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EVOLUTION OF INTESTINAL FLORA OF CHILDREN IN HEALTH AND DISEASE

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Despite material advances in intestinal microbiology during the past decade, difficulties persist. Proper understanding of relationships among components of the microbial flora is pertinent, both in health and disease. This has particular significance because the etiology of a great part of the acute and chronic diarrheal diseases of the general population is still unknown. Yet, the biological significance of the intestinal flora in nutrition, in host defense and other important ways has been well documented in germfree and conventional animals (Luckey, 1965; Dubos, Schaedler & Costello, 1963). That the intestinal flora of necessity also has important functions in the human host is a wholly reasonable assumption. The present need is for fundamental information on the evolution of man's gastrointestinal flora in health and the changes incident to genesis of disease.

Population. The data derive mostly from long-term prospective observations of Mayan-Indian children from a Guatemalan highland village (Mata, Urrutia & García, 1967). Infants were recruited at birth and followed

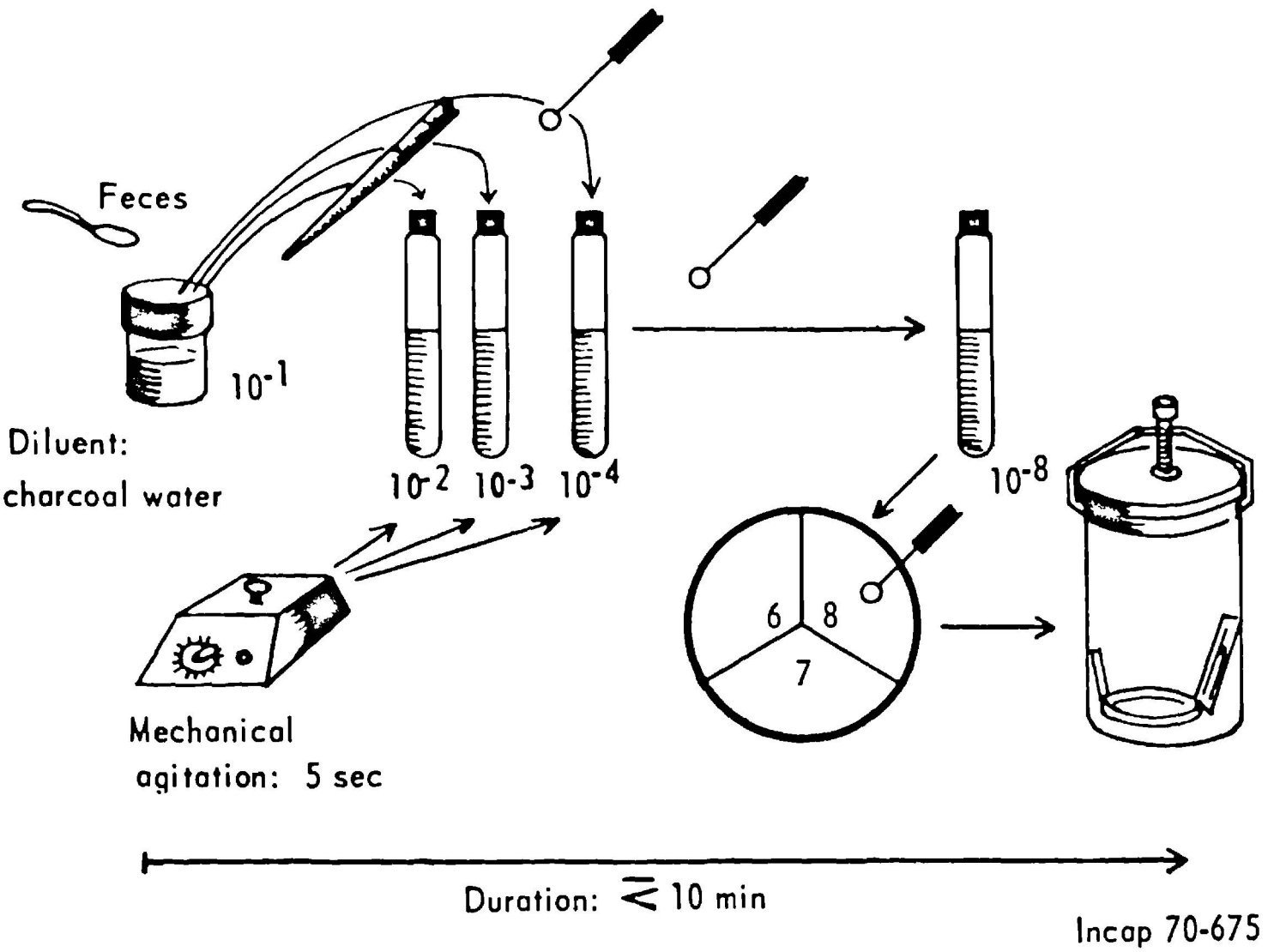
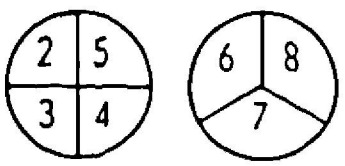
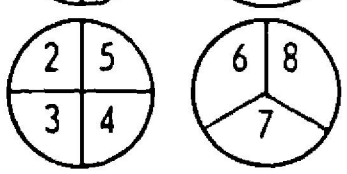
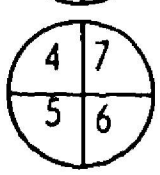
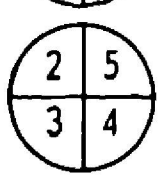
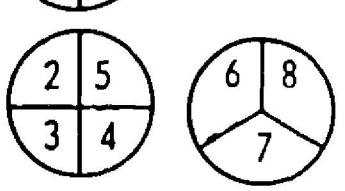


Figure 1. Procedure to quantitate the intestinal flora. Feces are measured with a calibrated plastic spoon; aspirates with a pipette. Dilutions 10^{-2} and 10^{-3} are made with a pipette; dilutions 10^{-3} to 10^{-4} with calibrated (0.01 ml) platinum-rhodium loops. Agar areas are thoroughly streaked with calibrated loops.

