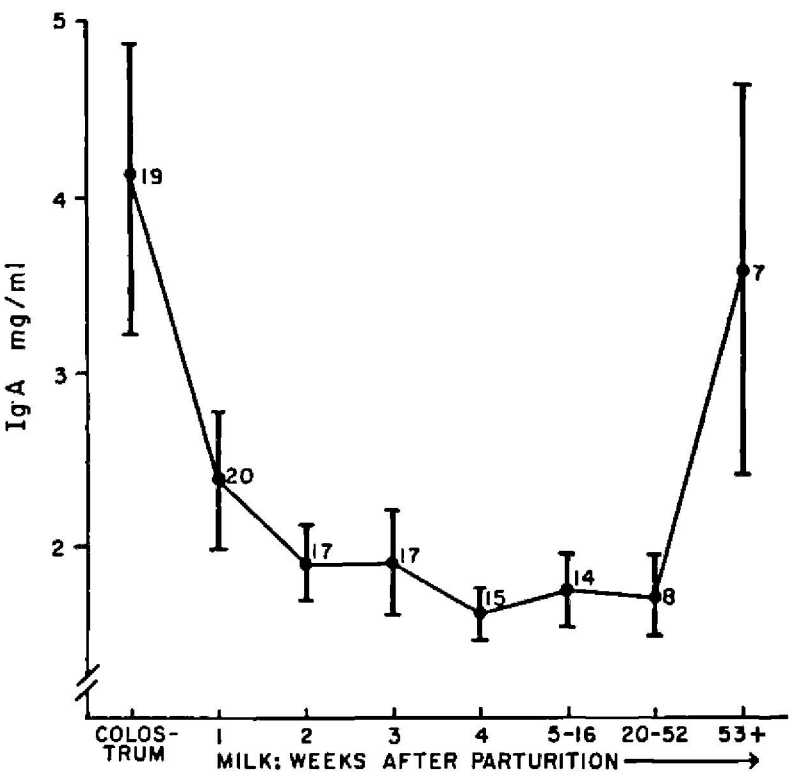

FIG. 1. Concentrations (mean ± 1 SD) of 11-S IgA in colostrum and milk of Mayan Indian women, Santa María Cauqué, Guatemala, 1968. Numbers of women studied are indicated in curve.

In contrast to the high concentration of secretory IgA in breast milk, only small amounts of IgG are present in the preponderant serum immunoglobulin. Reported values are 0.4 mg/ml in initial colostrum, and 0.04 mg/ml in 4-day colostrum (20). IgM is present in human milk but in lesser concentrations than IgA, with values of 1.6 mg/ml in initial colostrum and 0.1 mg/ml in 4-day colostrum (20).

Immunoglobulins were investigated by radial immunodiffusion in colostrum and milk of Mayan Indian women. A standard technique adapted to local conditions was used (22). Commercial plates and reagents (Hyland) were used to measure IgG, IgM, and the C'3 component of complement. An 11-S serum was used to quantitate secretory IgA. Average values are shown in Fig. 1 and Table 1. IgA (11-S) was found in average concentrations of 4.1 mg/ml in 2- to 4-day colostrum. IgA decreased to an average 1.8 mg/ml 2 weeks after parturition, remaining at similar concentrations throughout the period of intensive breast feeding (Fig. 1). The pattern in Guatemalan Indian villages is to

TABLE 1
Total solids, IgG, IgM, and C'3 component of complement in colostrum and milk of Mayan Indian women[a]

	Total solids, g/100 ml	IgG, mg/ml	IgM, mg/ml	C'3, mg/ml
Colostrum (1–4 days)	8.4[b] (7.3–9.8)	0.063 (0.025–0.185)	0.095 (0–0.340)	0.044 (0.028–0.070)
Milk				
1 week	8.9 (6.7–13.0)	0.063 (0.030–0.096)	0.017 (0–0.035)	0.011 (0–0.028)
2 weeks	7.8 (6.3–9.0)	0.074 (0.025–0.103)	0.007 (0–0.035)	0
3 weeks	7.7 (6.8–9.1)	0.063 (0.025–0.102)	0.011 (0 0.055)	0.006 (0 0.028)
4 weeks	7.5 (6.9–8.2)	0.090 (0.025–0.150)	0	0

[a] All determinations were made in five women from whom colostrum and four samples of milk could be collected.
[b] Average; range in parentheses.

have prolonged breast feeding, occasionally 4 years. In milk samples collected from seven women after 1 year of lactation, IgA was present in a concentration of 2.4 ± 0.4 mg/ml (mean ± SE).

