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Clinical Effects of *Bifidobacterium* Preparations on Pediatric Intractable Diarrhea

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Abstract

We investigated the effects of administration of *Bifidobacterium* preparations and commercially available Bifidus Yogurt on infantile intractable diarrhea. Fifteen patients with intractable diarrhea were treated from 1982 to 1985. These patients (11 boys and 4 girls) ranged in age from 1 month to 15 years (mean 2.5 years) and received antibiotic therapy for the treatment of such diseases as septicemia and respiratory tract infections. During treatment, watery diarrhea appeared and lasted for 1 to 10 weeks (mean 25 days) with deterioration of the general condition. Conservative therapy such as diet control, infusion and drug therapy could not cure the diarrhea. The antibiotics used included cepheems, penicillins and aminoglycosides. In most cases, abnormal microflora was observed: *Candida* or *Enterococcus* often predominated with a marked decrease of anaerobes and aerobes in the stool flora. During the disease, we could not detect any pathogens or toxins responsible for the diarrhea. In all patients, the stool frequency and appearance were dramatically improved within 3 to 7 days after oral administration of *Bifidobacterium* preparations of bifidus yogurt. The intestinal microflora of all subjects also became normal with predominance of resident *Bifidobacterium* or administered *B. breve*.

Key words: intractable diarrhea, *Bifidobacterium*, intestinal microflora

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Introduction

Pediatric intractable diarrhea includes diarrhea that satisfies all of the following conditions: underminable cause, intractable clinical course and high mortality rate.^{1,2} So-called intractable pediatric diarrhea, which was the subject of this study, resisted dietary therapy and treatment with transfusion and antidiarrheal agents, producing a deteriorating systemic condition that resulted in an intractable clinical course. However, we found that treatment with *Bifidobacterium* preparations and bifidus yogurt had excellent clinical effects on this type of diarrhea. Herein we describe representative cases and discuss the mechanisms of these preparations as a cure for diarrhea.

Materials and Methods

1. Subjects

Thirteen inpatients treated at hospitals connected with the Department of Pediatrics of School of Medicine, Keio University and two other patients, for a total of 15 patients were subjects. The study period was between December 1982 and October 1985. The mean age of the patients was about 2.5 years (six cases, below 1 year, seven cases from 1 to 5 years, and two cases over 6 years); there were 11 boys and four girls. Ten of the 15 patients had the following underlying diseases: periodic granulocytopenia, Kawasaki disease, congenital nephrosis, chronic nephritis, megacolon, milk allergy, hemophilia B (two cases), ventricular septal defect and Reye syndrome.

2. Collection of Specimens

Rectal feces was collected using our original hard-glass fecal collection tube. Immediately after collection, the fecal material was put into a test tube (16 mm × 160 mm), which was maintained under anaerobic conditions with CO₂ saturation. The test tube, which was kept at a low temperature (0–5°C) was promptly transported so that specimens could be examined within three hours in all cases.

3. Bacteriology and Examination of Diarrheal Toxins

Modified VL-G medium was used to measure the total numbers of organisms.³ Modified VL-G medium, to which 80 µg/ml vancomycin and 1 µg/ml kanamycin were added, MPN medium³ and CW medium (Nissui, Tokyo), were used to detect *Bacteroidaceae*, *Bifidobacterium* and *Clostridium perfringens*, respectively. MPN medium, to which 3,000 µg/ml streptomycin and 1,000 µg/ml neomycin were added, was used to recover the administered organism *B. breve*. All of these media were prepared by the anaerobic roll tube method.⁴ The numbers of *Enterobacteriaceae*, *Enterococcus*, *Lactobacillus* and *Staphylococcus* were determined using either DHL medium (Nissui), KMN medium⁵ or Staphylo No. 110 medium (Nissui). For the isolation of fungi,

candida GS medium (Eiken Chemical Co., Tokyo) was used.