Agar Medium	Bacterial Group	Incubation at 37°C	Range of Bacterial count (log 10)
BC-I, BC LS 	Bacteroides, Clostridia Bifidobacteria, Lactobacilli Streptococci, Veillonellae	48 hr Anaerobic	4 - 12
L 	Microaerophilic Lactobacilli and Streptococci	48 hr Candle jar	4 - 12
E 	Enterococci	24 hr Aerobic	6 - 11
MS 	Micrococci, Staphylococci	24 hr Aerobic	4 - 9
T7T 	Enterobacteriaceae	24 hr Aerobic	4 - 11

systematically for various periods to determine the pattern by which bacterial groups were acquired, the evolution of the intestinal flora, and its varied reaction to different kinds of stress (Mata & Urrutia, 1970). All infants were breast-fed beginning as soon after birth, as milk became available, and for the next one to three years.

Weaning in this culture is a slow and prolonged process, starting at 3 to 4 months with introduction of small amounts of fluids or semisolid foods. Solids are introduced somewhat later, so that by one year of age the child, although still breast-fed, is receiving small quantities of a diet similar in nature to that of the adult. The nutritional state of the child starts to deteriorate at 4 to 5 months of age because the available maternal milk no longer meets the needs of the growing child. The nutritionally stressful period is the second year of life as the deficit in food supplements becomes more pronounced (Mata, Urrutia & Lechtig, 1970).

Another set of data was from children with severe diarrhea or with protein-caloric malnutrition, drawn from several Guatemalan villages.

Methods

Eight to 10-fold dilutions of feces or fluid aspirates (according to the specimen being studied), were prepared. Amounts of 0.01 ml of certain dilutions were plated on areas of Schaedler media (Schaedler, Dubos & Costello, 1965) as modified in this laboratory (Mata, Carrillo & Villatoro, 1969) (Fig. 1). Gaspak disposable generators were used to produce anaerobiosis (Brewer & Allgeier, 1966).

The bacterial groups shown in Table I, were identified by a combination of criteria such as sensitivity to oxygen, microscopic and colony morphology, and growth in various agar media (Dale & Mata, 1968; Mata, Carrillo & Villatoro, 1969; Nelson & Mata, 1970).

Selected isolates from the highest dilutions were subcultivated in pre-reduced media (Moore, 1966) and some of their fermentation products analyzed by silicic-acid and gas-liquid chromatography (Moore, Cato & Holdeman, 1969; Cato, Cummins, Holdeman, Johnson, Moore, Smibert & Smith, 1970).

Results

A. Fecal Flora of the Breast-Fed Infant

The neonatal period. Fifty village newborns were observed. Under normal conditions, the intestinal tract of newborn infants is free of microorganisms. Bacteria occasionally were cultivated from meconium four hours after birth. Six of nine samples passed 4 to 7 hours after birth contained facultative bacteria, mainly micrococci, streptococci, and enterobacteriaceae.

Figure 2 shows the progressive appearance of bacteria during the neonatal period. Half of the infants had *Escherichia coli* in concentrations of 10^8 to 10^{11} per gram of feces during the first 24 hours of life. By the second day, all infants had *E. coli*, with counts ranging from 10^5 to 10^{11} per gram.

A few infants had bifidobacteria on the first day and a third by the second day of life, in concentrations that ranged between 10^8 and 10^{10} per gram. Frequencies increased with age, so that by the end of the first week, all infants had these bacteria in concentrations ranging from 10^{10} to 10^{11} per gram (Fig. 2).

Eighty-three per cent of newborn infants showed anaerobic streptococci on the first day in concentrations of 10^6 to 10^{10} per gram of wet feces. By the second day the counts were 10^9 to 10^{11} per gram. Streptococci and *E. coli*, then more frequent than bifidobacteria in the first days of life, decreased by the end of the neonatal period to low frequencies. When found, concentrations ranged from 10^7 to 10^{11} per gram.

Clostridia appeared in a few infants from the first day, to reach high titers (10^8 to 10^{11}) on the second day. Few infants, however, had these bacteria on the third day or thereafter.

Bacteroides and veillonellae were not detected on the first day in dilutions of 10^{-4}. Few infants were colonized with these bacteria during the neonatal period; those that were had them from the second day. When

