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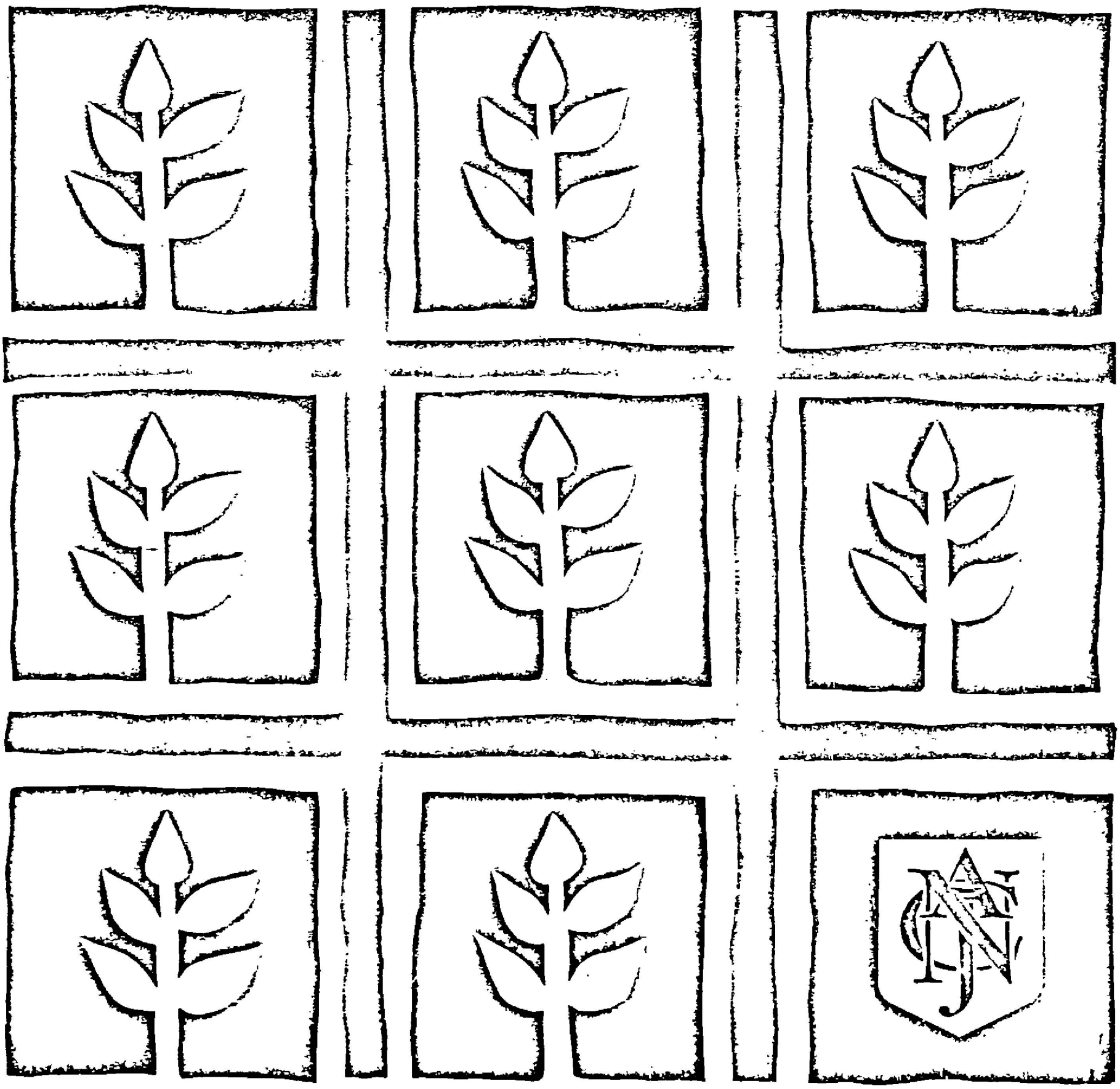

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special issue

Studies on the indigenous gastrointestinal flora of Guatemalan children[1]

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The main health problems in Latin America and other less developed regions of the world derive from inadequate nutrition and excessive infection. In conjunction, both factors are the most important forces leading to malnutrition, high morbidity rates, and increased mortality (1). In such poor ecosystems, the newborn is rapidly exposed to microbial colonization in a fashion similar to that known in industrialized societies (2). However, differences are observed, inasmuch as the newborn may have an altered immunologic endowment as a result of intrauterine stresses and poor fetal growth, apparently more common in less developed than in industrialized countries (3). In poor environments, the child becomes exposed to negative environmental forces at all stages of development. In the first place, the food intake of the child, adequate in the 1st weeks of life, due to his being breast-fed almost excusively, soon becomes insufficient to meet the demands of the growing child (4). Milk from malnourished mothers often is produced in suboptimal volume and may be deficient in essential components. Supplemental feeding is definitely inadequate in most preindustrial societies and consists of small amounts of foods of low nutritional value, and given under unhygienic conditions (4–6). Furthermore, the child is progressively exposed to infection by undesirable agents (bacterial pathogens, intestinal parasites, and viruses) often associated with infectious disease (3, 6, 7).