In the detection of pathogens from loose stools, *Shigella*, *Salmonella* and *Yersinia* were examined using SS medium (Nissui), *Vibrio cholerae* and *Vibrio parahaemolyticus* with TCBS medium (Eiken), and *Campylobacter* with Skirrow's medium (Nissui). At least five strains of *Clostridium difficile* and enteropathogenic *Escherichia coli* were examined from each specimen using CCFA medium⁶ and DHL medium (Nissui), respectively, and the presence or absence of toxin production was examined for each bacterium. Cytotoxin and enterotoxin produced by *C. difficile* were examined using fecal material at 1:10 dilution and culture supernatant as follows: cytotoxin was determined by means of cytopathogenic effects (CPE) on HeLa cells and CPE neutralization by anti-*Clostridium sordarii* antibody⁷ (kindly provided by Prof. Nakamura of Kanazawa University); enterotoxin was determined by means of reversed-passive latex agglutination using a kit for enterotoxin detection (kindly provided by Denka Biological Laboratories, Tokyo). Heat-labile toxin (LT) and heat-stable toxin (ST) of enteropathogenic *E. coli* were detected using a commercial kit for enterotoxin detection (Denka Biological Laboratories) and by intragastric administration into young mice,⁸ respectively.

4. Oral Administration of Viable Bacterial Preparations

Patients received a preparation (BBG-01) containing *Bifidobacterium breve* at 10^9 /g, which was isolated from breast-fed healthy infants, and another preparation (BLG-B) containing both *Lactobacillus casei* at 10^{10} /g and *B. breve* at 10^9 /g. Bacteria contained in these viable bacterial preparations are all present in commercial dairy products. In addition to these preparations, some of the patients were given bifidus yogurt (commercial name: Milmil, Yakult Honsha, Tokyo) that contained *B. breve*, *Bifidobacterium bifidum* and *Lactobacillus acidophilus* at 10^{10} /100 ml, 10^{10} /100 ml and 10^9 /100 ml, respectively. These preparations were administered at a mean daily dose of 3 g (divided by 3). A daily amount of 60–600 ml of bifidus yogurt was combined in some of the patients, based on their respective clinical states.

Results

1. Clinical Results

Table 1 shows the clinical results for the 15 patients who were given viable bacterial preparations. All these patients were being administered antibiotics for treatment of infections, none of which originated in the gastrointestinal tract, except for one case of *Salmonella* enteritis. The most frequently found infectious diseases in these patients were septicemia (two cases) and suspected septicemia (six cases), and respiratory infections (four cases). Peritonitis, furuncles and *Salmonella* enteritis were found in one case each. The antibiotics administered to the patients included cepheems, penicillins

Table 1 Summary of Clinical Responses of Infants with Intractable Diarrhea

Patients No.	Age Sex	Underlying disease	Symptoms	Antibiotics	Bacterio-therapy	Duration of diarrhea (days)	
						before treatment	after treatment
1.	2y F	periodic granulocytopenia	furuncle	CCL,CET,GM,PIPC	BLG-B	7	7
2.	2y10m M		sepsis	KM,GM,ABPC,CBPC, CEZ,CMZ,CTX	BLG-B MTTMIL	30	14
3.	1y M	Kawasaki disease	bronchitis	CEX	MILMIL	35	7
4.	1y M		sepsis ?	LMOX,CET	BBG-01	5	7
5.	1y8m F	nephrosis	salmonellosis	ABPC,ST,KM,FOM	BLG-B	35	8
6.	1m M		bronchitis	ABPC,AMP	BBG-OL	25	7
7.	15y M	chronic nephritis	peritonitis	TOB,CET	BBG-01	11	6
8.	15m M	Hirshsprung	sepsis ?	TOB,CBPC,CTX,FOM, Gm,CAZ	BBG-01	10	3
9.	1m F	milk allergy	sepsis ?	ABPC,CEZ,KM	BBG-01	25	7
10.	3y M	hemophilia B	sepsis ?	CTX,CLDM,TOB	BBG-01	9	4
11.	6y M	hemophilia B	URTI	CTX,CXM	BLG-B	70	10
12.	1m M		sepsis ?	ABPC,LMOX	BBG-01	7	4
13.	3m M	ventricular septal defect	sepsis	CTX,MCIPC,ABPC, LMOX,PIPC,CP	BBG-01	30	10
14.	4y6m F	Reye syndrome	broncho-pneumonia pulmonary edema	ABPC,PIPC,CET,GM	BBG-01	40	7
15.	3m M		sepsis ?	CMZ	BBG-01	40	4