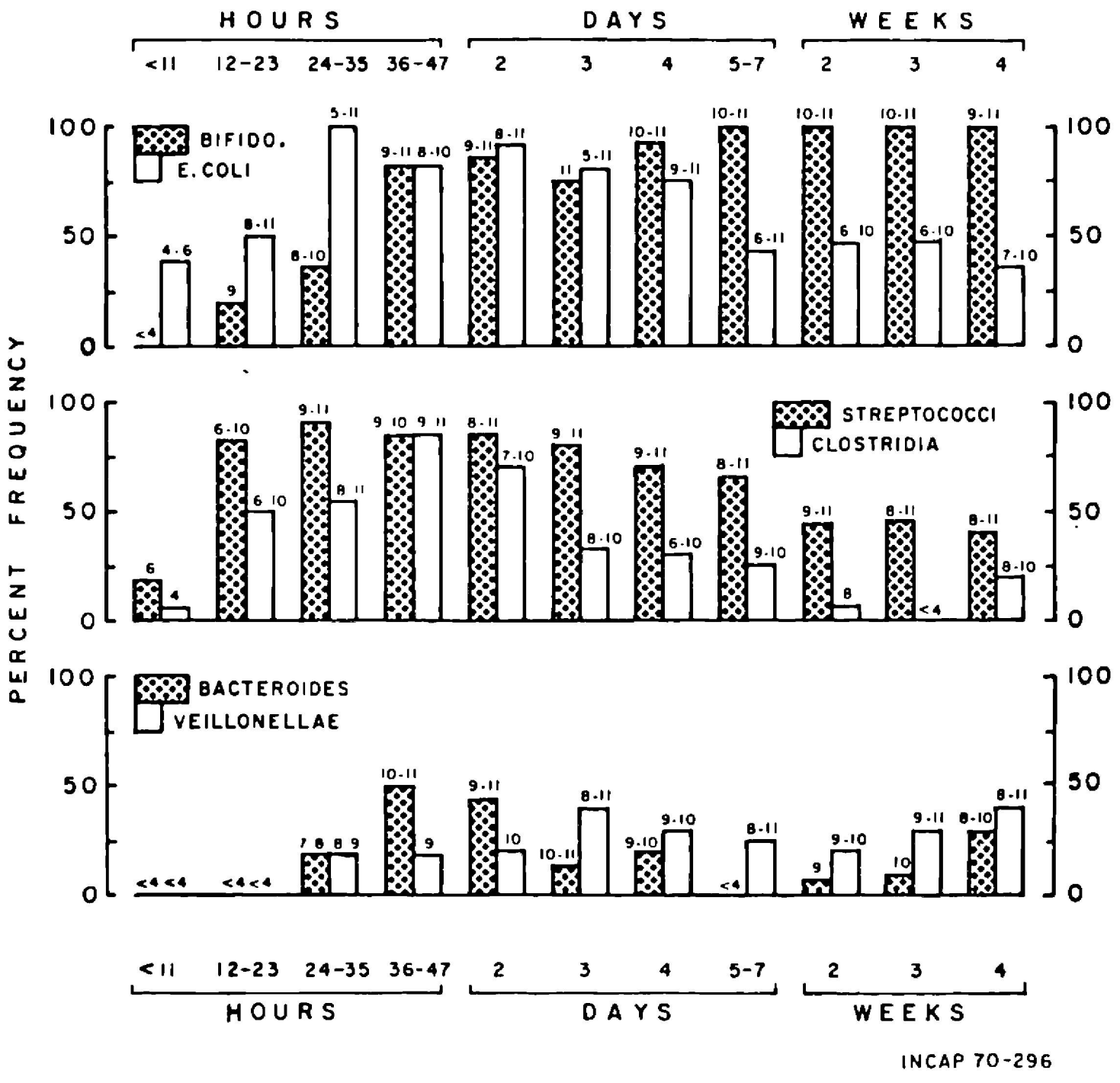


Figure 2. Bacterial groups in meconium and feces of breast-fed Mayan-Indian neonates, Santa María Cauqué, Guatemala, 1967-1968. Numbers on top of columns indicate range of bacterial counts, as reciprocal of $\log_{10}$ of concentration per gram of wet meconium or feces (from Mata & Urrutia, 1970).

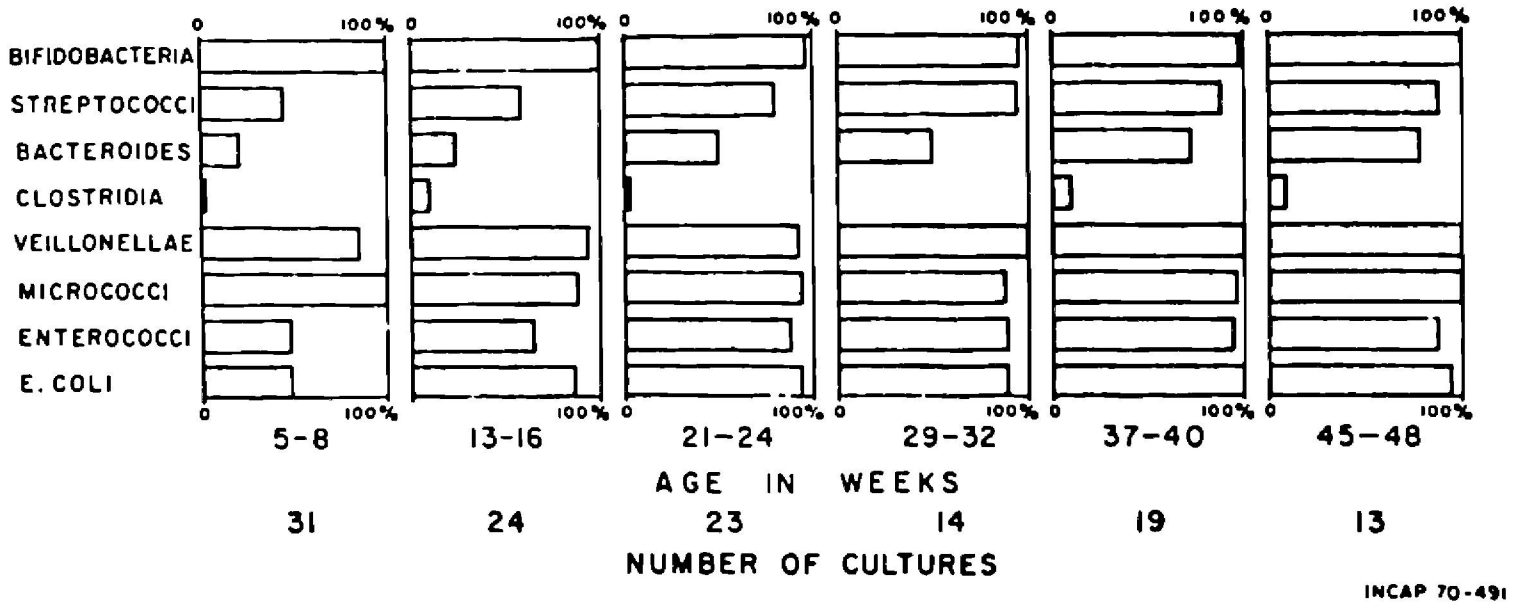


Figure 3. Bacterial groups in 12 breast-fed Mayan-Indian infants studied from birth to one year of age, during disease-free periods, Santa María Cauqué, Guatemala, 1967-1968. Minimum bacterial count values detected are: 10^8 for bifidobacteria, streptococci, bacteroides, clostridia and veillonellae; 10^6 for enterococci; and 10^4 for micrococci and *E. coli* (from Mata & Urrutia, 1970).

present, concentrations ranged from 10^8 to 10^{11} per gram.

The post-neonatal period. Twelve breast-fed infants were observed throughout the first year of life. Bifidobacteria continued as the most frequent group, in concentrations of 10^{10} to 10^{11} per gram (Fig. 3). Streptococci, veillonellae, and bacteroides, increasing progressively with age, were present in more than 75 per cent of the study group by the end of the first year.

The frequency of anaerobic bacteria cultured in concentrations of 10^8 per gram or more during the first 9 months of life is summarized in Table II. In breast-fed infants, bifidobacteria definitely were commonest. Eubacteria, catenabacteria, and other anaerobes were detected irregularly.

Enterobacteriaceae were always present at all ages noted. *E. coli*, however, was not present, in concentrations of 10^6 or more, in all children within the neonatal period, but increased steadily with age. At two months, this bacterial group was found in all infants; and, in most instances where cultures were made. Enterobacteriaceae and other aerobic or facultative bacteria were always present in concentrations 2 to 3 logs below the level attained by bifidobacteria.

