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The intestinal ecosystem in chronic functional constipation

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Chronic functional constipation is common in infants, and the bacterial composition of stools in this condition is not known. The study aims were to: (i) investigate the composition of the intestinal ecosystem in chronic functional constipation; (ii) establish whether the addition of the water-holding agent calcium polycarbophil to the diet induces an improvement in constipation; and (iii) determine the composition of the intestinal ecosystem after the use of this agent. In total, 42 children (20F, 22M; mean age: 8.6 ± 2.9 y) were studied. Twenty-eight children with functional chronic constipation without anatomical disorders were treated double-blind in random sequence for 1 month with an oral preparation of calcium polycarbophil (0.62 g /twice daily) or placebo. Intestinal flora composition was evaluated by standard microbiological methods and biochemical assays on faecal samples collected before and after treatment. Fourteen healthy children were studied as controls. The results show that (i) the constipated children presented a significant increase in clostridia and bifidobacteria in faeces compared to healthy subjects—different species of clostridia and enterobacteriaceae were frequently isolated; no generalized overgrowth was observed; Clostridia outnumbered bacteroides and *E. coli* mean counts by 2–3log, while bacteroides and *E. coli* counts were similar (5–6 $\log_{10}$/g fresh faeces); these intestinal disturbances could be defined as a dysbiosis, i.e. a quantitative alteration in the relative proportions of certain intestinal bacterial species. (ii) Clinical resolution of constipation was achieved only in 43% of treated children and an improvement in 21% (one bowel movement every 2 d). (iii) Calcium polycarbophil treatment induced no significant changes in the composition of the intestinal ecosystem, nor in blood chemistry parameters. □ *Calcium polycarbophil, children, chronic constipation, dysbiosis, intestinal ecosystem*

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Chronic functional constipation is common in infants. Constipation may be defined as a ''delay or difficulty in defecation sufficient to cause significant distress to the patients'' (i.e. less than one bowel action/48 h) (1).

Normal values for the weight of stool output show substantial fluctuations as a consequence of dietary variation and seem to be reduced in constipated infants, particularly if the daily fibre intake is low (1). Fibre is known to induce an increase in faecal mass and may accelerate bowel transit with disappearance of chronic constipation. In 1982, it was demonstrated that not all types of fibre may be ideal in paediatric patients: wheat bran, for example, induces a decrease in blood levels of calcium, phosphate and trace elements and also promotes a predominance of proteolytic over saccharolytic faecal flora (2).

It is well known that the bacterial content accounts for almost 50% of stool bulk. To the best of our knowledge, however, the bacterial composition of stools in chronic functional constipation in children is not known.

Water-holding agents such as calcium polycarbophil are used in the treatment of diarrhoea (3), constipation (4, 5) and irritable bowel syndrome (6). The resin is capable of absorbing 60–100 times its weight in water, thereby adding soft bulk to faeces (7). In adults, constipation disappears 3 d after intake in about 90% of cases treated (4). As no studies of the use of this polyacrylic resin have yet been conducted in infants and children, more information on the subject would be particularly welcome in view of the fact that it is easy to use and well tolerated.

The aims of this study were: (i) to investigate the composition of the intestinal ecosystem (i.e. bacterial composition of stools) in chronic functional constipation; (ii) to establish whether the addition to the diet of a water-holding agent such as calcium polycarbophil induces an improvement in constipation; and (iii) to assay the composition of the intestinal ecosystem after use of this agent.

Material and methods

Subjects

Forty-two children (20F, 22M; mean age 8.6 ± 2.9 y; range 5–14 y) were studied. Habits, lifestyle, diet and socioeconomic condition (school attenders) were similar for all children studied.

Twenty-eight of them (12F, 16M; mean age 9.5 ± 3.0 y) were suffering from functional chronic constipation (stool frequency less than one per 48 h and hard stool consistency)

with no evidence of anatomical disorders, no encopresis and/or soiling. Fourteen normal healthy children (8F, 6M, mean age 7.9 ± 2.9 y) seen in the hospital as outpatients for non-gastroenterological problems (radiological examinations, ECG, EEG, etc.) were used as controls. The exclusion criteria were laxative use at any time, pharmacological treatment for 2 months prior to entry into the study, and presence of infectious diseases.