Relatively small amounts of IgG and IgM were detected in colostrum and milk, Table 1. The C'3 component of complement, important in the lysis of bacteria, was also detected in colostrum. The low levels of IgG, IgM, and C'3 in colostrum decrease significantly 2 weeks after parturition. This change is not correlated with the slight decrease observed in the concentration of total solids (Table 1).

Specific antibody activity. All immunoglobulins have antibody activity, yet a certain disagreement persists as to types of antibody, by serological tests, in relation to classes of immunoglobulins. Serum IgA contains antibodies of all types. IgG has a wide variety of antibodies to viruses, rickettsiae, protozoa, H antigens of *Salmonella,* as well as bacterial antitoxins and incomplete Rh antibodies. The IgM fraction contains Rh agglutinins, syphilis "reagins," cryoagglutinins, and antibodies to the O antigens of *Enterobacteriaceae.* Allergic "reagins" have been reported in IgE. Recently, antibody activity was also demonstrated in IgD (23, 24).

The main localization of antibodies in human milk is in the IgA fraction. Hodes et al. (25), for example, determined that polio virus neutralizing activity of colostrum had a different sedimentation rate than serum antibody, and furthermore, was destroyed by 0.1 M mercaptoethanol. Immunologically, this colostral antibody was a beta 2A globulin (IgA). Treatment with 0.2 M mercaptoethanol also decreased the hemagglutinating titer to O polysaccharides of *Enterobacteriaceae* by one to three dilutions (unpublished observations). It has been shown that the hemagglutinin to *Escherichia coli* in human colostrum is in the bulk of the IgA fraction after DEAE(diethylaminoethyl)-cellulose chromatography; in contrast, the hemagglutinating activity of human serum was associated with the IgM fraction (26). Finally, purified IgA obtained from colostrum possessed antigenic structures characteristic of serum antibodies (27).

Vahlquist (28) reviewed the limited literature on antibodies present in human colostrum and milk. Sabin and Fieldsteel (29) described an antipolio virus active principle in human milk from American women that could not be differentiated from serum polio virus antibody. Also, colostrum from women from an industrialized society contained neutralizing antibodies to at least one of eight enteroviruses tested (polio 1, 2 and 3; Cox-

sackie B1 and B5; Coxsackie B9; and Echo 6 and 9) (30). Antipolio virus antibody to all types was also reported by others (31, 32).

Human milk in the first week of lactation strongly inhibited the hemagglutinating capacity of influenza virus (33); cow's milk lacked that capacity at any stage of lactation. Similarily, breast milk of women from industrial societies possessed passive hemagglutinating antibodies to several serotypes of *Escherichia coli* (25, 26, 34).

In general, high titers characterize initial colostrum and decline progressively with time to low or undetectable levels 2 weeks after parturition (30), a phenomenon paralleling the known decrease in immunoglobulin content between the 3rd and 7th day (35).

The findings in milk of women from industrialized countries resemble those of women living in a highly contaminated environment. Viral neutralizing antibodies and passive hemagglutinating antibodies to polysaccharide O of *Enterobacteriaceae* were demonstrated at INCAP laboratories in colostrum and milk from Mayan Indian women (Tables 2 and 3). These antibodies were frequently present in colostrum in high titers, but declined thereafter. However, antibody could be detected sporadically by neutralization tests even 3 or 4 years after parturition (Table 2). Antibodies to O antigens of enteric bacteria were also detected in significant titers by the microhemagglutination test (36). In a series of 45 mothers from whom colostrum and at least three weekly milk samples were collected, 25 (55%) had antibody titers of at least 1:32 and 7 (15%) had titers of 1:64 to one or more antigens. The patterns of antibody in breast milk of five women chosen as examples are presented in Table 3. As with viral antibody. titers declined shortly after parturition but less rapidly, being still detectable in 4-week milk from several mothers. Furthermore, the mature milk of some mothers had greater antibody activity than colostrum, as exemplified by case 242 of Table 3.