Clostridia was rarely found in the post-neonatal period in concentrations of 10^8 or more per gram. Along with other rare bacteria, they may have been present more consistently, although in low and unimportant numbers.

Bifidobacteria are anaerobic at first isolation, although they become somewhat tolerant to low concentrations of oxygen on continued cultivation. They are non-sporulated, strongly gram-positive straight rods in palisade-like arrangement. Branching is not observed in all primary cultures or upon passage, but all village strains exhibit branching if grown in liquid media with 0.35 M NaCl, as recommended by Kojima, Suda, Hotta & Hamada (1968).

More elaborated methods were applied to selected cultures in order to identify the various bacterial groups more precisely. Figures 4 and 5 show the fermentation patterns as analyzed by silicic-acid and gas-liquid chromatography. In general, *Bifidobacterium*,

Table II

Frequency of anaerobic bacteria in 262 fecal samples of healthy infants, during the first nine months of age, Santa María Cauqué, Guatemala 1967 — 1968

Bacterial group	Number of samples positive*	Percentage
Bifidobacteria	258	98.5
Veillonellae	157	59.9
Streptococci	152	58.0
Bacteroides	75	28.6
Lactobacilli	27	10.3
Clostridia	17	6.5

* At the dilution 10^{-8} or greater.

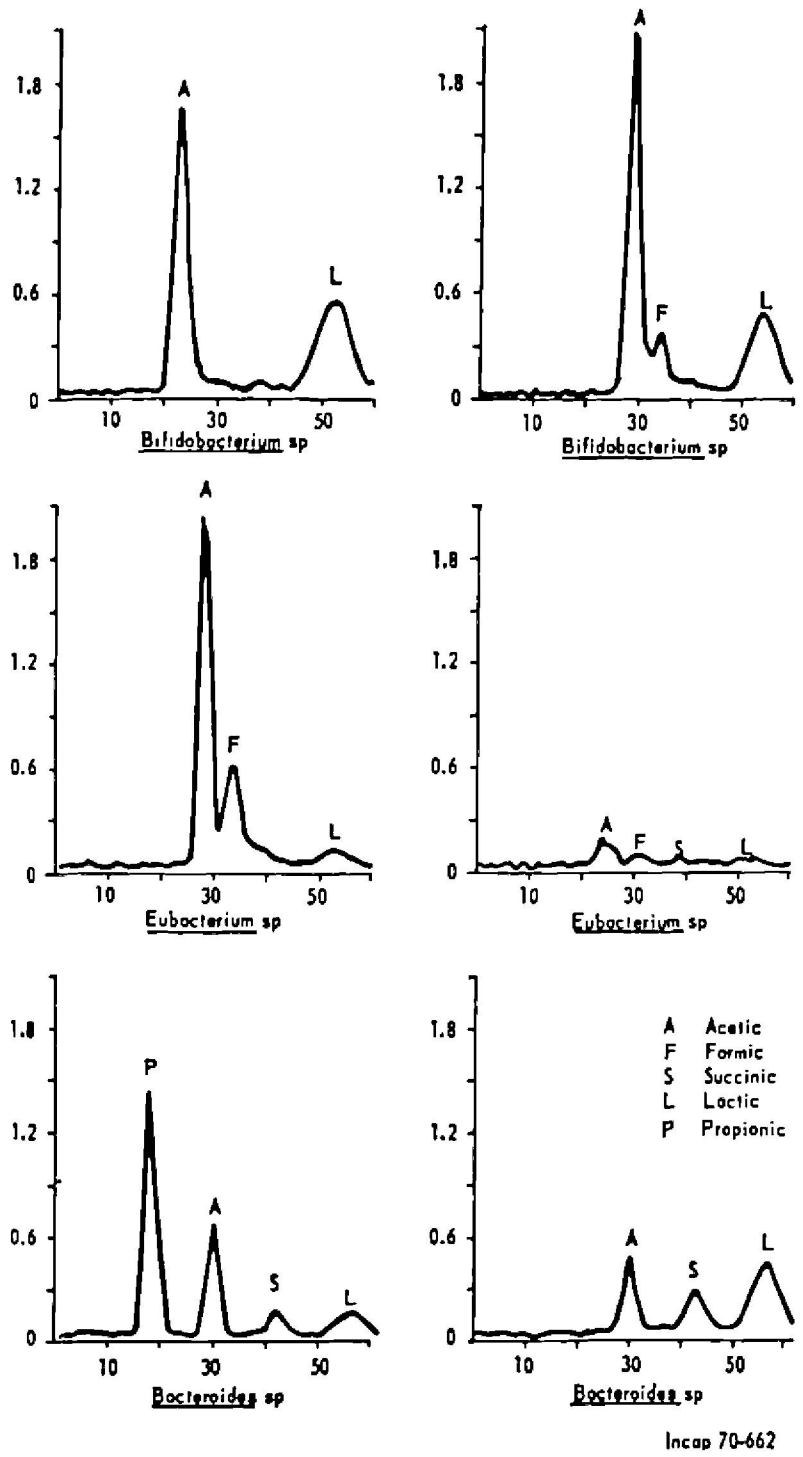


Figure 4. Silicic-acid chromatography of cultures of indigenous bacteria isolated from breast-fed infants, Santa María Cauqué, Guatemala, 1970.

Bacteroides and *Veillonella*, were confirmed by these methods. *Eubacterium* may have been confused with *Bifidobacterium*, but few were isolated from breast-fed infants, compared with bifidobacteria.

Shift to an adult fecal flora. The "simple" flora of breast-fed infants, almost wholly of bifidobacteria, remains thus during the period of exclusive breast-feeding (from birth to 3 months of age). This is related to a stimulating factor present in maternal milk in large concentrations (György, 1957). During said period, the average concentration of *E. coli* is low (Fig. 6). As food supplements are introduced, streptococci and bacteroides become more frequent, in concentrations above 10^8 per gram. Proliferation of facultative gram-negative bacilli and enterococci also occurs. More particularly, concentrations of facultative bacteria became relatively greater during the periods 13 to 16 and 21 to 32 weeks of age. This component was almost exclusively enterobacteriaceae, of which *E. coli* was the dominant group.

Microorganisms present in breast-fed infants, weaned children and adults of the area are compared in Table III. Nearly all culturable bacteria in breast-fed infants were bifidobacteria. In the three groups, the anaerobic component always outnumbered the aerobic by two to three logs. During weaning anaerobes decreased by one log. In the adult, bacteroides were more frequent than bifidobacteria, outnumbering all other bacterial groups.