Treatment

The chronically constipated children were treated at home in random sequence using double-blind technique for 1 month with an oral preparation of calcium polycarbophil (dosage 0.62/g thrice daily) ($n = 14$) or placebo ($n = 14$) consisting of the same amount of soluble starch.

During the study all the children received a balanced daily diet supplying an amount of energy of 80 kCal kg/d in accordance with age and the recommended safe levels of nutrients of the Italian Society of Human Nutrition. The diet was typically Mediterranean, including fresh whole cow's milk, biscuits, spaghetti, meat, bread, vegetables and fruit.

The study protocol was approved by the internal hospital Ethical Committee.

Informed consent for the study was obtained from the parents before the start.

Blood chemistry

The following haematological and chemical parameters were determined in all subjects before and the day after the end of treatment or placebo administration: haemoglobin, haematocrit, total protein, cholesterol, triglycerides, calcium, phosphorus, alkaline phosphatase, iron, magnesium, copper, zinc, vitamin B_{12}, folate and ammonia. These parameters were automatically determined with the use of a Technicon-SMAC high-speed computer-controlled biochemical analyser (Tarrytown, NY). Magnesium, copper, zinc, vitamin B_{12}, and folate levels were determined manually (2).

Microbiological studies

At the same time as the blood tests, stool samples were collected from all subjects. The stool specimens were collected immediately after emission in sterile plastic containers, gassed with CO_2 and frozen at −70°C, stored at the same temperature until analysis and subsequently tested for microbial composition within 4 weeks of collection. The samples collected after treatment were analysed within a few days, so as to analyse the two samples from each subject in the same experiment. The control samples were processed during the study along with the samples from children with constipation. In these conditions, freezing at −70°C induced a decrease in the number of bacteria but yielded more reliable results than those obtained at −24°C, while the storage time would not appear to be a critical factor. Analysis of samples before and after freezing (in our experimental conditions) has shown that aerobic and anaerobic cocci, lactobacilli and bifidobacteria counts remain stable, while enterobacteriaceae, total anaerobic count and clostridia may decrease after freezing. The decrease is not constant or generalized, but can affect some samples only (20–30% of cases) in an unpredictable way. Many results from the literature are actually based on determinations carried out on stool samples frozen at −70°C (8–11) and are comparable. Our standard methods (10) were used to quantify and identify aerobic and anaerobic bacteria. The samples analysis was performed in an anaerobic cabinet. Briefly, 11 10-fold serial dilutions were prepared after homogenization of 1 g of faeces in 10 ml of prereduced sterile saline. Each dilution was seeded on the following selective and non-selective culture media: blood agar for total aerobic and anaerobic count; Schaedler agar (total anaerobic count); kanamycin–vancomycin Schaedler agar (bacteroides); reinforced clostridial agar (clostridia); mitis salivarius agar (aerobic and anaerobic cocci); tomato juice agar and Rogosa SL Agar (bifidobacteria and lactobacilli); bile esculine azide Agar (enterococci); mannitol salt agar (staphylococci); McConkey agar and SS agar (enterobacteriaceae); and Sabouraud dextrose agar (yeasts). After incubation in aerobiosis (37°C, 24 h) and anaerobiosis (85% N_2, 10% CO_2, 5% H_2 gas mixture) for 48–72 h at 37°C in an anaerobic chamber, different colony types were enumerated, isolated and identified by morphological and biochemical analysis (API System: 20E, 50 CHL, rapid ID 32 A, Strep, Staph, C AUX; bioMérieux, Marcy l'Etoile, France). The lower limit of detection was 10^2 microorganisms/g of faeces. The growth on selective medium was considered insufficient to identify different species of microbial flora. The quantitative determination was based on the identification of microorganisms grown on the last dilutions of different media. Results were expressed as $\log_{10}$ of the number of CFU per gram of fresh faeces (arithmetic mean ± SD).

Stool pH was measured with a glass pH electrode, though polyacrylic resin is known not to modify pH in adult patients.

We utilized a semiquantitative micromethod to detect a range of microbial enzyme activities in faecal samples. Enzyme activity determination was done at the same time as microbiological analysis on the 3rd dilution of each sample using the API-ZYM System (API Products). Suspensions (60 μl) of each sample were added to cups with different substrates and incubated for 4 h at 37°C. Enzyme activity was detected by visual estimation of chromophoric products; the reactions were graded from 0 to 5 (API units), depending on colour intensity. One API unit corresponds to 5 nmol of hydrolysed substrate per cup; 2 units to 10 nmol; 3 to 20 nmol, 4 to 30 nmol, and 5 to ≥40 nmol. Results are expressed as nmol/g fresh faeces (arithmetic mean ± SD). Preliminary studies revealed no interference from the third dilution of the faecal suspension with colour development in the assay. This method showed

good agreement with standard and more recent fluorimetric determinations of faecal bacterial enzymes (12, 13).

