Gastrointestinal flora of children with protein–calorie malnutrition[1, 2, 3]

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Protein–calorie malnutrition (PCM) is the principal nutritional problem in most preindustrial areas of the world (1). Malabsorption is observed in children with PCM, particularly in severe cases. Malabsorption is also a characteristic feature of the blind loop syndrome and tropical sprue (2, 3). Proliferation of bacteria in the small intestine to higher concentrations than normally present has been consistently reported in patients with these diseases. Treatment with antibiotics exerts a beneficial effect, whereas withdrawal of the drugs may be accompanied by a return of the symptoms (4). Also, bacterial overgrowth in the small bowel has been reported in adults with PCM (5). Therefore, the study of the gastrointestinal flora of children in the acute stage of PCM and during nutritional therapy seems indicated. This is necessary for the understanding of the nature of malabsorption in PCM and is a step in the exploration of ways for treatment and control.

Materials and methods

Population

Thirteen children with acute protein calorie malnutrition and four normal children (controls) were admitted to the Clinical Center of the Institute of Nutrition of Central America and Panama between the period July 1969 and May 1971. PCM cases were representative of the kwashiokor–marasmus type and fulfilled the following criteria to be admitted to the study: male, between 1 and 6 years of age, not treated in the preceding month in any medical center, and without concurrent serious infectious diseases such as meningitis, pneumonia, whooping cough, or exanthemata.

The main characteristics of cases are summarized in Table 1. All children presented a marked growth retardation, low total serum proteins, low hematocrit, low creatinine/height index, changes in skin and hair, and muscular atrophy. They were from the rural area or from the slums of Guatemala City. One of the children died shortly after admission, and one was excluded because antibiotics had to be given. Eleven children were treated for nutritional deficiency disease and seven had been fully treated at the time this report was prepared. The four control subjects were between 10 and 22 months of age, and did not present the signs and symptoms of severe PCM or any overt infectious disease. They were from the poor areas of Guatemala City.

Nutritional recuperation

Dietary regimen. During the first 2 days of hospitalization (acute period), the children were maintained on a low protein, low calorie gruel similar in nutritive value to the diets they received at home. Electrolytes were also provided. Then, and for 10 days thereafter (stabilization period), the children were given a vegetable protein mixture (Incaparina) (6) in amounts providing 1 g of high quality protein per kilogram body weight per day. The diet contributed approximately 70 calories/kg per day, of which 20 to 30% was derived from fat. After that, and for approximately 3 to 4 weeks (recuperation period), their intake was progressively raised to 3 to 4 g protein and 120 to 150 kcal/kg body wt. The children were then maintained on 4 g protein and 120 to 150 kcal/kg daily. Electrolytes and vitamins were provided at all stages of treatment. The results of treatment were monitored by measurements of weight, serum albumin, and hematocrit, and determination of the creatinine/height index (7).

Intubation. Intubation for gastric, duodenal, and jejunal aspirates was done 1 to 2 days after admission (acute), while the child was on the low protein, low calorie diet. After the first intubation, dietary therapy was begun, and the child was intubated again ap-

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TABLE 1

Characteristics of children with protein-calorie malnutrition

No.	Age, years and months	Weight, kg	Total blood protein, (g/100 ml)	Hematocrit, %	CHI[a]	Diarrhea	Days to attain CHI = 0.85	Intestinal parasites	Enteric pathogenic bacteria	Enteric viruses
PC-213	3, 10	11.745	3.3	25	0.51	+[b]	Antibiotics given	*Giardia*	–[b]	Enterovirus (jejunum)
PC-223	4, 3	8.840	3.5	33		+	Died	–	*S. flexneri 2*	–
PC 215	3, 6	9.126	4.0	33	0.55	+	50	*E. coli, Trichomonas*	–	–
PC-215r	4, 7	12.490	3.5	33	0.30	+	80	*Enteromonas, Ascaris*	–	–
PC 222	5, 1	12.940	3.6	35	0.42	+	105	*Giardia, Ascaris,* Hookworm	–	Adenovirus (feces)
PC-226	2, 8	6.230	3.5	36	0.42	+	145	*Giardia, Trichomonas*	–	–
PC-237	4, 6	5.140	3.5	31	0.65	–	125	*Giardia, Trichuris,* Hookworm	–	–
PC-235	1, 11	8.180	4.1	33	0.50	–	45	*Giardia, Trichuris, Strongyloides*	–	–
PC-241	2, 6	8.990	4.4	36	0.65	+	110	*Giardia, Trichomonas*	*Salmonella*	–
PC-249	2, 6	8.345	3.8	26	0.39	+	Pending	*Giardia*	–	–
PC-250	6, 3	13.040	3.3	18	0.53	+	Pending	*E. histolytica, E. coli, Giardia, Strongyloides,* Hookworm, *Trichuris*	*Edwardsiella*	Enterovirus (duodenum)
PC-252	1, 6	5.329	3.9	32	0.37	–	Pending	*Giardia*	–	–
PC-253	1, 5	3.919	4.2	21	0.39	+	Pending	–	–	–