Significance of antibody derived from human milk. Certain antibodies are transferred from mother to child in utero. Those that readily cross the placenta are to be found

TABLE 2

Viral neutralizing antibody in colostrum and milk[a] of Mayan Indian women

Lactation time	Number of specimens	Polio virus 1		Coxsackie virus B5	
		Positive	Titers[b]	Positive	Titers[b]
1 to 3 day (colostrum)	9	8	10–160	6	10–640
4 to 28 days	15	1	10	1	10
29 days to 52 weeks	20	1	10	0	
181 and 201 weeks	5	2	10	0	
Total	49	12		7	

[a] Measured in whey. [b] Reciprocal.

in the IgG fraction, but not in significant amounts in the IgM or IgA components.

Normally, antibodies in homologous and heterologous colostrum and milk are not absorbed by the human gastrointestinal mucosa in significant amounts (37), yet have a role in local immunity of importance to intestinal infection. For example, Lepow et al. (38) reported diminished effectiveness of oral polio virus vaccination in breast-fed American infants, and Warren et al. (32) observed viral interference when milk antibody levels were above 1:16. Similarly, the rate of infection with attenuated polio virus in Mulago infants was 31% when the colostral antibody titer was 1:256 or more, as opposed to 71% when antibodies were at 1:64 or less (39). Thus, a protective effect against viral infection correlated with the presence of polio virus antibody in breast milk. Furthermore, Katz and Plotkin (40) demonstrated that administration per os of antipolio virus type 1 serum a few hours before and after infection with attenuated polio virus 1, interfered with virus replication. None of ten children became infected when the serum was administered 2 hr before and 2 hr after inoculation; all of eight children became infected when serum was given 6 hr before or 6 hr after inoculation. This study demonstrated the capacity of immunoglobulins to maintain antibody activity along the gastrointestinal tract. Similarly, Kenny et al. (34)

TABLE 3
Passive hemagglutinating antibody to polysaccharides of enterobacteriaceae in colostrum and milk[a] of Mayan Indian women

Mother serial number	Lactation time, weeks	Reciprocal of antibody titer					
		Shigella dysenteriae 2	*Shigella flexneri 1*	*Shigella flexneri 6*	*Shigella sonnei*	*Escherichia coli 0111:B4*	*Salmonella panama*
240	Colostrum	64	256	256	0	4	2
	1	8	64	16	16	0	0
	2	4	32	32	8	0	0
	3	0	64	32	8	0	0
	4	0	32	16	8	0	0
241	Colostrum	32	32	2	0	4	16
	1	4	8	8	0	0	0
	2	0	4	8	0	0	0
	3	0	16	8	0	0	8
	4	0	8	4	0	2	0
242	Colostrum	8	8	0	4	0	0
	1	0	4	4	0	0	0
	2	0	8	4	0	0	0
	3	0	16	4	0	0	0
	4	8	32	16	0	0	0
246	Colostrum	0	256	8	0	0	8
	1	0	32	4	0	0	0
	2	0	32	2	0	0	0
	3	0	64	4	0	0	2
	4	0	16	2	0	0	0
247	Colostrum	16	32	4	4	4	2
	1	8	8	4	0	0	0
	2	8	8	2	0	0	0
	3	4	4	0	0	0	0
	4	8	4	2	0	0	0

[a] Measured in whey.

found that antibodies to *Escherichia coli* in breast milk traverse the gastrointestinal tract without appreciable change. This was concluded from the similarity of titers of "copro-antibody" and those of milk of the preceding day.

This review suggests an incomplete understanding of the significance of antibodies in human colostrum and milk, although no doubt exists of their participation in host defense, particularly derived from their presence in the gastrointestinal lumen. Antibodies with capacity to act locally are essential to protection from continued exposure of young children to infection, particularly in preindustrial countries. Environmental fecal contamination could be so great that even breast milk often carries coliforms and other enteric bacteria (41). That antibodies in colostrum and milk act importantly in control of invading agents is a reasonable deduction.