The following enzyme activities associated with human faecal bacteria were evaluated: esterase-lipase, leucine-arylamidase, α- and β-glucosidase, α- and β-galactosidase and β-glucuronidase.

Statistical analysis

The results were analysed statistically using Student's *t*-test for comparison of the mean values. The Mann-Whitney *U* test was used where appropriate.

Results

Clinical results

Haematological and chemical parameters were within the normal ranges for both controls and patients; calcium polycarbophil induced no modifications.

The clinical results of calcium polycarbophil treatment were the following:

1. Six children (43%) showed disappearance of constipation, i.e. they started to have one bowel movement a day after 1 week of therapy, this pattern then continuing for the following 3 weeks until the end of the 1-month observation period; the stools were soft and brown.
2. Five children (36%) had a slight but significant reduction of constipation, i.e. they started to have one bowel movement every 2 d after 1 week of therapy throughout the month, but the stools remained hard and evacuation difficult.
3. Three children (21%) presented no changes in symptoms during the month of observation.

The patients treated with placebo showed no changes in bowel habits.

Microbiological results

The composition of the intestinal ecosystem in healthy subjects and in constipated children before treatment is reported in Table 1. The mean total anaerobic count was similar for both groups, corresponding to $\log_{10}$ 10.2 ± 2.5 (mean ± SD) and $\log_{10}$ 10.5 ± 4.3 in children with constipation and controls, respectively. Aerobic and anaerobic Gram-positive components outnumbered Gram-negative species in all children with constipation as well as in control children. Children with chronic functional constipation showed higher bacterial counts for different species of anaerobes in comparison to healthy children, with a significant increase in clostridia ($p < 0.001$) and bifidobacteria ($p < 0.02$).

In the majority of constipated children (26/28) clostridia outnumbered bacteroides and *E. coli* mean counts by 2–3 log, while the bacteroides and *E. coli* counts were similar ($\log_{10}$6.3 and $\log_{10}$5.9/g fresh faeces, respectively). These abnormalities of the bacterial microflora could be defined as a dysbiosis, i.e. a quantitative alteration in the relative proportions of certain intestinal bacterial species in the same subject. In healthy children clostridia, bacteroides and bifidobacteria presented similar mean counts (Table 1), showing a balanced microbiota. Great intersubject variability was observed among the children and there were occasional differences on a periodic sampling basis as shown by the range of the counts. These variations are consistent with the characteristics of the intestinal ecosystem and may be induced by various different factors.

Bacteroides presented low mean counts; the species most frequently identified in the controls and in the cases before treatment were *B. bivius*, *B. uniformis* and *B. buccae*.

Clostridium species were identified in large numbers in chronic constipation: *C. sporogenes*, *C. paraputrificum*, *C. fallax* and *C. innocuum* were the predominant species

Table 1. Composition of the intestinal ecosystem in chronic functional constipation. Values are expressed as $\log_{10}$ no. bacteria /g fresh faeces (mean ± SD).

	Healthy subjects ($n = 14$)		Subjects with constipation ($n = 28$)	
Microorganisms	Mean count	Range	Mean count	Range
Total aerobic count	8.1 ± 2.6	4–12	7.4 ± 1.7	5–10
E. coli	6.0 ± 3.6	2–9	5.9 ± 1.8	3–8
Enterobacteria (other)	2.8 ± 1.1 [33.3]	2–4	3.1 ± 1.5 [53.6]	2–5
Enterococci	7.5 ± 4.7	3–12	6.9 ± 2.5	4–10
Lactobacilli	4.3 ± 2.3 [80]	2–6	6.4 ± 3.0 [60]	3–12
Staphylococci	2.8 ± 0.4 [77.7]	2–4	3.7 ± 1.5 [66.6]	2–7
Fungi	5.0 ± 2.2	3–10	3.8 ± 2.2	2–8
Total anaerobic count	10.5 ± 4.3	8–12	10.2 ± 2.5	6–12
Bacteroides	5.9 ± 1.3	3–8	6.3 ± 2.4	3–10
Clostridia	6.6 ± 2.4	4–12	9.3 ± 2.5**	4–12
Bifidobacteria	5.5 ± 1.3	3–7	8.2 ± 3.1*	3–12
L. acidophilus	4.0 ± 1.2	3–6	6.0 ± 2.5	4–11
An. cocci	7.5 ± 2.7	4–12	7.6 ± 2.4	4–12
pH	6.5 ± 0.4	5.7–7.0	6.2 ± 0.2	5.7–6.7