[a] Creatinine/height index. See (7). [b] + = present; – = negative or absent.

proximately 13 days after admission (stabilization). Children were again intubated approximately 37 and 60 days after admission, corresponding, respectively, to the initial recuperation and total recuperation periods. Attempts were made to follow this schedule exactly, but spacing between intubations varied due to individual responses to nutritional therapy.

The intubation technique was developed by Schneider and Viteri (unpublished observations). Briefly, three small radiopaque tubes were attached to each other with their openings separated by varying lengths of tube to reach the stomach, duodenum, and upper jejunum 10 cm past the ligament of Treitz. The evening preceding collection of the aspirates, tubes were introduced through the nasopharynx after local anesthesia of the mucosa, and were conducted to the stomach. During the night, tubes were advanced to a mark to reach the jejunum. The position of the tubes was checked the next morning under fluoroscopy; if found to be correct, the sealed proximal end of the stomach tube was opened and a gastric aspirate was obtained. Similarly, aspirates from duodenum and jejunum were obtained that morning. The total time for sample collection ranged from 15 to 45 min. The time for aspiration of each sample was from 2 to 5 min. All specimens were obtained aseptically and anaerobically.

The four control subjects, who had received a hospital diet, were fasted overnight and intubated in a manner similar to that described for children with PCM. Only one intubation was performed on each control subject. Aspirates from both patients and control subjects were collected approximately 14 hr after fasting.

Microbiological techniques

Gastrointestinal flora. The quantitative data on bacteria presented here were obtained with the plate technique of Schaedler, Dubos and Costello (8), as described elsewhere for fecal flora (9). Aspirates were placed in a sterile vial and transported within 3 min from the bedside to the laboratory (Fig. 1). Dilutions (10^{-1} through 10^{-6}) were prepared using charcoal water (8) as a diluent. Selected dilutions were streaked onto the surface of the agar media described in Table 2, using calibrated platinum loops (0.01 ml). Plates were incubated immediately in jars and anaerobiosis was produced with disposable generators (10). On rare occasions, anaerobiosis was not obtained within

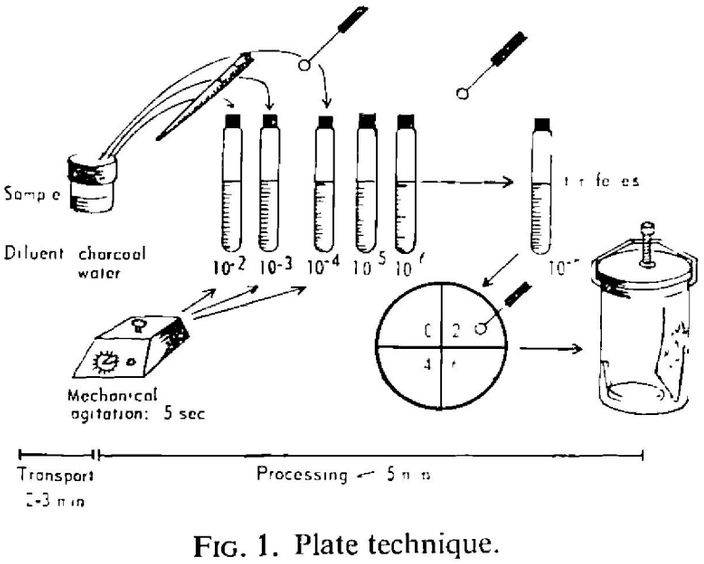

FIG. 1. Plate technique.