It becomes extremely difficult, if not impossible, to separate the contribution of the two negative environmental forces, i.e., deficient diet and frequent infection. Therefore, a practical derivation is to recognize both interrelated factors as similarly important in the genesis of acute and chronic diarrhea, malabsorption, and finally malnutrition and deficient growth and development (1, 3).

In order to know more about these interrelationships, observations on the intestinal flora were begun in 1966 in infants and preschool children belonging to an ecosystem of low socioeconomic conditions, and therefore characterized by overwhelming infection and deficient nutrient intakes. Some studies were made in a typical Guatemalan highland village (8), whereas others were in children from rural and slum areas of Guatemala, admitted to a metabolic ward with a diagnosis of diarrhea or severe protein–calorie malnutrition, or both (9). Studies were also made in hospitalized children and adults from the same ecosystems, but without malnutrition or diarrhea (9, 10).

The field and laboratory procedures have been described before (5, 8–11). Mention will be made of the bacteriologic methods used when the findings are described. The criteria used to differentiate the various bacterial groups have been described elsewhere (8–10, 12, 13). Special emphasis was given to rapid collection, transport, and processing of specimens for bacteriological studies. A field laboratory was established to facilitate processing of specimens after collection. In the hospital studies, specimens were processed within a few minutes of collection (8, 10, 14).

Results

The flora of the healthy individual

Acquisition of bacteria by the breast-fed newborn. Thirty children were studied longitudinally during the neonatal period, 12 of

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whom were followed until the first birthday. The plate-dilution technique (5) was employed to count bacteria, and anaerobiosis was obtained in jars (15, 16). The method did not detect concentrations below 10^4/g wet meconium or feces. The colony counts were approximated to the nearest log (8).

Bacteria were found in meconium as early as 4 hr after birth (8). In contrast to the overwhelming fecal contamination at birth (7), only few bacterial groups could be demonstrated in significant numbers in the first days of life. Facultative micrococci, streptococci, and gram-negative bacilli were more readily demonstrated than anaerobes in the first few hours. In the 2nd day of life, almost all infants showed *Escherichia coli* in concentrations of 10^5 to 10^{11}/g. Anaerobes were detected less frequently than facultatives and only a few babies had bifidobacteria in the 1st day of life. By the 2nd day, one-third of the infants had been colonized by bifidobacteria that reached concentrations of 10^8 to 10^{10}/g (8). The rate of appearance of bifidobacteria in feces increased with age, and by the end of the 1st week all had these bacteria in concentrations of 10^{10} to 10^{11}/g. The flora appeared firmly established as the dominant component throughout the neonatal period and thereafter (8).

Individual children varied in both number and concentrations of bacteria acquired in early life. This was evident in the 30 newborns studied, and in three pairs of twins recruited in the same village in the last 5 years. Differences in the kinds of bacteria present were evident for twins. For example, one twin definitely had more *E. coli* and enterococci than did the other. However, similarities were found; twins of the same pair generally appeared colonized on the same day by the various groups of anaerobes (unpublished observations).

Fecal flora of the breast-fed child

Although there were qualitative and quantitative variations in fecal flora in the 18 breast-fed children (12 single births and 6 twins) during the 1st year of life, at least two features were common to all: *a*) the total concentration of bacteria was of the order of 10^9 to 10^{11}/g feces, a rather constant finding during health and most common episodes of disease; and *b*) the predominant component of the flora consisted almost exclusively of bifidobacteria.

TABLE 1

Fecal bacterial flora of three pairs of twins in the first 2 years of life[a]

Twin	Anaerobes	Facultatives
302		
a	10.9 ± 0.6 (21)[b] (9–11)[c]	9.6 ± 1.0 (22)[b] (6–10)[c]
b	10.9 ± 0.5 (21) (10–11)	9.6 ± 0.7 (22) (8–10)
304		
a	10.7 ± 0.7 (20) (9–11)	9.3 ± 0.9 (20) (7–10)
b	10.8 ± 0.6 (19) (9–11)	9.1 ± 1.1 (19) (6–10)
327		
a	10.5 ± 0.5 (18) (10–11)	9.7 ± 0.8 (18) (9–11)
b	10.9 ± 0.3 (18) (10–11)	9.3 ± 0.8 (18) (8–10)

[a] Study performed in Santa María Cauqué, Guatemala, 1967 to 1971. [b] Mean ± SD of $\log_{10}$ bacterial counts; numbers in parentheses refer to the number of specimens examined; specimens corresponded to periods of health. [c] Range of bacterial counts.