*$p \leq 0.05$; **$p \leq 0.01$ Student's *t*-test vs healthy subjects.
In brackets [ ] percentage of positive samples; in other cases all samples were positive [100%].

Table 2. Enzyme activity of intestinal microflora in healthy children and in children with chronic functional constipation. Values are expressed as nmol/g fresh faeces (mean ± SD).

Enzymes	Healthy subjects ($n = 14$)	Subjects with constipation ($n = 28$)
Esterase lipase	3.9 ± 1.3	3.3 ± 3.4
Leucine arylamidase	15.6 ± 12.9	11.3 ± 9.7
α-galactosidase	4.4 ± 2.4	6.4 ± 5.6
β-galactosidase	17.5 ± 9.4	18.9 ± 7.8
β-glucuronidase	1.6 ± 1.3	0.8 ± 1.8
α-glucosidase	17.0 ± 9.4	11.0 ± 5.1
β-glucosidase	9.2 ± 8.0	6.4 ± 5.4

in all the samples studied. *Clostridium perfringens* and *C. ramosum* were also isolated less frequently (21.4% and 14.2%, respectively). These species are usually present in healthy adult subjects. In our healthy children we isolated *C. beijkerinkii* and *C. tyrobutyricum* as predominant species; these species were not found either before or after treatment in children with constipation.

In the children with chronic constipation, the Gram-positive non-spore-forming isolates also included *E. limosum* (30%) and *Prop. granulosum* (7.1%) in addition to *L. acidophilus* and *Bifidobacterium. Propionibacterium granulosum* was frequently isolated in control children (44.4%).

In the healthy children, the enterobacteriaceae isolates were *E. coli* and *Klebsiella pneumoniae* only. Enterobacteriaceae other than *E. coli* were frequently isolated in the children with chronic constipation (53.6% of subjects), including *Enterobacter cloacae, E. agglomerans, Citrobacter freundii, K. oxytoca* and *Hafnia alvei.*

Faecal pH was similar in the controls and in the children with constipation.

Table 2 gives details of the enzymatic activities of the intestinal ecosystem in healthy children and in those with chronic functional constipation. In the latter there were slight differences in enzymatic activities in comparison to controls: α-galactosidase activity was higher than in control samples, while β-glucuronidase and leucine arylamidase activities were lower than in controls, though the differences were not statistically significant.

Table 3 gives the composition of the intestinal ecosystem in children before and after treatment with calcium polycarbophil or placebo.

Treatment for 1 month with calcium polycarbophil did not induce any significant changes in the microflora composition of the children with constipation, whereas slight qualitative changes were observed. Treatment reduced the number of enterobacteriaceae species isolated; *E. cloacae* and *K. oxytoca* were not identified. Enterococci (*Enterococcus faecium, E. faecalis, E. durans*) were isolated in all samples studied (both controls and children with constipation); after 1 month of therapy *Streptococcus milleri* and *Aerococcus viridans* were identified as new species in two patients.

The total number of clostridia remained practically unchanged before and after treatment. *C. perfringens* and *C. ramosum* were present before treatment in children with constipation, but were not isolated in the samples from children treated with calcium polycarbophil. Reduction of the presence of *C. perfringens*, a potential pathogenic microorganism and producer of enterotoxins, is considered a positive effect. The proportion of clostridia to bacteroides tended to change, with a slight increase in mean bacteroides counts. Among the anaerobic Gram-negative bacilli we identified *B. fragilis* (10%) after treatment.

In the treated children there was a reduction in the frequency of identification of Gram-positive non-spore-forming rods, namely bifidobacteria (70%) and lactobacilli (35%); *Lactobacillus rhamnosus* and *Arachnia propionica*, which were isolated prior to treatment in two children, were

Table 3. Composition of the intestinal microflora in children with chronic constipation before and after 1 month of treatment with calcium polycarbophil. Values are expressed as $\log_{10}$ no. bacteria/g fresh faeces (mean ± SD).