TABLE 2
Quantitation of gastrointestinal bacteria

Agar medium	Bacterial group	Incubation
BC-1 Schaedler's base BC BC-1 + placenta + neomycin	Bacteroides, clostridia Bifidobacteria, lactobacilli Streptococci, veillonellae	48 hr Anaerobic
BC-1	Gram-negative bacilli Gram-positive cocci	48 hr Aerobic
MS Manitol-salt	Micrococci, bacilli	24 hr Aerobic
H Peptone-glucose + antibiotics	Yeasts	5 days Aerobic
T7T Tergitol 7 + tetrazolium	Gram-negative bacilli	24 hr Aerobic
SS MacConkey	Gram-negative bacilli	24 hr Aerobic

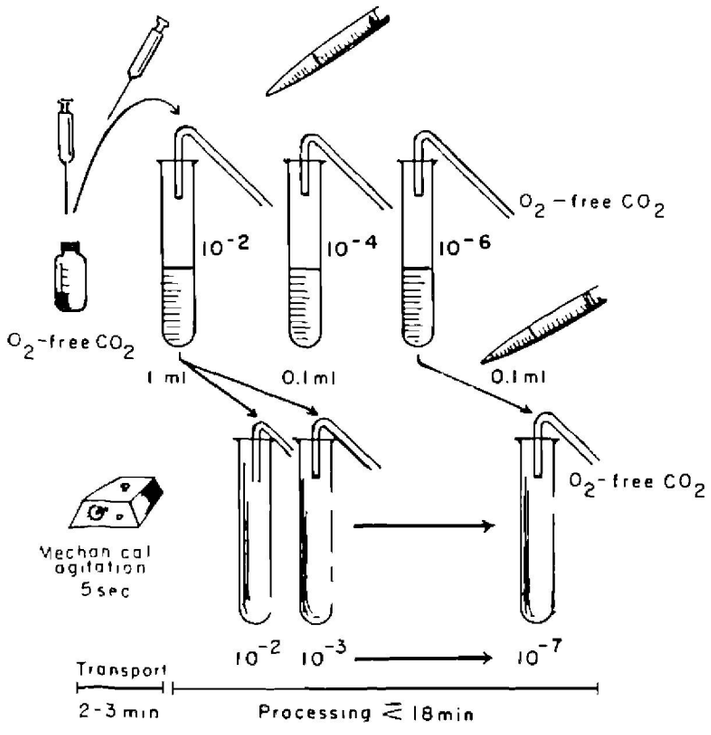

FIG. 2. Roll-tube technique.

30 min. These cases were not tabulated. This technique permitted estimation of bacterial concentrations between 10^2 and 10^9/ml. The time required to process each specimen was less than 5 min from the moment the sample arrived in the laboratory to the moment the anaerobic jar was sealed.

Certain aspirates were processed simultaneously by the roll-tube technique (11). The specimen was placed in a sealed, sterile bottle containing oxygen-free CO_2, transported to the laboratory within 3 min, and processed as illustrated in Fig. 2. Three dilutions (10^{-2}, 10^{-4}, and 10^{-6}) were prepared using prereduced peptone–yeast extract broth as a diluent (12). From these dilutions, six roll-tubes were made with prereduced Schaedler's agar base (8). The final dilutions obtained ranged from 10^{-2} through 10^{-7} and count estimates ranged from 10^2 to 10^9/ml. With this method specimens were not exposed to oxygen at any time.

In the plate technique, agar media were incubated for 48 hr, except for medium H which required a 5-day incubation period. The roll-tube cultures were incubated for 7 days.

Enteropathogenic bacteria. Agar plates of SS MacConkey and Tergitol 7 with 0.004% triphenyl-tetrazolium chloride were inoculated with undiluted aspirate. Identification of *Shigella*, *Salmonella*, and other enterobacteriaceae was done by standard procedures (13).