The transition of one flora into another is a slow and subtle process that begins with initiation of weaning, continues throughout

Table III

Fecal bacterial flora of healthy breast-fed children, and of adults
Santa María Cauqué, Guatemala, 1967 — 1969

Bacterial group	12 Breast-fed infants 5 to 8 weeks old	12 Weanlings 2 to 3 years old	12 Adults 13 to 37 years old
Bifidobacteria	11.1* (31/31)**	10.6 (12/12)	9.4 (9/12)
Bacteroides	9.6 (6/31)	9.2 (10/12)	10.3 (12/12)
Total anaerobes	11.5	11.0	10.5
Total facultative bacteria	8.0	9.0	8.8
Ratio anaerobes / Facultative	3160 / 1	100 / 1	50 / 1
% anaerobes in total	99.9	99	98

* Average $\log_{10}$ of bacterial counts per gram of wet feces.
** Number of cultures with 10^8 or more of the bacterial group in total number of cultures.
(Adapted from Mata & Urrutia, 1970).

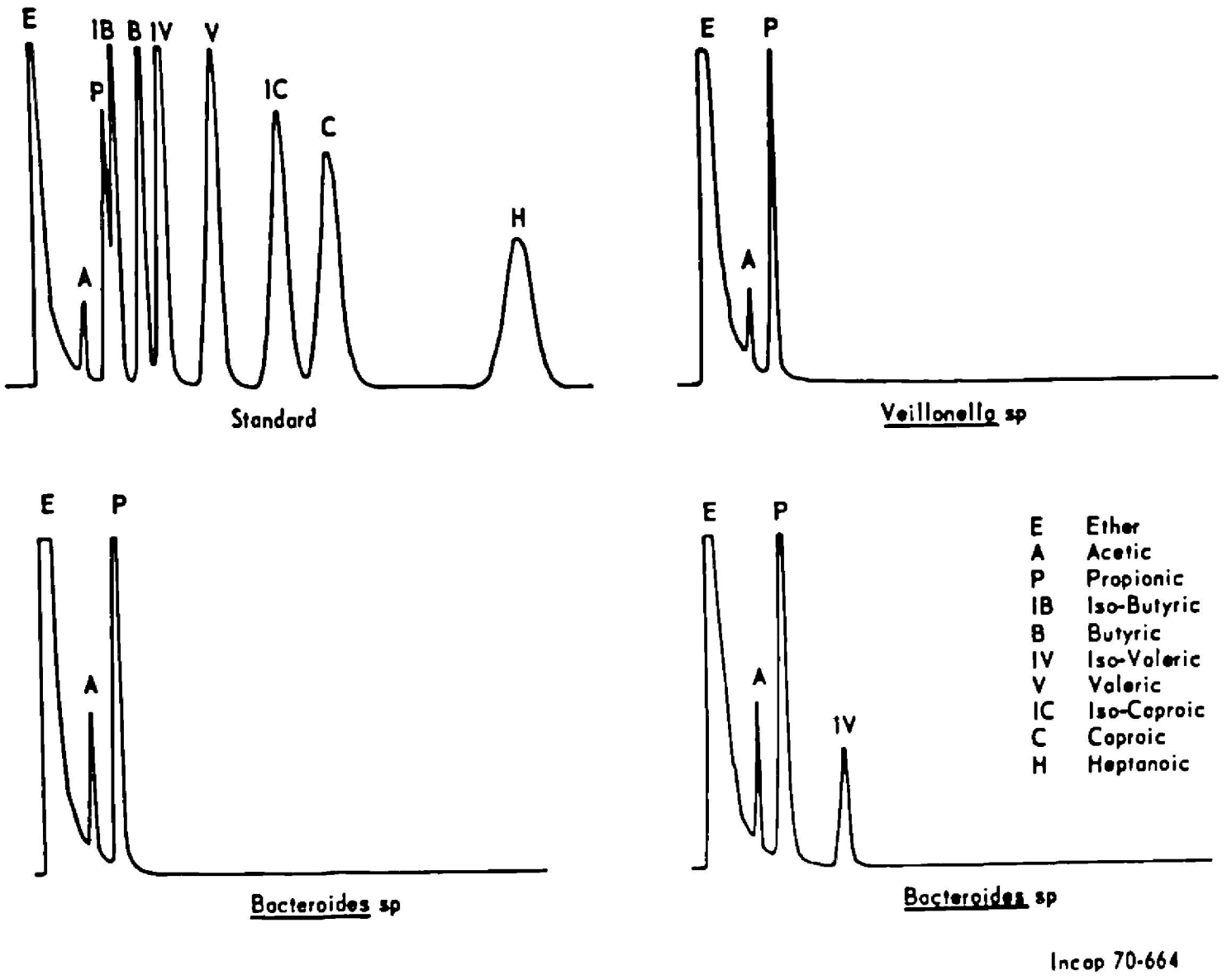


Figure 5. Gas-liquid chromatography of cultures of indigenous bacteria isolated from breast-fed infants, Santa María Cauqué, Guatemala, 1970.

that prolonged period, and is marked by higher rates of diarrheal disease (Mata, Fernández & Urrutia, 1969).

B. *Tre Intestinal Flora in Disease*

No qualitative or quantitative changes in the fecal flora were detected during respiratory infections, uncomplicated measles and other exanthems, and skin infections.

Diarrheal disease. Data on the flora in diarrheal disease derives from the cohort of 12 children studied throughout the first year of life. Figure 7 gives average concentrations of facultative and anaerobic bacteria. No differences were noted in the concentration of total aerobes and anaerobes before onset of symptoms, at onset, or during the following 5 days. However, qualitative changes occurred: a) bifidobacteria were not detected in

Table IV

Fecal bacterial flora of one-year-old breast-fed children, healthy and with severe diarrhea

Bacterial group	n = 10 Healthy	n = 10 Diarrheic
Facultative, total	9.2* (6-10)	9.7 (8-11)
Anaerobic, total	11.5 (11-12)	8.3 (<8-10)**
Bifidobacteria	11.2 (11-12)	(<8-10)***
Coliforms	9.2 (6-10)	9.4 (8-11)

* Average of $\log_{10}$ of bacterial concentration per gram of wet feces.
** One case had less than 10^8 anaerobic bacteria per gram.
*** Five cases had less than 10^8 bifidobacteria per gram.