The bifidus factor

Early in the history of medical bacteriology, Tissier (42) isolated from the feces of breast-fed infants a Gram-positive, nonmotile anaerobic bacillus (*Bacterium bifidum*) that predominated over all other species. Many others confirmed that the flora of breast-fed infants differs from that of bottle-fed infants, mainly in an almost exclusive content of bifidobacteria.

A principle in human milk (the bifidus factor, or factors) promotes development of the characteristic microflora. György (43) added breast milk to a culture medium rendering it capable of supporting the growth of a fastidious variety of the bifidus bacillus,

namely, *Bifidobacterium bifidum* var. *Pennsylvanicum*. Increasing amounts of breast milk promoted acid production by this bacillus to a maximum level. Human milk was 40 to 100 times more active than cow's milk; other substances such as yeast, carbohydrates, and vegetable extracts failed to exhibit this capacity.

Highly active concentrates of the bifidus factor subjected to acid or enzymatic hydrolysis produced *N*-acetyl-*d*-glucosamine, L-fucose and D-galactose, but no amino acids. The bifidus factor was characterized as a dialyzable nitrogen containing carbohydrate (methyl-*N*-acetyl-D-glucosaminide) not present in appreciable concentrations in cow's milk treated similarly (43–47).

The pronounced effect of breast milk on the intestinal flora is universally recognized (48–53). The results of a prospective study of Mayan Indian children (54) are presented in illustration.

Children were studied in their natural environment from birth to 3 years. All were breast-fed exclusively for 3 to 9 months, after which small amounts of fluids were introduced. By the 1st birthday, gruels and solids were a regular supplement, although most children continued to be breast fed through the 2nd year of life. In this culture, the nutritive value of the supplement was practically nil during the 1st year, and especially deficient in protein of adequate biological value (54).

Although aerobic bacteria ordinarily were as abundant as anaerobes in the first days of life, by the end of the 1st week bifidobacteria predominated over all other culturable organisms to reach average concentrations of 10^{11}/g wet feces. Coliforms were less numerous at values between 10^{6} and 10^{10}/g feces. During the wholly breast feeding period and as supplemental feeding began, bifidobacteria constituted more than 99% of the total flora. A change occurred, coincidental with greater amounts of gruels and solid foods (55, 56). Other indigenous organisms, such as *Bacteroides, Veillonella,* and *Streptococcus* (Fig. 2), and *Enterobacteriaceae,* mainly the *Escherichia coli* group (Fig. 3), progressively increased in numbers. The fully weaned child exhibited a mixed population of bifidobacteria and Gram-negative anaerobic organisms (*Bacteroides*). The latter are the predominant component of the fecal flora of the adult. Bifidobacteria are present in the intestinal tract of man at all ages; they outnumber other culturable bacteria only in breast-fed infants (53).

Bifidobacteria metabolize a variety of sugars, producing large amounts of acetic and lactic acid and trace amounts of formic and succinic acid (57, 58). These products are responsible for the low pH of feces of wholly breast-fed infants. The relationship of bifidus flora and pH has been derived from several studies. Dehnert (59) placed five healthy infants for 2 weeks on a formula of cow's milk, rice gruel, and sugar, and then transferred them abruptly to sterile pooled human milk.