Viruses. Aspirates and feces were suspended in a buffer salt solution made of Hanks with skimmed milk and antibiotics. After high-speed centrifugation at 4 C, supernates were inoculated into two tubes of primary human kidney and two of HEp-2 cells growing in Melnick's tissue culture fluid with calf serum and penicillin and streptomycin. Cell cultures were observed for at least 2 weeks for cytopathic effects. Fluid and cells from positive or suspected cultures were passed into the same kind of cell cultures.

Intestinal parasites. Direct examination of fresh aspirates and feces was made. More than one-half the specimens were preserved in polyvinyl alcohol-Schaudinn fixative (PVA), and stained with trichrome (14).

Results

The plate and roll-tube techniques for quantitation of bacteria

Table 3 illustrates the total bacterial counts obtained using the two techniques on the same specimens. No significant differences in total bacterial counts in aspirates from three different sites were found. However, the roll tube permits isolation of certain highly O_2-sensitive bacteria (unpublished observations). The results that follow were obtained with the plate technique.

TABLE 3
Total bacterial counts of gastrointestinal bacteria in children with acute PCM by two culturing techniques

Site	No. of specimens	Plate	Roll tube
Stomach	8	5.5 ± 1.7[a] (2–7)	4.4 ± 2.0 (<2–7.3)
Duodenum	8	4.6 ± 2.4 (<2–8)	4.5 ± 2.6 (<2–7.7)
Jejunum	6	5.0 ± 1.5 (3–6)	6.2 ± 1.5 (3.3–7.6)

[a] Mean ± SD of $\log_{10}$ of total bacterial count per milliliter of aspirate. The figures in parentheses indicate the range.

Gastrointestinal flora of control children

Table 4 shows the total anaerobes and facultative bacteria expressed as $\log_{10}$ of concentration per milliliter or gram of specimen, of the normal children. Large numbers of bacteria were detected in the stomach and jejunum of three children. The duodenal aspirate of one child had less than 10^2 bacteria per ml, but 10^3 to 10^7/ml were detected in the other three. Two children had high counts in the jejunum, one with 10^8 *Escherichia coli* and 10^5 bacteroides/ml. The fecal specimens revealed a flora of predominantly anaerobes (streptococci and bacteroides), as described for normal children of the area (9, 15). The bacteria found in the stomach, duodenum, and jejunum were mainly streptococci.

Gastrointestinal flora of children with severe PCM

Ten of thirteen children with severe PCM had diarrhea upon admission. The average bacterial concentration in the gastrointestinal tract is shown in Table 5. No differences in the average concentration of anaerobic and facultative bacteria of the stomach contents were noted between patients (Table 5) and controls (Table 4). All 13 children with PCM had bacteria in the duodenum, whereas 1 out of 4 controls had less than 10^2/ml. Several children with PCM had as many as 10^7 and 10^8 bacteria/ml in the jejunum. In feces, facultatives were as abundant, or even outnumbered the anaerobic flora in all cases. Bifidobacteria, generally found in feces of normal children, were present in only one-third of the PCM cases. Many children were colonized by *Proteus*, *Pseudomonas* and other not typically indigenous Gram-negative bacilli. Bacteroides and veillonellae were absent from many cases.

Protein–calorie malnourished children with diarrhea had a tendency to show more bacteria in the stomach and the jejunum than those without diarrhea. The bacterial groups found in malnourished children with and without diarrhea were basically the same (Tables 6 and 7), although there was a tendency for gram-negative anaerobic bacilli (bacteroides) to be more common in the jejunum in the absence of diarrhea. There was no consistent pattern of bacterial groups

TABLE 4
Gastrointestinal flora of children without malnutrition

Child	Component	Stomach	Duodenum	Jejunum	Colon
JHCh	Anaerobic	6[a]	<2	6	Not done
	Facultative	7	<2	5	Not done
DGR	Anaerobic	7	5	5	10
	Facultative	6	3	8	9
JGE	Anaerobic	7	6	7	10
	Facultative	7	4	5	9
MGM	Anaerobic	4	7	3	10
	Facultative	2	7	2	9
Mean ± SD	Anaerobic	6.0 ± 1.4	6.0 ± 1.0	5.3 ± 1.7	10
	Facultative	5.5 ± 2.4	4.7 ± 2.1	5.0 ± 2.4	9

[a] Log_{10} bacterial count per milliliter or gram of material.