The similarities in flora among individuals are illustrated in Table 1 containing the total bacterial counts for six twins studied during the first 2 years of life. Only monthly specimens collected during health were tabulated. The magnitude of the standard deviations indicate the stability of the bacterial concentration throughout time. This was also the case when the various bacterial groups were examined separately, as shown in Table 2, although individual differences were apparent among infants, twins included. For example, twin "a" in one pair regularly showed veillonellae during the first 32 weeks of life, whereas twin "b" did not show this group of anaerobes at the level of 10^6/g. The twin in question became negative for veillonellae, but was positive again in the 60th and 68th weeks of life; veillonellae were not cultured thereafter for at least 14 weeks (Fig. 1). The other twin, not overtly colonized by veillonellae in the first 75 weeks of life, showed this group of bacteria in significant numbers (more than 10^6/g) for a period of approxi-

TABLE 2

Bacterial groups in feces of three pairs of twins studied in the first 2 years of life[a]

Group	Twins 302		Twins 303		Twins 327	
	a	b	a	b	a	b
	$n = 22$[b]		$n = 20$		$n = 18$	
Bifidobacteria	10.7 ± 0.7[c]	10.7 ± 0.7	10.4 ± 0.6	10.6 ± 0.7	10.8 ± 0.6	11.0
Bacteroides	10.2 ± 0.6	10.4 ± 0.5	10.2 ± 1.1	10.4 ± 0.6	10.6 ± 0.5	10.6 ± 0.5
Streptococci	10.1 ± 0.8	10.1 ± 0.7	9.9 ± 0.7	9.9 ± 0.5	10.0 ± 0.4	10.4 ± 0.5
Veillonellae	9.8 ± 0.8	9.8 ± 0.7	9.6 ± 0.7	10.1 ± 0.4	9.6 ± 0.8	10.4 ± 0.9
Enterococci	8.1 ± 1.0	7.6 ± 1.3	7.9 ± 1.0	7.9 ± 1.2	8.4 ± 1.3	8.7 ± 1.2
Escherichia coli	9.1 ± 1.0	9.3 ± 0.5	8.8 ± 1.0	9.2 ± 0.8	9.5 ± 0.8	8.7 ± 1.2

[a] Study performed in Santa María Cauqué, Guatemala, 1967 to 1971. [b] Number of specimens examined; specimens corresponded to periods of health. [c] Mean ± SD of $\log_{10}$ bacterial counts.