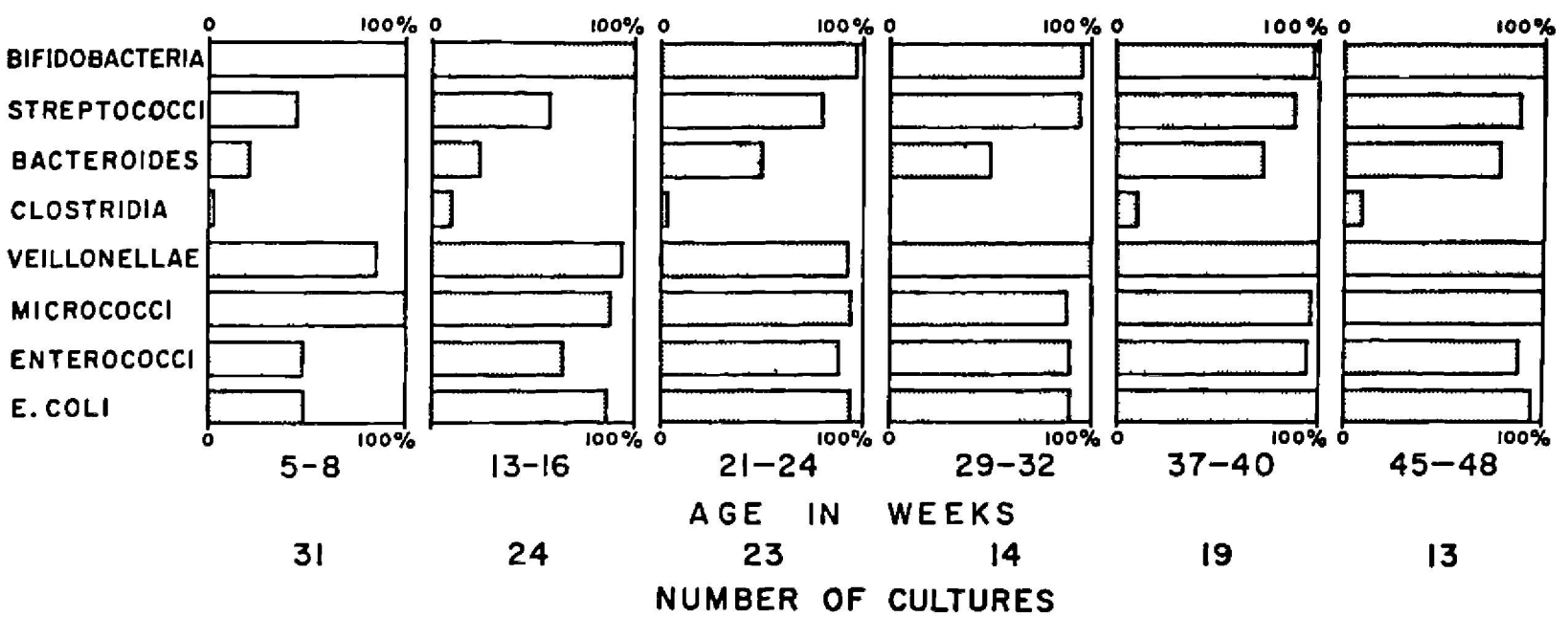

FIG. 2. Occurrence of indigenous microorganisms in a cohort of breast-fed Mayan Indian infants studied from birth to 11 months of age, Santa María Cauqué, Guatemala, 1967–1968. Bacterial counts of 10^{8}/g feces or greater were used to calculate rates (56).