TABLE 5
Gastrointestinal flora of children with PCM

Chronic diarrhea	No. of children	Component	Stomach	Duodenum	Jejunum	Colon
Yes	10	Anaerobic	6.1 ± 2.5[a] (2–8)	4.6 ± 2.1 (2–8)	6.1 ± 1.6 (3–8)	9.4 ± 1.0 (8–11)
		Facultative	6.3 ± 1.4 (4–8)	4.3 ± 2.2 (2–8)	6.5 ± 1.7 (5–8)	9.9 ± 0.8 (8–11)
No	3	Anaerobic	4.0 (2–8)	4.0 (2–8)	4.7 (2–6)	11.0 (11)
		Facultative	6.0 (4–8)	5.3 (4–8)	6.0 (4.8)	10.3 (10–11)

[a] Mean ± SD of $\log_{10}$ bacterial count per milliliter or gram of material. The figures in parentheses indicate the range.

TABLE 6
Frequency of bacterial groups in the GI tract of 10 children with PCM and chronic diarrhea

	Stomach	Duodenum	Jejunum	Colon
Number of specimens	10	9	8	9
Gram-positive non-spore-forming bacilli[a]	3[b]	0	1	3
Bacteroides	4	3	0	5
Streptococci	8	8	8	9
Veillonellae	6	7	3	4
Yeasts	8	7	6	4
Enterobacteriaceae	6	5	5	9
Escherichia coli	4	5	4	7

[a] Mainly bifidobacteria. [b] Specimens with at least 10^2 bacteria/ml or g of material.

TABLE 7
Frequency of bacterial groups in the GI tract of three children with severe PCM but without diarrhea

	Stomach	Duodenum	Jejunum	Colon
Number of specimens	3	3	3	3
Gram-positive non-spore-forming bacilli[a]	1[b]	1	1	3
Bacteroides	1	1	2	3
Streptococci	2	1	2	3
Veillonellae	1	0	1	2
Yeasts	3	3	3	1
Enterobacteriaceae	1	2	3	3
Escherichia coli	1	1	1	3

[a] Mainly bifidobacteria. [b] Specimens with at least 10^2 bacteria/ml or g of material.

in aspirates; streptococci were found in most aspirates from all sites.

Detailed study of bacterial isolates from well-nourished and malnourished children, using prereduced and chromatographic techniques, showed that although bacterial groups were the same, rare subgroups and species of bacteroides, for example, are found in malnourished children (unpublished observations).

Changes in the gastrointestinal flora with dietary treatment

Qualitative and quantitative changes were observed in the gastrointestinal flora of most children during nutritional recuperation. Table 8 and Figs. 3 to 6 illustrate bacterial counts obtained on four different occasions during hospitalization. There was a trend for the flora of the jejunum to decrease during nutritional recuperation. In feces, the abnormal inversion of the ratio of anaerobes to aerobes was corrected, with an increase in total anaerobes. Also, there was a 1-log decrease in fecal facultative bacteria. These changes are illustrated in Figs. 3 and 4 for two particular children, and in Fig. 5 for all subjects with PCM and diarrhea.

In children with severe PCM but without diarrhea, a similar phenomenon was noted regarding jejunal aspirates (Fig. 6). However, bacterial counts remained the same in the stomach and the duodenum. Feces of the malnourished children without diarrhea did not present an altered anaerobe-aerobe ratio but facultatives also decreased one log after nutritional recovery.

Qualitative changes in the flora were also observed in association with nutritional recuperation. For example, there was a reduc-

TABLE 8

Jejunal and colonic flora of three children with severe PCM and chronic diarrhea

Child no.	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	11	9
PC-241	<2	<2	9	9
Recuperation phase I				
PC-222	<2	3	11	10
PC-226	6	6	10	9
PC-241	<2	<2	11	9
Recuperation phase II				
PC-222	3	2	11	10
PC-226	5	4	11	8
PC-241	<2	2	10	9

[a] Logarithm$_{10}$ of bacterial count per milliliter or gram of material.

tion or disappearance of the predominant bacterial group such as *Escherichia coli* from the jejunum. Also, in one malnourished child with diarrhea, bifidobacteria appeared in the feces during recuperation. *Proteus*, absent in the feces of two children, appeared in one of them during recuperation. However, most children remained with a qualitatively abnormal fecal flora after treatment, lacking bifidobacteria and without an overt predominance of anaerobes.