BLG-B ; Combined preparation of *Bifidobacterium breve* (10^9 /g) and *Lactobacillus casei* (10^{10} /g).

BBG-01 ; Preparation of *B.breve* (10^9 /g).

MILMIL ; *Bifidobacterium* yogurt : containing 10^{10} of viable *B.breve*,*B.bifidum* and 10^8 of *L.acidophilus* per 100ml of bottle.

and aminoglycosides, either singly or in combination.

The duration of diarrhea prior to the administration of viable bacterial preparations was an average of 25.3 days (ranging from 5–70 days). Diarrheal passages returned to normal after a mean of 7.0 days (3–14 days). Diarrhea was green to yellow-green and watery or mucous. The number of defecations varied from five to several dozen per day.

Out of the 15 patients, 10 patients received treatment with BBG-01 (*B. breve* at 10^9 /g); three a combined preparation, BLG-B (*B. breve* at 10^9 /g and *L. casei* at 10^{10} /g); the remaining two either a combination of BLG-B and bifidus yogurt (Milmil) or bifidus yogurt alone.

2. Changes in Enteric Flora

Table 2 shows changes in enteric flora before (drastic period) and during the treatment with viable bacterial preparations (recovery period). Enteric flora could be examined in 13 of the 15 patients. The total number of bacteria before the treatment had clearly decreased, to 8.51 ± 1.77 /g (mean log value $\pm$ S.D. per 1 g wet fecus weight), but this number returned to the normal level at 10.21 ± 0.26 /g during the treatment,

Table 2 Summary of Fecal Flora Changes of Infants with Intractable Diarrhea

Patients No.	Antibiotics	Bacteriotherapy	Total bacteria	Bacteroidaceae	Bifidobacterium (Dosed B.breve)	Enterobacteriaceae	Enterococcus	Candida	Clostr. difficile	Fecal Toxin, CT ET
1.	CCL, CET, GM, PIPC	*B	6.46	5.30	<2.30	<3.30	<3.30	6.46	NT	
		†A	10.61	9.43	10.61 (7.85)	9.33	8.99	5.79		
2.	KM, GM, ABPC, CBPC, CEZ, CMZ, CTX	B	5.69	4.30	<2.30	<2.30	2.30	5.69	NT	
		A	9.75	8.23	9.49 (9.36)	8.43	8.09	3.41		
5.	ABPC, ST, KM, FOH	B	9.72	<4.30	<2.30	4.66	9.72	7.16	<3.30	<3.30
		A	10.23	9.81	9.81 (9.15)	9.94	8.08	5.76		
6.	ABPC, AMPC	B	7.40	7.25	<1.81	7.39	3.77	<2.81	<2.81	<2.81
		A	10.26	8.58	10.15 (9.76)	8.48	7.60	<2.32		
7.	TOB, CET	B	9.55	7.85	8.86	7.75	5.67	<2.30	<2.30	<2.30
		A	10.51	7.00	10.04 (9.00)	6.56	7.08	<2.30		
8.	TOB, CBPC, CTX, FOM, GM, CAZ	B	9.04	9.00	<2.30	7.34	7.43	7.79	<2.30	NT
		A	10.34	7.77	9.88 (9.88)	9.23	8.48	2.30		
9.	ABPC, CEZ, KM	B	9.29	8.92	6.32	9.29	<2.67	<2.67	<3.67	<3.67
		A	10.55	9.38	10.53 (9.77)	9.53	7.68	<2.34		
10.	CTX, CLDM, TOB	B	9.62	8.72	5.91	9.62	6.48	<2.30	<3.30	<3.30
		A	10.08	9.87	9.89 (9.60)	8.97	8.68	<2.30		
11.	CTX, CXM	B	9.58	8.06	6.31	9.56	8.33	5.39	<3.30	<3.30
		A	10.68	9.11	10.26 (9.93)	9.06	10.00	5.19		
12.	ABPC, LMOX	B	5.26	<1.30	<1.30	5.26	<2.30	<2.30	<2.30	<2.30
		A	10.32	5.82	10.32 (8.73)	8.83	5.82	<2.30		
13.	CTX, MCIPC, ABPC, LMOX, PIPC, CP	B	8.15	<3.96	<2.96	<2.96	3.45	8.15	<2.96	- -
		A	10.19	9.36	9.26 (9.26)	9.71	9.89	<2.96		
14.	ABPC, PIPC, GM, CET, AMK	B	10.52	<8.29	<5.67	8.85	9.63	4.35	2.44	- -
		A	10.58	8.51	10.35 (7.20)	9.19	9.48	5.60		
15.	CEZ	B	10.38	<6.30	<4.30	10.04	9.87	<2.30	<2.30	- +
		A	10.18	9.49	9.66 (9.30)	9.61	9.04	<2.30		