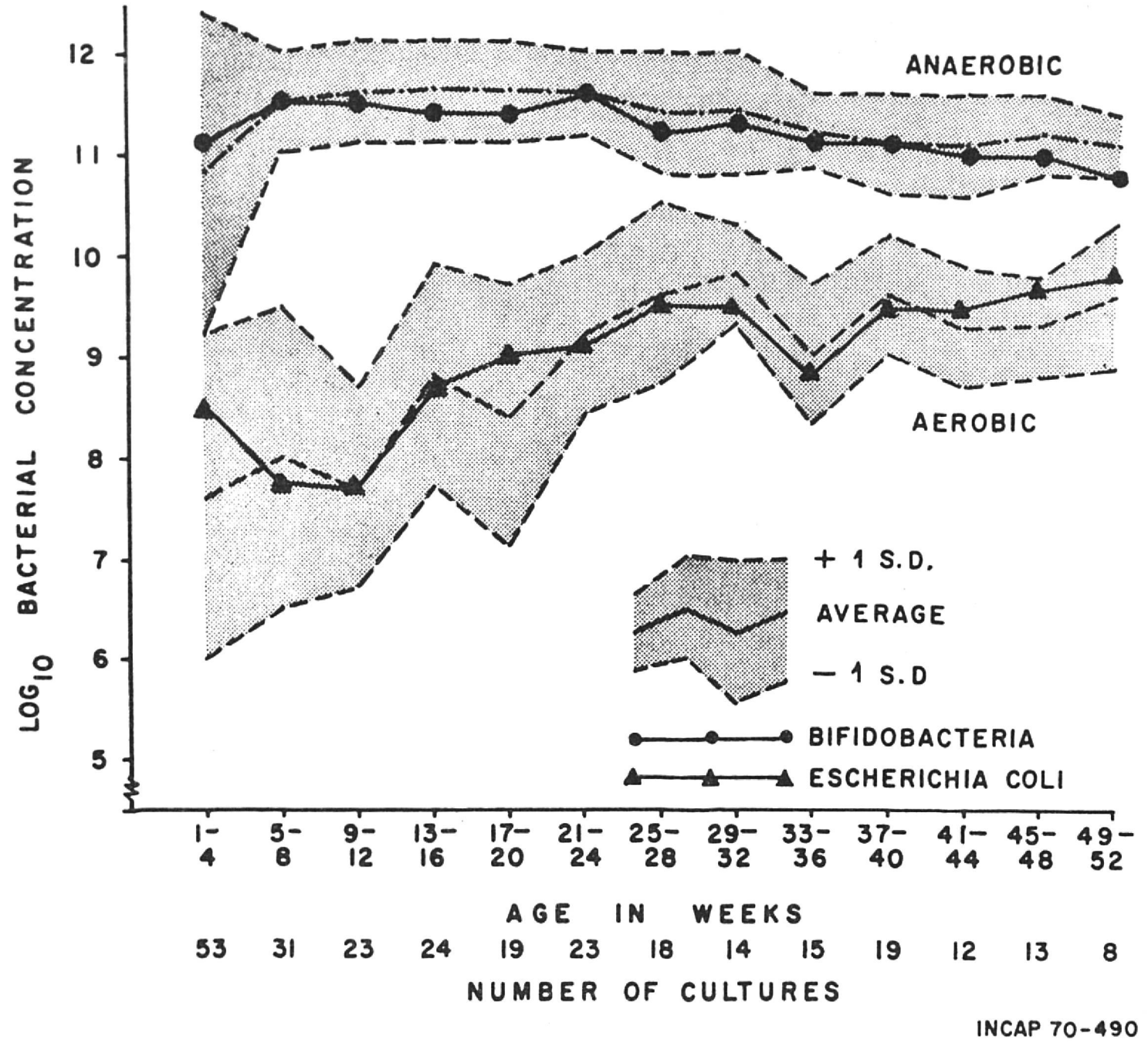


Figure 6. Anaerobic and facultative fecal flora of 12 breast-fed infants studied from birth to one year, during disease-free periods, Santa María Cauqué, Guatemala, 1967-1968 (from Mata & Urrutia, 1970).

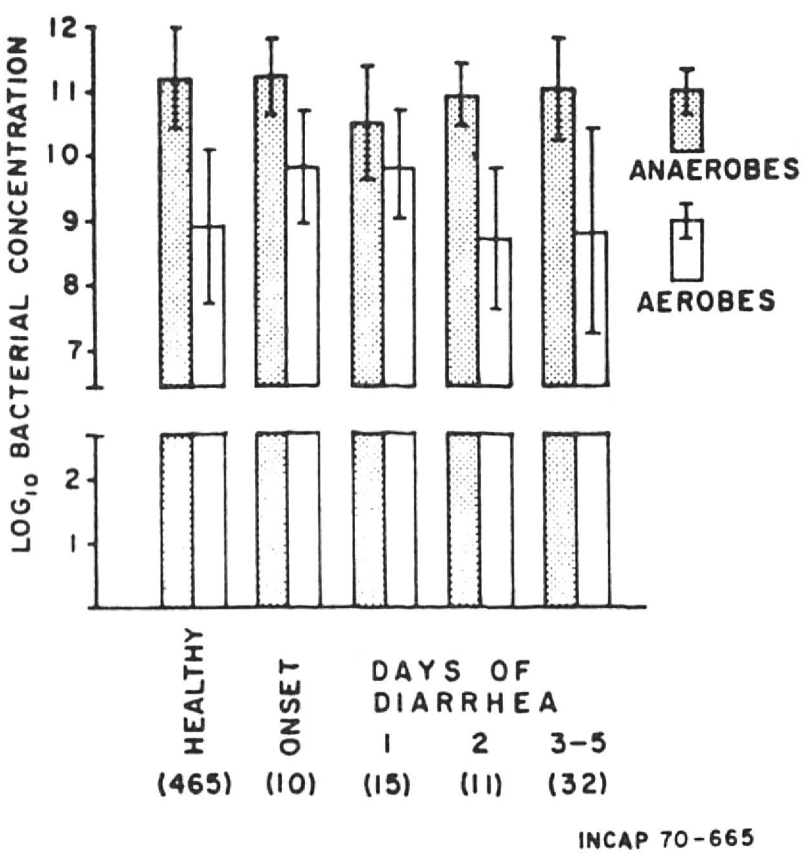


Figure 7. Bacterial fecal flora of infants in relation to onset of diarrhea, Santa María Cauqué, Guatemala, 1967-1968. Bars represent arithmetic averages and standard deviations of $\log_{10}$ total bacterial counts. Figures in parentheses are numbers of specimens studied.

the feces of several patients with diarrhea at a dilution of 10^{-8}; b) in some cases, streptococci became the dominant flora, replacing bifidobacteria; c) certain groups of gram-negative facultative bacilli, such as *Proteus* and *Providence*, predominated over the usual *E. coli*.