Pathogenic enterobacteriaceae

Among the PCM children, one had *Edwardsiella*; one, *Shigella flexneri 2*; and another, *Salmonella* sp. (Table 1). The child with *Shigella* died shortly after admission. No enteric pathogens were found in the control subjects.

Enteroviruses

Three children with PCM had virus infections during the acute phase of the dis-

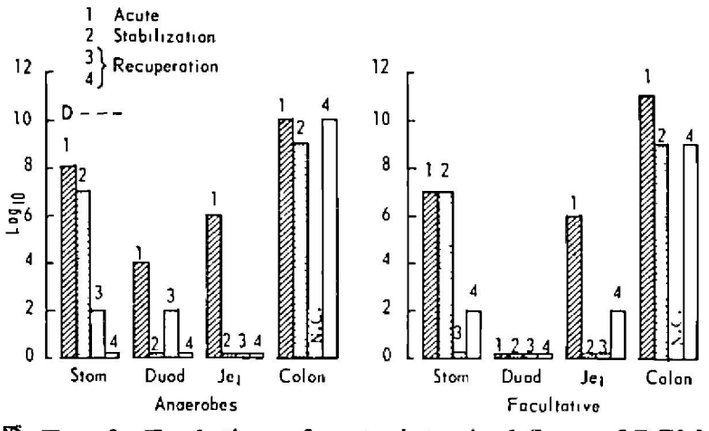

FIG. 3. Evolution of gastrointestinal flora of PCM child PC-241 during dietary treatment. Diarrhea disappeared shortly after initiation of dietary therapy.

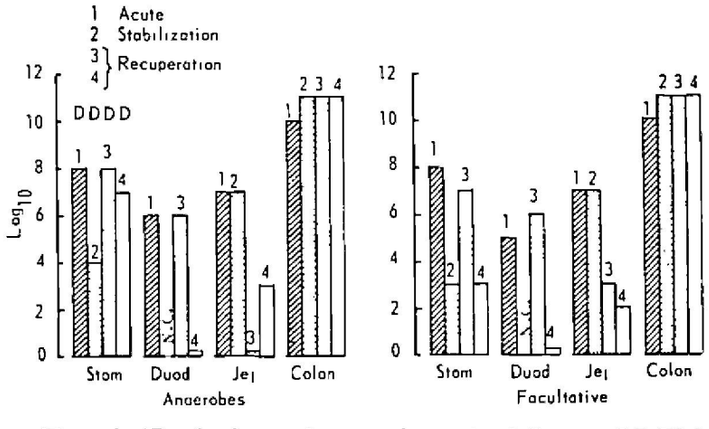

FIG. 4. Evolution of gastrointestinal flora of PCM child PC-222 during dietary treatment. Diarrhea persisted throughout dietary treatment.

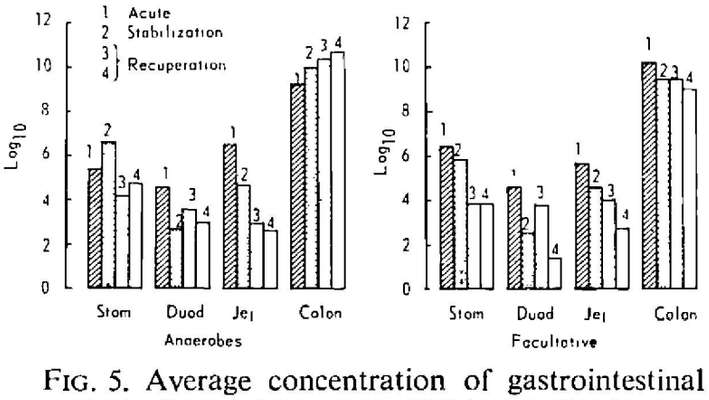

FIG. 5. Average concentration of gastrointestinal bacteria in five children with PCM and diarrhea, at four stages of hospitalization.