* B; before bacteriotherapy

† A; after bacteriotherapy

the time at which diarrhea was being cured. The most dominant bacteria before treatment included facultative anaerobes (*E. coli* group in six cases, *Enterococcus* in two) and *Candida* (three cases). *Bifidobacterium* were not detected in nine of the 13 patients; the number of cells was decreased to $6.97 \pm 1.31/g$ even in the four patients with *Bifidobacterium*, indicating eradication or marked decreases of *Bifidobacterium*. After diarrhea was cured by treatment with viable bacterial preparations, *Bifidobacterium* predominated over other bacteria in the intestinal tract in 11 of the 13 patients, *Bifidobacterium* together with other bacteria (*E. coli* group and *Enterococcus* in one patient each) predominated in the remaining two patients, with the number of *Bifidobacterium* returning to the normal level of $9.82 \pm 0.96/g$ ($n=13$). The administered *B. breve* was recovered from all of the 13 patients, at a level of $9.68 \pm 0.96/g$.

From these results, decreases in anaerobes, especially decreases or eradication of *Bifidobacterium*, were markedly observed during the drastic period, while increases in facultative anaerobes, including the *E. coli* group and *Enterococcus*, and *Candida* were characteristic of this period. After diarrhea was cured by treatment with vital bacterial preparations, normal flora, consisting of *Bifidobacterium* for the most part, was formed. Representative cases are given below:

3. Case Reports

Case 1 (Figs. 1 and 2):

Case 1 (1983) was a 2-year-old girl suffering from periodic granulocytopenia as an underlying disease. There were furuncles on her neck and external genitalia. The patient had fever two days before hospitalization, and although she received cefaclor (CCL) orally at 500 mg/day, she was not relieved, but had very severe watery diarrhea at a frequency of more than 14 times a day after hospitalization. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated from the external genitalia, which exhibited gangrenous lesions. Watery diarrhea at a rate of 14–17 times per day persisted for about one week, and was resistant dietary therapy such as prohibition of eating or drinking and administration of 1/2 cow milk. At this time, we started to give her the combination preparation BLG-B containing *B. breve* and *L. casei*. Although treatment with cephalothin (CET), gentamicin (GM) and piperacillin (PIPC) was continued, the frequency and state of defecations showed improvement at the fifth day of treatment with BLG-B and returned to normal at the seventh day (the day after discontinuation

Fig. 1 Clinical course of patient No. 1.