Severe diarrhea. Since most diarrheas of breast-fed infants are clinically mild, older children with severe diarrhea (diarrhea with blood and mucus in the stools) were investigated. A profound alteration of the fecal flora, primarily a marked decrease in anaerobes, particularly bifidobacteria and bacteroides, was observed in these cases (Table IV). This was often accompanied by a proliferation of facultative gram-negatives, but these were sometimes reduced.

Shigellosis. Similar alterations were observed in severe diarrhea associated to *Shigella* infection. The anaerobic flora decreased to levels comparable to the facultative flora or even less (Table V). Bifidobacteria were not found. *E. coli* and other enterobacteriaceae formed most of the facultative flora. Shigellae

Table V

Fecal flora of patients with shigellosis in relation to antimicrobial treatment, Santa María, Cauqué, Guatemala, 1970

Days after treatment →	*S. dysenteriae* 1		*S. dysenteriae* 2			*S. sonnei*			
	−1	2	6	−2	3	6	−1	2	5	7
Total Anaerobes	7*	←10	11	9	11	11	8	11	11	11
Bifidobacteria and Lactobacilli	0**	← 0	0	0	10	10	0	9	11	11
Bacteroides	7	←10	11	8	10	0	8	9	0	0
Streptococci	0	← 0	0	9	11	11	0	10	10	9
Veillonellae	0	← 0	0	9	10	10	0	0	10	0
Clostridia	0	← 0	9	0	0	9	0	0	0	0
Total Facultative Bacteria	8	10	10	9	7	6	9	10	10	9
Shigellae	7	0	0	8	0	0	8	0	0	0
Enterobacteriaceae	6	9	9	9	7	6	9	10	10	7
Enterococci	7	6	8	7	7	8	6	9	10	7
Micrococci	4	4	5	4	7	0	5	6	6	5

* Numbers are $\log_{10}$ of bacterial concentration per gram (or ml) of feces.
** 0 = Bacteria not found at dilution 10^{-4}.

were present in concentrations of 10^6 to 10^8 per gram.

Patients with Shiga dysentery (*S. dysenteriae*, type 1) had a flora even more extensively altered. This variety of shigellosis was characterized by marked pathological changes in the intestinal mucosa, extending frequently from the small intestine to the rectum, leaving no segments of the mucosa intact. Pathological alterations induced by *S. dysenteriae* 1 account for the severe clinical manifestations and high lethality of this disease entity (Mata, Gangarosa, Cáceres, Perera & Mejicanos, 1970).

The administration of an effective drug such as nalidixic acid, was followed by a reduction in numbers of shigellae and a rapid increase in anaerobes (Table V). The altered flora persisted for weeks or months.

Severe protein-calorie malnutrition. Children with acute protein-calorie malnutrition were admitted to the hospital and intubated according to a special technique (Schneider, Viteri & Mata, 1970). After fasting for 12 hours, specimens were obtained under fluoroscopy from stomach, duodenum and jejunum (about 10 cm past the ligament of Treitz). Aspirates were diluted, plated and incubated under anaerobiosis within 5 to 8 minutes.

Of 9 children, three had an apparently normal flora (Table VI), except for large numbers of bacteria in the stomach (10^6 to 10^8 per ml) in two of the cases, and in jejunum in one case.

The remaining 6 children had an abnormal fecal flora (Table VII). Bacteria were cultured from the stomach in concentrations of 10^6 to 10^8 per ml. The numbers in the duodenum were relatively great, particularly child G. Z., 10^8 per ml. The same was true of the proximal small intestine of child J. F. O., which exhibited 10^8 bacteria per ml of aspirate.

According to Moore *et al.* (1969), numbers of bacteria in stomachs of fasting subjects are in the order of 10 to 100 per gram and with ingestion of food, numbers reach 10^6 to 10^9. INCAP studies of children recovered from malnutrition also showed low numbers of bacteria in the small intestine.

Children with severe protein-caloric malnutrition usually had an altered fecal flora. The usual normal preponderance of anaerobes was not observed. In general, the anaerobic flora was one log less than that normal for children of the area, in all instances constituting definite abnormalities.

The distribution of bacterial groups in the gastrointestinal tract of the 9 children did not, in general, follow the pattern for adults of the area. Streptococci predominated in the stomach, duodenum and jejunum. Bifidobacteria were found in feces of only 2 of the 9 cases.

Table VI

Gastrointestinal microflora of children with severe protein-calorie malnutrition, Guatemala, 1969-1970

Child		Stomach		Duodenum		Jejunum		Feces	
E. R. C.	FAC*	10^4	Yeasts Corynebacteria	10^4	Yeasts E. coli	10^4	Yeasts Klebsiella	10^{10}	E. coli Klebsiella
	ANA	10^2	Bacteroides Streptococci	10^2	Lactobacilli	10^2	Bacteroides	10^{11}	Bacteroides Bifidobacteria Streptococci Veillonellae
N. G.	FAC	10^6	Streptococci		$<10^2$	10^2	Streptococci	10^9	E. coli Streptococci
	ANA	10^6	Streptococci Veillonellae		$<10^2$		$<10^2$	10^{10}	Bifidobacteria Streptococci
J. A. L.	FAC	10^7	Micrococci Yeasts	10^4	Yeasts	10^6	E. coli Yeasts Streptococci	10^{10}	E. coli Klebsiella
	ANA	10^8	Bacteroides Streptococci Veillonellae	10^5	Streptococci Veillonellae	10^6	Streptococci Veillonellae	10^{11}	Bifidobacteria Bacteroides Streptococci Lactobacilli

* FAC = Facultative; ANA = Anaerobic.