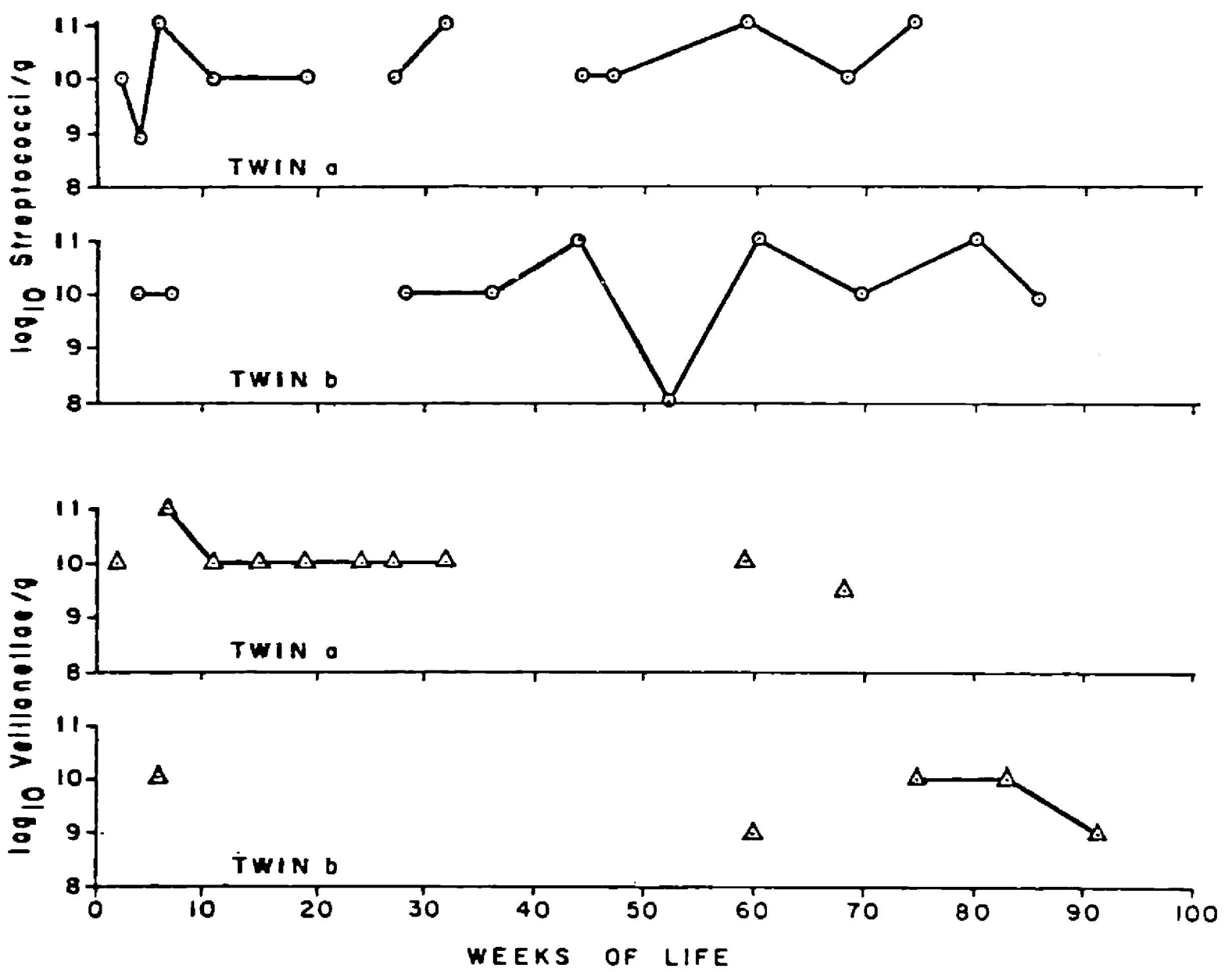

FIG. 1. Counts of streptococci and veillonellae in a pair of twins during the first 2 years of life, Santa María Cauqué, Guatemala, 1967 to 1971.

mately 4 months after the initial 75 weeks mentioned. It should be pointed out that this and other bacterial groups probably are present at all times under normal conditions, but in low concentrations that preclude their demonstration under the laboratory conditions employed.

Similar findings were observed regarding *Bacteroides*, streptococci, *E. coli*, and enterococci in this and other pairs of twins (Tables 2 and 3). However, the ratio of concentration of anaerobes to facultatives remained rather constant in each pair, as well as the predominance of anaerobes, for example bifido-

TABLE 3

Frequency of fecal bacterial groups in three pairs of twins in the first 2 years of life[a]

	Lowest concentration detected	Twins 302		Twins 304		Twins 327	
		a	b	a	b	a	b
		n = 22[b]		*n* = 20		*n* = 18	
Bifidobacteria	9	91[c]	91	90	100	83	94
Streptococci	8	82	73	80	74	94	83
Bacteroides	7	77	73	80	74	94	89
Veillonellae	8	55	50	55	42	94	28
Clostridia	8	9	5	0	11	17	6
Escherichia coli	6	91	82	100	89	94	100
Enterococci	6	82	82	75	74	83	94

[a] Study performed in Santa María Cauqué, Guatemala, 1967 to 1971. [b] Number of specimens examined; specimens corresponded to periods of health. [c] Percentage of specimens positive in total examined.

bacteria and enterobacteriaceae (Tables 1 and 2).

In addition to bifidobacteria, other groups such as veillonellae, streptococci, and *Bacteroides* were demonstrated, particularly with increasing age, although their concentrations during the 1st year of life were usually lower than those of bifidobacteria. The relative frequency of the various bacterial groups is presented in Table 4 for 12 infants whose feces were cultured fortnightly during the 1st year of life. The results correspond to periods of good health only (8). Lactobacilli and clostridia were relatively rare. *E. coli*, in concentrations of at least 10^6, was not common before 2 months, but after this age, it was present in concentrations of 10^6 to 10^{10}.

Changes in fecal flora during weaning

Concomitant with the increasing amounts of supplemental foods provided to the breast-fed child, a protracted shift of fecal flora from the almost exclusive bifidobacteria, to that more typical of the adult was observed with as many or more *Bacteroides* than bifidobacteria. When the child was fully weaned in the 2nd or 3rd years of life, the flora became very similar to that of the adult (Table 5) (8).

Acquisition of "undesirable agents" during weaning

The subtle shift in fecal flora observed with progressive weaning correlated with the intestinal invasion by enteric pathogenic bacteria, parasites, and viruses (3, 6–8). For example, weekly stool cultures in children

TABLE 4

Frequency of bacterial groups in 262 fecal samples of 12 healthy infants during the first 9 months of age[a]

	Lowest concentration detected	Number of specimens positive	Percentage
Bifidobacteria	9[b]	258	98.5
Veillonellae	8	157	59.9
Streptococci	8	152	58.0
Bacteroides	7	75	28.6
Lactobacilli	8	27	10.3
Clostridia	8	17	6.5
Micrococci	4	255	97.3
Enterobacteriaceae	6	233	88.9
Enterococci	6	184	70.2

[a] Study performed in Santa María Cauqué, Guatemala, 1967 to 1968 (8). [b] Log_{10} of bacterial concentration per gram of wet feces.

studied from birth to 3 years of age (5, 6) revealed *Shigella* infections in neonates, likely originating from early contamination with maternal feces (17). However, infections during the exclusive breast-feeding period were rare (8, 18), despite the obvious opportunities for infections. Later, at the end of the 1st year of life, infections increased, and during the 2nd year, more than the 10% of all children, on the average, were shedding shigellae (18). Many of these *Shigella* infections, contrary to behavior in well-nourished populations, were chronic. For example, in a cohort studied weekly during the first 3 years of life, 35% were symptomatically ill for 1 week; 36% lasted at least 5 weeks; and 19%