Fig. 2 Feecal flora of patient No. 1.

of chemotherapy).

At the time of first examination of enteric flora, her fecal state gradually improved from watery diarrhea to muddy stool with particles, in spite of ongoing treatment of her gangrenous external genitalia with CET, GM and PIPC. *Candida albicans* was seen only at 6.0 (log cell number per 1 g wet fecal weight), in the intestinal tract, while both anaerobes and facultative anaerobes were markedly decreased, even though administered

bacteria were not observed at the fifty day of the treatment with BLG-G, possibly due to chemotherapy. On the fourth day after the termination of chemotherapy (12th day of treatment with BLG-B), her fecal state had already returned to normal, and indigenous *Bifidobacterium* predominated among enteric flora. After that, this patient received CET for the treatment of tonsillitis, but bifidus flora was stable. Administered *B. breve* was detected at a level of 8.0.

Case 2 (Figs. 3 and 4):

This patient was a 22-month-old boy in 1983. He was hospitalized for vomiting and the occurrence of diarrhea more than 14 times a day. Immediately after admission, drinking and eating were prohibited, and kanamycin syrup, lactase and commercially available *Lactobacillus* preparation were administered. He had ileus at the fifth day of hospitalization and developed septicemia at the 12th day. The patient was then treated with ampicillin (ABPC), gentamicin (GM) and cefazolin (CEZ). *Klebsiella pneumoniae* was detected in his blood. After the start of treatment for septicemia,

*LacB: *Lactobacillus* preparation

Fig. 3 Clinical course of patient No. 2.

Fig. 4 Fecal flora of patient No. 2.

jaundice appeared, and pyothorax was an additional complication. In order to treat the pyothorax, carbenicillin (CBPC), GM, cefotaxime (CTX) and cefmetazole (CMZ) were administered in addition to other treatments, including thoracic drainage. Because the patient's condition was in a very critical and he also developed heart failure at the time of the onset of pyothorax following septicemia, adrenal cortex hormones and digoxin were administered. Furthermore, watery diarrhea occurring 5–10 times a day persisted for about one month. During this period, because diarrhea was not reduced in spite of dietary therapy such as prohibition of drinking and eating and intravenous hyperalimentation (IVH), combination therapy with BLG-B and bifidus yogurt was started at the fifth day of treatment for pyothorax. At the second day of administration

of bifidus preparations, not only had his green watery stool changed to yellow, muddy stool, but there was also sudden improvement in his systemic status. The number of defecations and fecal state returned to normal in about two weeks, although GM and ceftizoxime (CZX) were still being administered at this time.

At the time of the first examination for enteric flora, green watery stool was seen about ten times a day. Immediately after the collection of feces, the administration of both BLG-B and bifidus yogurt was started. Fecal cultures revealed abnormal flora, including *Candida* at a level of about 6.0 and *Lactobacillus*. At the second examination, performed in the first week of treatment with BLG-B and bifidus yogurt, *Enterococcus* predominated at a level of 8.0, and *B. breve*, which was administered, was found at a level of 4.0 together with *Lactobacillus*, in spite of the continuation of treatment with GM and CZX. Fecal consistency changed to yellow muddy from watery stool, and there was a reduction of defecations to four times a day, as well. *B. breve* at a level of 9.0 was detected together with *Enterococcus* as the predominant bacteria two weeks after treatment with bifidus preparations. At that time, GM was discontinued. Diarrhea was completely resolved, as his feces had changed to a normal, solid state, and defecations were reduced to twice a day. At the final examination performed after about one month, CZX was discontinued. *Bifidus* flora, consisting predominantly of administered *B. breve*, was observed. Thereupon, the patient was discharged.