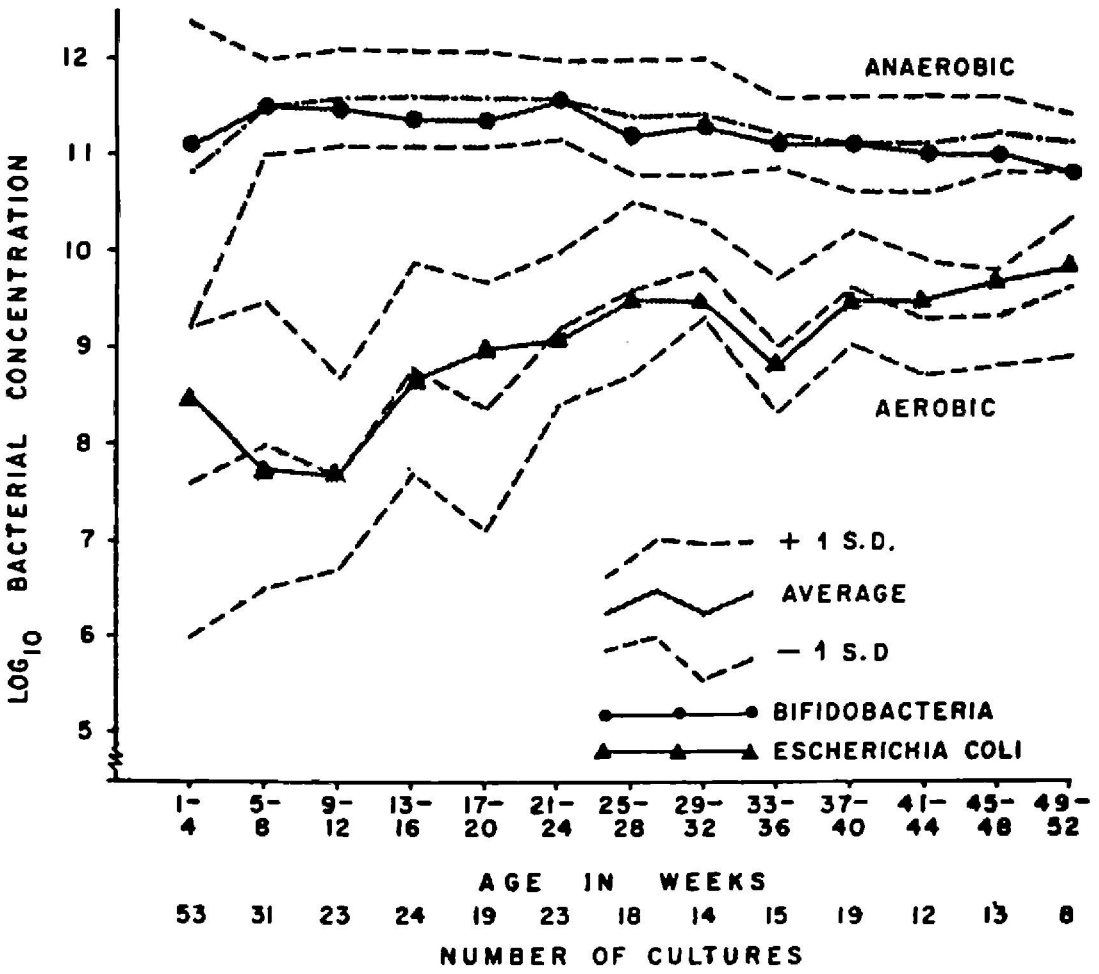

FIG. 3. Fecal indigenous microflora in a cohort of breast-fed Mayan Indian infants studied from birth to 1 year of age, Santa María Cauqué, Guatemala, 1967–1968 (56).

During the 2 weeks on cow's milk formula, the flora was predominantly nonbifidus; it reverted abruptly to a bifidus flora with breast milk. This was interpreted as not indicative of a promoting effect by human milk on bifidobacteria (60), but as suppression of putrefactive organisms present in the intestinal tract.

Leuterer (61) observed that 12 of 13 premature infants fed pooled, boiled breast milk developed a bifidus flora with mean pH value of feces of 5.3. Only three of 26 prematures fed cow's milk showed a predominant bifidus flora, with fecal pH ranging from 5.9 to 7.8. The relationship between breast feeding, type of flora, and fecal pH was also demonstrated by Gyllenberg and Roine (48), who found bifidobacteria to represent as much as 99% of the fecal flora of breast-fed infants, in contrast to less than 60% for infants under regimens of boiled human milk or cow's milk. Coliforms and enterococci proliferated to levels comparable to those of bifidobacteria under artificial feeding. Fecal pH increased when cow's milk formulae were given.

Significance of the bifidus flora. That a human milk diet leads to bifidobacteria flora is certain, although mechanisms remain unclear. The intestinal tract of wholly breast-fed infants is resistant to infection by pathogens such as *Shigella,* and intestinal protozoa, as reviewed by Mata and Urrutia (56). In rural Egypt and Guatemala, where prolonged breast feeding is customary and *Shigellae* are common, infants had few or no infections in early months (62, 63). As weaning progresses, incidence increases to reach a maximum shortly after completed weaning, as determined in a long-term pro-

TABLE 4
Incidence of shigella infection in a cohort of breast-fed children studied from birth to 3 years of age