TABLE 5
Fecal bacterial flora in breast-fed children and in adults, Santa María Cauqué, Guatemala

Bacterial group	12 Breast-fed children, age in weeks				12 Weanlings 2 to 3 years old	12 Adults 13 to 37 years old
	5–8	13–16	21–24	45–48		
Bifidobacteria	11.1[a] (31/31)[b]	11.4 (24/24)	11.6 (22/23)	11.0 (13/13)	10.6 (12/12)	9.4 (9/12)
Bacteroides	9.6 (6/31)	10.3 (6/24)	10.2 (11/23)	9.9 (10/13)	9.2 (10/12)	10.3 (12/12)
Total anaerobes	11.5	11.6	11.6	11.2	11.0	10.5
Total acrobes	8.0	8.8	9.2	9.3	9.0	8.8
Ratio $\frac{\text{anaerobes}}{\text{acrobes}}$	$\frac{3160}{1}$	$\frac{630}{1}$	$\frac{250}{1}$	$\frac{79}{1}$	$\frac{100}{1}$	$\frac{50}{1}$
Percent anaerobes in total	>99.9	>99.8	99.7	98.8	99	98

[a] Average $\log_{10}$ of bacterial counts per gram of wet feces. [b] Number of cultures with 10^8 or more of the bacterial groups in total number of cultures (8).

at least 9 weeks. In contrast, enteropathogenic *E. coli* and *Salmonella* infections ran the usual rapid course (8, 18). Certain parasitic agents like *Giardia* manifested a similar behavior to that of *Shigella*.

Flora in the stomach and small intestine

In a previous investigation of gastrointestinal biopsies, it was found that the stomach and small intestine of the adult were populated by significant numbers of bacteria (10). These were mainly anaerobic streptococci, lactobacilli, bifidobacteria, *Bacteroides*, veillonellae, aerobic cocci, and enterobacteriaceae. Streptococci were found at all levels; bifidobacteria and *Bacteroides* were more typical of the colon. Special histologic techniques (19) showed bacteria embedded in the tags of mucus attached to the mucosa, especially at the tips of villi folds (10).

TABLE 6
Bacterial flora in healthy children from low socioeconomic level, Guatemala, 1970 to 1972

Method and date	Child	Stomach	Duodenum	Jejunum
Plate (1970)	1	10^{7}[a]	10^{2}	10^{6}
	2	10^{7}	10^{5}	10^{8}
	3	10^{7}	10^{6}	10^{7}
	4	10^{4}	10^{7}	10^{3}
Roll tube (1972)	1	3×10^{5}	3×10^{2}	10^{6}
	2	3×10^{2}	10^{6}	10^{6}
	3	3×10^{5}	10^{4}	3×10^{6}

[a] Bacterial count per gram.

Furthermore, four children of the low socioeconomic strata of Guatemala were intubated using a three-lumen radioopaque tube (9), permitting collection of aspirates from stomach, duodenum, and jejunum (10 cm after the ligament of Treitz). Aspirates obtained after overnight fasting were studied by the plate dilution technique, revealing bacterial populations of 10^6/ml in the stomach, duodenum, and jejunum (9, 12) (Table 6). One child showed as many as 10^8/ml.

Recently, three additional healthy children were intubated in the same manner, but the aspirates were collected under anaerobiosis and were processed using the roll-tube technique and prereduced media (20, 21). For quantitation of bacteria, aspirates were diluted (10^{-1} to 10^{-7}) in prereduced peptone–yeast (21) using pipettes. Dilutions were inoculated in roll tubes containing Schaedler's agar, modified (13), and were incubated at 37 C for 3 to 5 days. Colonies were counted; 10 colonies from the highest dilution were transferred to chopped meat medium (21), and incubated at 37 C for 6 or 7 days. Subcultures to blood agar were made at 24 hr to characterize facultatives. All anaerobic strains were studied for aerotolerance, purity, and production of organic acids as detected by gas–liquid chromatography (21). Cultures were grouped in the various genera on the basis of these tests alone (12, 21).

Few bacteria (10^2/ml) were found in the stomach of one child; the other two had ap-

proximately 10^5 bacteria/ml (Table 6). The bacterial concentration in duodenum varied from 10^2 to 10^6, and in jejunum it was 10^6/ml. In the flora of healthy children there was a predominance of anaerobic genera (*Veillonella, Peptococcus, Propionibacterium, Eubacterium,* and *Fusobacterium).* Anaerobes accounted in two cases for 80% of the total duodenal bacteria, and for 50% in the third case. In jejunum, anaerobes represented 100% of the total flora in one child, 70% in another, and 40% in the third child. These results are in agreement with previous observations indicating a variety of anaerobic genera, with facultatives virtually limited to streptococci and gram-negative bacilli, and in fewer numbers than anaerobes (8, 10).