	Calcium polycarbophil (14 patients)		Placebo (14 patients)	
Microorganisms	Before	After	Before	After
Total aerobic count	7.2 ± 2.1	6.7 ± 1.9	7.6 ± 2.4	7.8 ± 2.0
E. coli	6.0 ± 2.1	6.2 ± 2.2	5.7 ± 1.5	6.4 ± 1.8
Enterobacteria (other)	3.0 ± 0.9 [35.7]	2.9 ± 0.7 [50]	3.3 ± 1.8 [71.4]	3.4 ± 1.2 [63.4]
Enterococci	6.7 ± 2.5	5.2 ± 3.0	7.1 ± 2.4	7.3 ± 3.0
Lactobacilli	5.5 ± 3.2 [55]	5.4 ± 2.6 [35]	7.3 ± 2.8 [65]	6.4 ± 3.5 [65]
Staphylococci	3.1 ± 1.3	3.1 ± 0.6	4.5 ± 1.8 [66.6]	4.3 ± 1.5 [33.3]
Fungi	3.6 ± 1.7	3.9 ± 1.8	4.0 ± 2.8	3.7 ± 1.3
Total anaerobic count	9.8 ± 2.1	9.4 ± 2.6	10.3 ± 2.9	10.4 ± 2.2
Bacteroides	5.2 ± 2.1	6.2 ± 3.1	7.4 ± 2.8	6.6 ± 3.2
Clostridia	8.6 ± 2.9	8.1 ± 3.3	10.0 ± 2.2	9.8 ± 2.0
Bifidobacteria	8.3 ± 3.0	8.6 ± 3.2 [70]	8.1 ± 3.1	8.5 ± 2.2
L. acidophilus	5.8 ± 2.9	5.3 ± 2.7	6.3 ± 2.2	6.8 ± 3.2
An. cocci	7.3 ± 2.4	6.9 ± 2.4	8.0 ± 2.6	8.3 ± 3.4
PH	6.21 ± 0.45	6.07 ± 0.36	6.20 ± 0.44	6.25 ± 0.65

In brackets [ ] percentage of positive samples; in other cases all samples were positive [100%].

Table 4. Enzyme activity of the intestinal microflora in children with chronic constipation before and after 1 month of treatment with calcium polycarbophil. Values are expressed as nmol/g fresh faeces (mean ± SD).

	Calcium polycarbophil (14 patients)		Placebo (14 patients)	
Enzymes	Before	After	Before	After
Esterase lipase	4.2 ± 4.2	2.0 ± 1.6	2.2 ± 1.9	1.7 ± 1.2
Leucine arylamidase	12.2 ± 10.4	15.2 ± 14.4	10.0 ± 9.3	16.4 ± 12.2
α-Galactosidase	7.5 ± 6.9	7.0 ± 5.9	5.3 ± 3.6	5.6 ± 1.7
β-Galactosidase	19.0 ± 8.9	17.0 ± 10.5	18.9 ± 6.9	20.6 ± 7.7
β-Glucuronidase	0.8 ± 1.2	1.0 ± 1.3	0.8 ± 1.2	1.1 ± 1.3
α-Glucosidase	12.5 ± 6.2	11.2 ± 4.1	11.1 ± 3.8	11.9 ± 5.3
β-Glucosidase	6.2 ± 7.2	6.5 ± 3.9	6.7 ± 2.5	5.0 ± 2.8

no longer detectable; *Propionibacterium granulosum* increased in frequency (14.3%).

Table 4 gives the data for the enzyme activity of faecal flora before and after 1 month of treatment with calcium polycarbophil and placebo. Treatment with the resin induced no significant changes.

pH in faecal samples showed no significant changes in the calcium polycarbophil-treated children.

Discussion

It is well known (4, 14) that conventional treatment, consisting in the addition of fibre to the diet, is successful only in 50% of children.

Our clinical results are similar to those reported in the literature. For the treatment of chronic functional constipation we chose the water-holding agent calcium polycarbophil instead of wheat bran, because the latter induces haematological changes as well as faecal excretion of bile salts and a predominance of proteolytic over saccharolytic faecal flora, as reported several years ago (2).

In fact, with this water-holding resin none of the haematological parameters exhibited any significant changes.