Age, weeks	Number of children	Weeks at risk	Number of infections	Rate per 100 person-weeks
0–25	81	1,783	1	0.06
26-51	65	1,546	11	0.7
52–77	52	1,192	20	1.7
78–103	42	942	32	3.4
104 129	26	559	15	2.7
130 155	12	247	10	4.0

spective study, Table 4 (5). Serial examinations of feces of newborns revealed early infection, although not necessarily infectious disease, with a variety of agents (6, 54). Neonatal shigellosis was detected in 4 of 109 infants. In one wholly breast-fed infant, it was transient and asymptomatic; in two receiving supplemental feeding, it was asymptomatic but of appreciable duration; and the fourth child who was born prematurely was inadequately nursed, received poor supplementary feeding, and was the only one with diarrhea (6). That a flora of bifidobacteria is antagonistic to certain pathogens is a valid observation (56).

Other factors

Resistance factor. Human milk has the capacity to enhance resistance of mice to infection (64, 65). Injection of breast milk mixed with sublethal doses of *Staphylococcus aureus* and followed by challenge with lethal doses of *S. aureus* 2 weeks later, resulted in lower fatality of animals compared with controls not injected with human milk. The responsible factor was described as nondialyzable, thermostable, and probably residing in the free fatty acid fraction of milk. This factor could be related to the greater resistance of breast-fed infants to parenteral infection, particularly due to *Staphylococcus.*

Lysozyme (*muramidase*). Human milk is known to contain a nonspecific antimicrobial factor, lysozyme (66), in concentrations as great as 0.2 mg/ml (67). This enzyme is bacteriolytic against *Enterbacteriaceae* and Gram-positive bacteria. The lysozyme from breast milk is thermostable (100 C) at acid pH, and twice as active as the lysozyme of egg white (68). Lysozyme is found in large concentrations in feces of breast-fed infants, but not in those fed cow's milk formula. It is then possible that lysozyme contributes to the development and maintenance of the special intestinal flora of breast-fed infants (66).

Complement. The C′3 component of complement, important for the lysis of bacteria bound to specific antibody, has been demonstrated in colostrum of Mayan women as mentioned above (Table 1).

Interferon. No references were found regarding interferon in human milk, but its presence is expected in analogy with findings in other body fluids.

Immune cells. Colostrum and milk contain cells with immunological capacity. Smith, Goldman and Murillo (69, 70) described significant numbers of lymphocytes capable of synthetizing IgA, and macrophages with the capacity to phagocytize, in human colostrum.

Comments

Lesser attack rates of infectious disease in breast-fed than in artificially fed infants is evidence that breast milk protects against infection. Protection is not only against agents that invade the gastrointestinal tract, but against others that induce parenteral infections. Clinical and epidemiological evidence and laboratory observations revealed substances and factors in colostrum and milk recognized as protective against infection. Mechanisms of action remain indeterminate.

Human colostrum and milk contain immunoglobulins acting against viruses and bacteria. Except for IgA, which is synthetized in the mammary gland and has special biological properties, all appear identical with serum immunoglobulins. Concentrations of immunoglobulins decrease regularly and rapidly after parturition, although levels of IgA and IgG persist in milk for several weeks. Secretory IgA, and viral and bacterial antibodies are detectable in significant levels in milk of Mayan women 1 to 4 years after parturition.

Other protective substances in human milk include the bifidus factor, itself related to predominance of bifidobacteria in the intestinal tract. These bacteria are intimately related to conditions unfavorable to implantation of protozoa and enteropathogenic bacteria. Lysozyme, the C′3 component of complement, antibody producing cells, and cells with phagocytic activity, present in colostrum and to a lesser degree in milk, likely play a role in the limitation of certain bacteria.

Human milk is definitely more effective

than cow's milk in protecting the newborn infant against infection through a greater concentration of secretory IgA, antibodies against human pathogenic bacteria and viruses, and a greater concentration of bifidus factor and lysozyme.

Future research is directed advantageously to better characterization of humoral substances in milk and investigation of protective mechanisms inherent in the gastrointestinal tract, as influenced by breast feeding. Studies of interferon and other nonspecific components will usefully complement the existing knowledge of the uniqueness of human milk.

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