Table VII

Gastrointestinal microflora of children with severe protein-caloric malnutrition, Guatemala, 1969-1970

Child		Stomach		Duodenum		Jejunum		Feces	
L. A.	FAC*	10^8	Streptococci			10^6	Streptococci	10^{11}	E. coli
	ANA	10^8	Lactobacilli Veillonellae	10^4	Streptococci	10^6	Streptococci	10^{10}	Streptococci Veillonellae
M. R. Q.	FAC	10^8	Streptococci Yeasts	10^6	E. coli Klebsiella Streptococci	10^7	E. coli Klebsiella	10^{10}	E. coli Klebsiella
	ANA	10^8	Streptococci Veillonellae	10^5	Streptococci Veillonellae	10^7	Streptococci Veillonellae	10^{10}	Bacteroides Streptococci Veillonellae
G. Z.	FAC	10^7	E. coli Streptococci	10^8	Streptococci E. coli Yeasts			10^{10}	E. coli Klebsiella
	ANA	10^7	Streptococci Veillonellae	10^8	Streptococci Veillonellae			10^9	Bacteroides Lactobacilli Streptococci
J. F. O.	FAC	10^6	Streptococci E. coli Yeasts	10^4	Streptococci E. coli	10^8	Streptococci Yeasts E. coli	10^8	E. coli Shigella Streptococci
	ANA	10^6	Streptococci Veillonellae	10^5	Streptococci Bacteroides Veillonellae	10^8	Streptococci	10^8	Bacteroides Streptococci Veillonellae
P. Ch.	FAC	10^8	Streptococci Corynebacteria			10^5	Streptococci Yeasts	10^{10}	E. coli Proteus
	ANA	10^8	Streptococci Lactobacilli Bacteroides			10^5	Streptococci	10^{10}	Bifidobacteria Bacteroides Streptococci
R. S.	FAC	10^4	Yeasts	10^4	Yeasts			10^{10}	E. coli Klebsiella
	ANA	$<10^2$		$<10^2$				10^8	Bifidobacteria Streptococci

* FAC = Facultative; ANA = Anaerobic.

Comment

The significance of the present investigation rests in the long-term prospective nature of the studies, conducted in a population exposed to an adverse environment in terms of nutrition and infectious disease.

Colonization of the infant's intestinal tract occurs early in life, and similarly to reported behavior in more advanced societies (Rosebury, 1962). Bifidobacteria were not the first to be excreted in the meconium and feces of breast-fed newborns, but they became the dominant element in the flora of the large intestine within the first week of life. Bifidobacteria accounted for more than 99% of the flora as observed in other global regions (Gyllenberg and Roine, 1957; Haenel, 1961). These bacteria produce large amounts of organic acids, accounting for the low pH of feces of newborns. This situation probably is responsible for the notorious resistance of the newborn to infection with protozoa, *Shigella*, and other agents (Mata & Urrutia, 1970).

The onset of weaning ordinarily marks an increased frequency of veillonellae, streptococci and bacteroides, and proliferation of the *E. coli* group. If maternal milk is discontinued and formulae substituted, changes in the intestinal flora rapidly follow (Gyllenberg & Roine, 1957). In breast-fed village infants, the situation differs because of the protracted weaning process, during 2 to 3 years. Shifts in the fecal flora are subtle and more difficult to evaluate, influenced as they are by frequent infection related to the highly contaminated environment (Mata, Fernández & Urrutia, 1969). The weaning period is characterized by an exaggerated incidence of diarrheal disease (Gordon,Chitkara & Wyon, 1963; Mata, Urrutia & Gordon, 1967).

During infancy, most diarrheas appear unassociated with infection by *Shigella* or other recognized pathogens. A common belief is that they are non-specific. Attempts to measure changes in fecal flora have not been productive. The only significant observed change has been a relative decrease in bifidobacteria, an increase in streptococci and in coliforms at the onset of diarrhea, and shortly thereafter. This does not mean that indigenous bacteria have no causal role. It may be that they proliferate abnormally in sites not normally extensively colonized, such as the small intestine. Koya, Kosaki and Fukasawa (1954), for example, demonstrated that enteropathogenic *Escherichia coli*, in order to cause diarrhea, has to colonize in the small intestine. This also is true in acute cholera (Gorbach, Banwell, Jacobs, Chattarjee, Mitra, Brigham & Neogy, 1970). The recently demonstrated significance of bacterial overgrowth in the small intestine, in the blind loop syndrome (Goldstein, Wirts & Kramer, 1961; Donaldson, 1964), and in tropical sprue (Gorbach, Banwell, Mitra, Chatterjee, Jacobs & Guha-Mazumder, 1969) suggests a similar situation in non-specific diarrheas of childhood.

In our studies marked abnormalities occurred in the fecal flora of children with severe diarrhea, especially a significant reduction in concentrations of anaerobic bacteria. Occasionally, anaerobes could not be demonstrated at the lowest dilution used. Facultative bacteria could remain at the same level or increase.

In dysentery due to *S. dysenteriae*, type 1 (the Shiga bacillus), the indigenous flora is practically absent, the only bacteria cultured being *Shigella* and *E. coli*, and sometimes bacteroides. Numbers of bacteria in rectal contents are of the order of 10^7 per gram or even less. In this particular shigellosis, virtually the whole mucosa of the ileum and large intestine results inflamed and damaged. A reasonable speculation is that a physiologically normal mucosa is necessary for the existence of the ordinary indigenous microflora. In this regard, children with severe protein-calorie malnutrition characteristically have abnormally large numbers of bacteria in the stomach, and frequently in the small intestine. The fecal flora also is significantly altered.

It has been shown that species of indigenous bacteria are capable of producing enterotoxins (Smith & Halls, 1967) and presenting certain pathogenic properties like *Shigella* and *Salmonella* (Ogawa, Nakamura & Sakazaki, 1968), of producing significant amounts of organic acids (Moore, Cato & Holdeman, 1969), of splitting bile acid conjugates (Shimada, Bricknell & Finegold, 1969), and competing with the host for certain nutrients (Donaldson, 1967). Biochemical capacities of this nature presumably may be related to the chronic diarrhea and malabsorption commonly present in malnourished infants.

Accumulating evidence indicates that the etiology of diarrheal diseases is not to be understood by a sole effort to demonstrate invading pathogens within the involved intestine. Host nutrition and the characteristics and interrelations of members of the usual microflora require consideration.

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