Case 3 (Figs. 5 and 6):

This patient was a 3-month-old boy in 1985. His parturient conditions were completely normal, mature delivery occurred at the 42nd week of gestation and birthweight was 4,145 g. Although he was bottle-fed, his suckling strength decreased, and vomiting and diarrhea occurred three months after birth. Therefore, he was hospitalized at a nearby hospital. At the time of admission, dehydration and almost shock-like symptoms were observed as well as fever and a strongly positive inflammatory reaction, suggesting septicemia. Cefmetazon (CMZ) at 300 mg/day was given for two weeks. During this time, watery diarrhea occurring 10–20 times/day continued. Thereafter, diarrhea was reduced to 5–7 times/day for a short time but again increased to 8–10 times/day four months after birth. The boy was then transferred to another public hospital. At that time, so-called malabsorption syndrome was suspected due to his thin layer of subcutaneous fat. A combination of transfusion and 1/2 Bonlact (soy bean milk, Wakodo Co., Tokyo) with BBG-01 rapidly improved his fecal consistency and the frequency of defecation. Normal feces was seen at the fifth day.

Enteric flora before the treatment with BBG-01 showed *E. coli* and *Enterococcus*, both at a level of 10.0, as the predominant bacteria; *Pseudomonas aeruginosa* was also detected at a level of 9.0. At that time, watery or soft stool was seen at a frequency of five times/day. At the fourth day of treatment with BBG-01, both *Bacteroidaceae*

Fig. 5 Clinical course of patient No. 15.

and *Bifidobacterium* were the most dominant, both at a level of 10.0; the administered *B. breve* was also seen at a level of 8.0. Soft or normal feces was observed at a rate of two times/day. *P. aeruginosa* had quickly decreased to a level of 4.0 from 9.0. At the final examination (second week of treatment with BBG-01), *Bifidobacterium*, *E. coli* group, *Bacteroidaceae* and the administered *B. breve* were seen as the predominant bacteria, typical of the flora found in bottle-fed infants.

Discussion

The most common definition of intractable diarrhea in the pediatric field was put forth by Avery, *et al.*,¹ who advocated the following criteria: 1) diarrhea continues for longer than two weeks; 2) it occurs within 3 months after birth; and 3) fecal cultures performed more than 3 times show no evidence of causative organisms for the

Fig. 6 Fecal flora of patient No. 15.

diarrhea. As symptomatic treatment, transfusion is considered to be an important tool.⁹ Strictly speaking, the patients in our study did not exactly fit Avery's criteria; however, our patients resisted dietary therapy and treatment with drip infusion therapy and drugs that are commonly used for the treatment of diarrhea in the pediatric field in Japan, and they showed intractable clinical courses, leading to poor systemic states. Therefore, it is impossible to have a suitable control group for intergroup comparison. Intra-

subject comparisons before bacteriotherapy were therefore done in these patients. Most of the patients were treated with antibiotics before the occurrence of diarrhea, suggesting that the onset of diarrhea was associated with treatment with antibiotics.

The occurrence of diarrhea is well known to accompany by treatment with antibiotics. Oral treatment with unabsorbable antibiotics affects enteric flora. Although antibiotics absorbed in the upper intestinal tract theoretically have no effect upon bacteria flora in the lower intestinal tract, this is not always true. Moreover, although parenteral treatment with antibiotics is thought to strongly inhibit the activities of enteric flora under good bile secretion, we often encounter strong inhibition of bacterial activities in the intestine even under poor bile secretion. This is because enteric flora is affected by antibiotics secreted from the intestinal mucosa. Therefore, enteric flora must be affected by treatment with antibiotics to some extent, regardless of the drug(s) being administered or the route(s) of administration. It cannot be denied that changes in enteric flora play some role in the onset of diarrhea.

Recently Larson, *et al.* reported that antibiotic-induced pseudomembranous enterocolitis is caused by toxin produced by *Clostridium difficile* due to changes in enteric flora.¹⁰ This finding has been confirmed by many investigators, leading to clarification of one of the mechanisms leading to the onset of antibiotic-induced diarrhea.