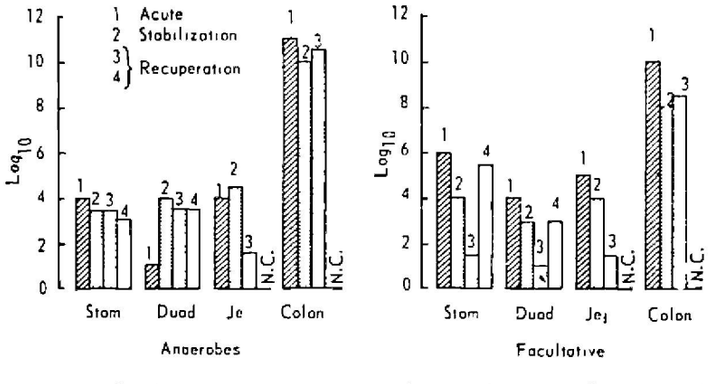

FIG. 6. Average concentration of gastrointestinal bacteria in two children with PCM without diarrhea, at four stages of hospitalization.

ease: two had enteroviruses (one in the duodenum and the other in the jejunum), and one an adenovirus in the feces (Table 1). One control child had an enterovirus in the feces.

Intestinal parasites

Only 2 children among 13 with PCM did not show intestinal parasites. Nine had *Giardia*; two had *Strongyloides*; and, three hookworm (Table 1). In general, no statement can be made as to the effect of nutritional recuperation on intestinal parasites; but in the majority of cases, they tended to disappear with nutritional therapy, although parasitic infections remained unaltered in a few cases. Among four control children, only one showed a parasite, *Giardia*, in gastric and duodenal aspirates.

Discussion

For many years, the small intestine of man was claimed to be sterile. Aseptic handling of the specimens revealed low amounts of bacteria in the duodenum and the upper jejunum under normal fasting conditions. In normal Guatemalan adults, high concentrations of bacteria were also detected in the mucus overlying the small intestinal mucosa (16), similar to what has been described for normal American subjects (17).

Reports on the blind loop syndrome and tropical sprue (18–21) suggest that bacterial overgrowth in the upper small bowel is a cause of malabsorption. The mechanism involved could be liberation of enterotoxins (22–24), production of bacterial metabolites (25–27), and deconjugation of bile salts (28–30).

Because children with acute protein–calorie malnutrition frequently present diarrhea and have an altered gastrointestinal function (31, 32), study of the microflora seems to be indicated. Observations in four normal children without signs of malabsorption indicated relatively large amounts of bacteria in the stomach, jejunum, and duodenum, although the fecal flora was unaltered.

Investigations in 13 children with severe PCM, using the same methodology, revealed similar bacterial concentrations in those sites. The only difference noted was that PCM children showed more enterobacteriaceae in the small intestine and an altered fecal flora, qualitatively and quantitatively. These findings could be interpreted as meaning that the gastrointestinal flora of the PCM child is similar to that of the normal. However, it is possible: *a*) that bacterial overgrowth is as common a finding in normal subjects as mucosal morphologic abnormalities (33, 34) and D-xylose malabsorption (35, 36) are in subjects of certain tropical areas; and *b*) that the bacterial concentrations detected in the small bowel of children with PCM, although not higher than those of controls, cause damage to the already altered mucosa. In this regard, distinct mucosal alterations were shown in the children from whom the present data were derived (37).

On the other hand, a significant decrease in the bacterial population of stomach, duodenum, and jejunum was observed during nutritional recuperation and normalization of gastrointestinal function, thus suggesting that such a bacterial population is abnormal. Therefore, the high bacterial counts in the small intestine of normal children should be considered abnormal, as is the presence of *Giardia* or other recognized pathogens. Bacterial overgrowth in normal subjects from low socioeconomic groups may reflect the prevailing low level of sanitation and dietary deficiencies, or may be related to subclinical malnutrition and malabsorption. Bacterial overgrowth in the small bowel of children from this area could be favored by the continuous viral, bacterial, and parasitic infections to which children are exposed in unsanitary environments (38). These could also be implicated in the establishment of malabsorption.

The marked alterations in the fecal flora of children with PCM are comparable to those observed in patients with acute diarrhea and shigellosis (39). A correction of the inverted anaerobe–facultative ratio in feces was observed with nutritional therapy in many PCM cases, similar in nature to that observed in patients with shigellosis during effective antibacterial treatment (39).

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