TABLE 7

Fecal bacterial flora of 1-year-old breast-fed children, healthy and with severe diarrhea[a]

Bacterial group	Healthy	Diarrheic
	n = 10	*n = 10*
Facultative, total	9.2[b] (6–10)	9.7 (8–11)
Anaerobic, total	11.5 (11–12)	8.3 (<8–10)[c]
Bifidobacteria	11.2 (11–12)	(<8–10)[d]
Coliforms	9.2 (6–10)	9.4 (8–11)

[a] From (12). [b] Mean of $\log_{10}$ of bacterial concentration per gram of wet feces, range in parentheses. [c] One case had less than 10^8 anaerobic bacteria per gram. [d] Five cases had less than 10^8 bifidobacteria per gram.

The flora in disease conditions

General morbidity. The long-term study of 12 single births during the 1st year of life permitted an evaluation of the fecal flora in relation to disease occurrence. No changes in fecal flora were noted in relation to respiratory infections, uncomplicated measles, and whooping cough, or in exanthematic diseases or skin infections (12). These results have been confirmed in six twins studied in the first 2 to 3 years of life.

Mild and moderate diarrhea. There were no changes in fecal flora during mild or moderate diarrheal disease, and the total concentration of anaerobes and facultatives was basically the same before onset, during disease, and in convalescence. Often, however, changes were observed consisting in *a*) decrease or absence of bifidobacteria at the dilution tested (10^{-6}), *b*) occasional proliferation of streptococci or micrococci to surpass the concentration of bifidobacteria, and *c*) substitution of the common *Escherichia coli* for *Proteus, Providence,* or *Pseudomonas* (12).

Severe diarrhea. Regarding severe diarrhea with dehydration, profound alterations of the fecal flora were revealed when using the plate-dilution technique. These consisted in a marked decrease in anaerobes, mainly bifidobacteria and *Bacteroides* (Table 7) (12). A similar phenomenon was observed in patients with shigellosis (12), particularly Shiga dysentery, a variety characterized by extensive inflammation and ulceration of the mucosa with passage of bloody stools. In this disease, the anaerobic component decreased to levels comparable to those of the facultative flora, or even lower. Bifidobacteria were rarely found, whereas *E. coli* and other enterobacteriaceae formed the bulk of the flora (unpublished observations). Shigellae were present in numbers as high or greater than those of the anaerobic component (Table 8). The administration of an effective drug to Shiga dysentery patients brought prompt relief after 2 to 3 days, as measured by a decrease of pain and discomfort, disappearance of mucus and blood from the stools, and partial or total correction of the flora abnormalities just described. Within 48 hr of administration of nalidixic acid, the total fecal bacterial counts increased, and the concentration of anaerobes returned to normal levels (10^{11}/g) (Table 9). Coliforms raised to 10^5 to 10^{10}, and shigellae were not detected at a 10^{-2} dilution (isolation streak plate). In many cases, anaerobic bacteria such as veillonellae, bifidobacteria, streptococci, and lactobacilli reappeared after treatment, whereas in other cases, these common bacterial groups were not readily cultured for a while. A similar response has been noted in other varieties of shigellosis (unpublished observations). Alterations in fecal flora have been recorded also during diarrheal bouts in chronic recurrent shigellosis. In such occasions, shigellae also increase in numbers (14).

TABLE 8
Fecal flora of Shiga dysentery patients, Guatemala, 1970

	8 Dysenteric patients		12 Healthy persons	
Anaerobes	75[a]	6.1 ± 2.8 (3–10)[b]	100	10–11[c]
Bifidobacteria and lactobacilli	25	2.8 ± 2.1 (3–8)	75	10–11
Bacteroides	50	4.4 ± 2.6 (3–7)	100	10–11
Clostridia	0		40	9–10
Streptococci	63	5.5 ± 3.1 (3–10)	75	10–11
Veillonellae	0		50	9–10
Facultatives and aerobes	100	7.2 ± 0.7 (6–8)	85	9–10
Enterococci	38	3.9 ± 2.6 (3–7)	9	7–8
Micrococci	63	3.6 ± 2.6 (3–6)	50	4–5
Yeasts	0		25	4–6
Coliforms	100	7.1 ± 0.6 (6–8)	100	9–10
Shiga bacillus	100	6.8 ± 0.5 (6–7)	0	

[a] Percentage of cases with at least 10^3 bacteria of the particular group per gram or milliliter. [b] Mean ± SD of $\log_{10}$ bacterial counts; range in parentheses. [c] Range.