Our microbiological data offer a general picture of colonic microbiota in children with constipation and a number of bacterial metabolic activities. The stools of the constipated children showed a disturbed microbiota characterized by a high clostridia count in comparison to other genera (bacteroides and *E. coli*) and a substantial frequency of clostridia and enterobacteriaceae species, which are rarely isolated in healthy children. Functional constipation in children would therefore seem on the whole to induce no bacterial overgrowth, but only changes in selected bacterial species.

The intestinal microbiota in constipated children seems to maintain a favourable proportion of anaerobic to aerobic components (1000:1), which is regarded as physiological (15), and it was maintained after treatment with calcium polycarbophil. In constipated children the total anaerobic count appeared to be lower than that generally observed in healthy adults (10, 15) and in younger children (11). Moreover, the mean counts of clostridia, enterococci and lactobacilli of constipated children are similar to those observed in elderly subjects with constipation (17). Uniform diet, habits, lifestyle and narrow range of age should reduce possible factors of variability in our samples (11, 16, 17).

A variety of factors may potentially affect the composition of the gut flora, including environmental and dietary influences, transit time of intestinal contents, pH, age and geographically considerations as well as the interactions between the bacteria themselves (18). Major differences were described in the composition of colonic microflora in 1-y-old Estonian and Swedish infants. The prevalence of clostridia and bacteroides over lactobacilli and eubacteria was observed in Swedish infants. The different lifestyle and diet of Swedish and Estonian infants were considered factors of variability (11).

However, in different healthy subjects the quantitative proportion of clostridia, bacteroides and bifidobacteria appeared close to 1 or with 1 log difference as shown by the literature data (11, 15): we consider this general, well-known behaviour as normal biota composition, i.e. a good balance. Therefore, in constipated children, the higher number of clostridia, in comparison to other bacterial species, namely bacteroides and enterobacteria, in addition to different species of enterobacteriaceae and clostridia, represents, in our opinion, a moderately disturbed intestinal microbiota, i.e. a dysbiosis. However, the prevalence of Gram-positive components, i.e. saccharolytic flora, is also maintained in children with functional constipation, confirming that these subjects can be considered healthy without any major disturbances.

Moreover, leucine arylamidase and the saccharolytic enzymes, α-galactosidase, β-glucosidase and β-glucuronidase, which should be an expression of the prevalence of Gram-positive flora, showed slight modifications in the children with constipation. These enzymes may play a role in the metabolism of different compounds and their activation to procarcinogens. Intestinal transit time may affect the digestibility of certain substrates of endogenous and exogenous origin and the metabolites production by faecal bacteria increases with increasing transit time (18, 19). β-glucuronidase is produced in large amounts by bacteroides and enterobacteriaceae (20) which are present

in low concentrations in constipated children. The low metabolic activity of β-glucuronidase may indicate that functional constipation in children cannot be a risk factor for the formation of procarcinogens, i.e. that the levels observed are equivalent to those of a healthy subject. In other pathological conditions such as premenstrual syndrome we have observed a number of changes in β-glucuronidase activity and a reduction following treatment with probiotics (12). The metabolic capacity of the bacteria can be considered an indicator preceding evident microflora alterations or the early effects of various treatments (12, 21). There were no significant changes in faecal microbial enzyme activity after treatment.

The administration of calcium polycarbophil contributes towards correcting certain aspects of constipation. Polycarbophil is an inert bulk-forming laxative agent with hydrophilic and mechanical properties (5). It is not utilized as a substrate by intestinal microflora and does not induce the formation of volatile short-chain fatty acids. As a result of these characteristics the laxative effect of calcium polycarbophil may appear to be of low intensity and delayed in comparison with other bulk-forming agents (6). This behaviour was also observed in our paediatric patients, where the normalization of bowel movements (number of evacuations/day) was obtained without any significant changes in intestinal microflora or in the activity of a number of representative enzymes. The effect of calcium polycarbophil may be ascribed to mechanical action, without any interference with the metabolic capacity of the faecal flora. The normalization of peristalsis may be enough to induce a general intestinal improvement and a tendency to re-establish a more physiological environment. The consequence may be a reduction in the number of enterobacteriaceae species, the disappearance of *C. perfringens* and a better relative proportion between clostridia and bacteroides mean counts (as observed in our children).

In contrast with the wheat bran previously studied (2), calcium polycarbophil does not induce any changes in blood values, nor does it bring about any additional disturbances in the intestinal ecosystem.

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