Protein–calorie malnutrition. A total of 13 children with severe protein–calorie malnutrition were studied, and again the plate-dilution technique was employed (9). The subjects were placed on a diet similar to the one received in their homes for 1 or 2 days. Aspirates were then obtained from the stomach, duodenum, and jejunum, after overnight fasting (9). No striking differences were noted in the total bacterial counts of the stomach when compared with control children who were not malnourished. All 13 cases had bacteria in the duodenum and as many as 10^7 to 10^8/ml in the jejunum, that is, not in excess of values in control subjects. However, significant alterations were noted in the fecal flora, consisting in proliferation of facultatives (often outnumbering the anaerobes), frequent absence of bifidobacteria, and colonization with *Proteus*, *Pseudomonas*, and other gram-negative bacilli.

A definite decrease in total bacterial counts in stomach and small intestine was observed upon dietary treatment. Also, the inversion of the ratio anaerobes/facultatives, found in feces, was corrected (Table 10). The control children could not be kept in the hospital for prospective study under similar conditions.

Additional observations were made in four children with protein–calorie malnutrition from whom aspirates were collected under anaerobiosis, for inoculation in prereduced roll tubes. Again, relatively high counts of bacteria were found in the duodenum and jejunum of three children; these were about 1 log higher than those of children from the same ecosystem, without malnutrition, studied at the same time and under the same laboratory conditions (Table 11). The other child was colonized with *Candida* in the stomach and small intestine, with no other microorganism cultured from these sites. It was notable that in severely malnourished

TABLE 9
Fecal flora of Shiga dysentery patients in relation to treatment with nalidixic acid, Guatemala, 1970

Bacterial group	Prior to treatment	Days of treatment		Normal flora
		3 to 5	6 to 10	
	n = 8	*n* = 6[a]	*n* = 5[a]	
Anaerobes	6.1 ±2.8[b]	10.3 ±0.5	10.2 ±0.8	11
Bifidobacteria + lactobacilli	2.8 ±2.1	6.8 ±3.8	6.6 ±4.3	9–11
Bacteroides	4.4 ±2.6	10.0 ±0.6	8.4 ±3.6	9–11
Streptococci	5.5 ±3.1	8.8 ±3.4	8.0 ±3.4	9
Veillonellae	<2[c]	4.0 ±3.2	3.4 ±3.1	9–11
Facultatives	7.2 ±0.7	8.5 ±0.8	7.8 ±2.3	9–10
Enterococci	3.9 ±2.6	7.7 ±1.0	6.6 ±2.6	6–7
Micrococci	3.6 ±1.5	4.8 ±2.7	3.0 ±1.4	4–7
Coliforms	7.1 ±0.6	7.2 ±1.5	4.8 ±3.1	7–10
Shiga bacillus	6.8 ±0.5	<2	<2	<2

[a] Attrition in samples due to refusal to cooperate. [b] Mean ± SD. [c] Less than 10^2 bacteria per gram or milliliter.

TABLE 10
Jejunal and fecal flora of three children with severe PCM during nutritional treatment, Guatemala, 1970 to 1971

Child number	Jejunum		Colon	
	Anaerobic	Facultative	Anaerobic	Facultative
Upon admission				
PC-222	7[a]	7	10	10
PC-226	7	5	9	10
PC-241	6	6	10	11
Stabilization phase				
PC-222	7	7	11	10
PC-226	6	6	10	9
PC-241	<2	<2	11	9
Recuperation phase 1				
PC-222	<2	3	11	10
PC-226	6	6	10	9
PC-241	<2	<2	11	9
Recuperation phase 2				
PC-222	3	2	11	10
PC-226	5	4	11	8
PC-241	<2	2	10	9

[a] Log_{10} of bacterial counts per milliliter (or gram) of specimen.

children, fewer groups or genera of bacteria were detected in the small bowel than in controls without PCM, and that the small bowel flora of malnourished children had fewer anaerobes and relatively more facultatives. The genera cultured from the small intestine of children with protein–calorie malnutrition were *Bacteroides, Lactobacillus, Veillonella, Propionibacterium, Escherichia,* and *Klebsiella.*

Discussion

The renewed interest in the microbiota in the last decade has resulted in the accumulation of information (22, 23) which in many respects, only seems to mark the beginning of an understanding of the significance of the flora for the human host. That the intestinal microbiota, in particular, has important homeostatic functions is a well-accepted concept (24–26). However, much needs to be learned with regard to its development, associations with mucosal tissues, and its anatomic, physiologic, and immunologic significance for the human host. This would appear important in terms of application of knowledge, particularly in areas where man is exposed to malnutrition and increased infection with pathogens.

The present paper summarizes long-term observations made during the last 7 years in children of low socioeconomic condition, studied in their ecosystem, and in most instances with minimal intervention. The development of the intestinal microflora early in life, its stability during breast-feeding, and the striking similarities among all subjects became obvious after prospective study of infants in their first years of life. The fecal flora of infants was remarkably similar, although differences were noted even between twins of the same pair. The gross similarities in flora among individuals of the same ecosystem are indicative of the existence of host and environmental factors (the chemical microenvironment, diet, and infection with pathogens) determining and influencing the composition and characteristics of the flora.

It is accepted that the predominant bifidobacteria are responsible for the low frequency of *Shigella* and other enteropathogens during breast-feeding (8, 27), probably as a result of various factors among which bacterial interactions play a role (28). The particular characteristics of human milk (concentration of

TABLE 11
Bacterial flora in children from a low socioeconomic level, roll-tube method, Guatemala, 1971 to 1972

	Children	Stomach	Duodenum	Jejunum
With protein-calorie malnutrition	PC 250	5.1×10^{4}[a]	2.5×10^{5}	5.4×10^{6}
	PC 252	4.0×10^{4}	10^{7}	4.0×10^{7}
	PC 256	6.7×10^{6}	1.5×10^{6}	1.5×10^{6}
Without protein-calorie malnutrition	1	3.0×10^{5}	3.0×10^{2}	10^{6}
	2	3.0×10^{2}	10^{6}	10^{6}
	3	3.0×10^{5}	10^{4}	3.0×10^{6}

[a] Count per gram.

lysozyme and secretory IgA) also appear important in intestinal resistance (29). Bifidobacteria decrease significantly with weaning, a protracted process in the study area favoring deterioration of nutrition and acquisition of pathogenic agents. Changes in diet composition, the progressive attrition of "bifidus" factor and lysozyme, and the increased frequency of pathogenic infection are likely related to the subtle shift in the flora of the breast-fed child to that more characteristic of the adult. Gram-negative anaerobes become more frequent, whereas bifidobacteria decrease in numbers, a process accompanied by a rise in the rate of diarrheal disease (8, 11).

The intestinal microflora is in close contact with the mucosal epithelium. Various kinds of bacteria are visualized embedded in the mucus overlay of the intestinal epithelium of man (10, 30). The bacteria cultured from these specimens have been the same as those isolated from the lumen by employing similar bacteriologic techniques. It is expected that the flora exerts some influence on host nutrition processes, cell turnover, and maintenance of the mucosa, although readily stained and/or cultured bacteria do not form the thick plaques described in rodents (19, 31). In the Guatemalan subjects, bacteria were found at the sites of desquamation of epithelial cells, and may stimulate shedding, or accumulate in the tags as a result of the pumping action of the villi.

It may be expected that alterations in the flora lead to mucosal abnormalities; on the other hand, mucosal lesions alter the microbiota. The finding of significant numbers of bacteria in the small bowel of apparently healthy children living in poor environments, as well as in the small intestine of children with malabsorption or PCM from the same ecosystem, suggests that bacteria play a role in the genesis of these syndromes or in the maintenance of the status quo (9). It should be kept in mind that all children studied were originally breast-fed and underwent a slow weaning process. During the course of weaning, parasites and pathogenic bacteria gained entrance and often became established. Many children were virtually "colonized" by *Shigella* for weeks or months (3, 18). The acute and chronic infection with undesirable agents is a source of continuous insult and damage to the intestinal mucosa and its function. Malnutrition is an underlining factor. The summation of all these stresses appear to be responsible for the maintenance of an increased "physiologic" inflammation of the intestinal mucosa, so often reported in less developed societies (32). With malnutrition, intestinal motility diminishes, and it is not hard to visualize the occurrence of bacterial overgrowth with its many complicating effects (33, 34). Large numbers of bacteria, particularly entertobacteriaceae, in unusual sites may aggravate malabsorption in the course of malnutrition. The action of indigenous bacteria would be through release of enterotoxin, splitting of bile acid conjugates, production of harmful metabolites, or direct penetration into the mucosa (9).

On the other hand, more evidence is available regarding alterations of the flora as a consequence of damage to the intestinal mucosa. The typical example is shigellosis in which severe mucosal inflammation and ulceration is followed by an almost virtual disappearance of some components of the flora. This alteration was rapidly corrected by blocking *Shigella* activity with an appropriate drug. Thus, the arrest of *Shigella* mutiplication stops the agent-induced phenomena responsible for the pathogenesis of the disease.

Current evidence shows the unique characteristics of the bacterial flora of breast-fed infants, the interrelation of flora and diet, the protective nature of the flora against infection, and the indispensable need of a healthy mucosa for the maintenance of a protective flora. Epidemiologic studies have shown that deterioration of the nutritional status, changes in composition of the flora, increased frequency of pathogenic infection, and increased rate of diarrheal disease are interrelated phenomena. The interplay of all these factors lead to chronic malabsorption and malnutrition.

Many questions still require scrutiny. More knowledge is needed on the role of bacteria in the nutrition processes, and on the significance of individual bacterial species or groups in terms of mucosal physiology and

defense against infection. The beneficial effect of bifidobacteria as a predominant flora in breast-fed babies is recognized, but it is not known how these bacteria could be established in the absence of human milk.

These and other questions need investigation, although it appears that some solutions can be proposed on the basis of existing knowledge. Measures must consider improvement of the nutritional status and reduction of the risk of infection. These will not be as effective if applied independently. How to implement measures will depend on specific or particular situations and on the application of appropriate knowledge in a rational way.

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