As shown in Table 2, 10 of 11 patients in whom we were able to examine the presence or absence of *C. difficile* exhibited negative results. In the three patients in whom enterotoxin (ET) and cytotoxin (CT) of *C. difficile* were examined, no relationship to diarrhea was seen. Although hemorrhagic colitis, one of the antibiotic-induced types of diarrhea, was found to be related to *Klebsiella oxytoca* by Totani, *et al.*,¹¹ its pathogenic association with diarrhea has come under question. Suzuki, *et al.* pointed out that ischemic enteritis caused by immediate allergy in the intestinal tract in situ is counted as one of the mechanisms in the onset of hemorrhagic colitis.¹² However, clinical symptoms are different from those found in our patients, as this type of hemorrhage occurs abruptly, and diarrhea accompanied by the hemorrhage disappears rapidly after discontinuation of treatment with antibiotics.

Fig. 7 shows a chart describing the mechanisms involved in the onset of antibiotic-induced diarrhea and in the recovery from diarrhea with viable bacterial preparations, mainly bifidus preparations. Enteric flora has various metabolic activities that are essential to maintenance of the health and physiological conditions of the host. From the standpoint of enteric physiology as it relates to the onset of diarrhea, inhibition of anaerobes, which predominate in the large intestine, means that bile acid metabolism and organic acid fermentation, which requires unabsorbed vegetable polysaccharides and mucin as its substrates, are largely affected.^{13,14} Among these metabolic activities mainly led by enteric anaerobes, decreases in the activity of 7 α -dehydroxylase, which converts primary bile acids to secondary bile acids, indicate inhibition of the conver-

Fig. 7 Some possible causes of antibiotic associated diarrhea and cure mechanisms of *Bifidobacterium* therapy on infantile intractable diarrhea.

sion of deoxycholic acid and chenodeoxycholic acid into lithocholic acid. Needless to say, the amount of primary bile acids will be increased if this metabolic activity of anaerobes is suppressed by treatment with antibiotics. From the standpoint of bile acid-induced diarrhea, it is especially noted that chenodeoxycholic acid plays an important role in the onset of diarrhea, due to its detergent effects causing injury to mucous epithelial cells.¹⁵

Since products of short chain fatty acids such as acetic acid, propionic acid and butyric acid result from the main energy metabolisms of enteric flora, the production of these organic acids is reduced as a consequence of the inhibition of activities of enteric anaerobes by treatment with antibiotics. It was revealed that short chain fatty acids in the large intestine usually facilitate absorption of Na^+ and H_2O .¹⁶ Therefore, it can be said that the inhibition of anaerobes in conjunction with treatment with antibiotics possibly reduces the production of these short chain fatty acids, resulting in malabsorption of water.

Mucin secreted from the large intestine is a very important source of carbon for enteric flora.¹⁷ Although mucin is usually decomposed quickly, this substance is accumulated in the intestinal tract at a high level if enteric flora is suppressed by treatment with antibiotics, possibly inducing diarrhea as a result of elevated osmotic pres-

sure.¹⁸

The cecum of germ-free animals is greatly swelled by water retention.¹⁹ Treatment with antibiotics frequently causes the same phenomenon in germ-free animals. The inhibition of bile acid metabolism, organic acid fermentation and utilization of mucin decomposition seems to be closely related to water retention in the cecum of germ-free animals, resulting in the occurrence of diarrhea.

Our study revealed that viable bacterial therapy, mainly using bifidus preparations, quickly induced enteric flora to return to normal states, in which *Bifidobacterium* sp. predominate. This meant that metabolic activities of enteric flora returned to physiological conditions and the balance between the host and its enteric physiology returned to normal. For example, when enteric flora returns to normal, malabsorption of water and electrolytes is corrected through short chain fatty acids produced by the normal flora, resulting in a cure of diarrhea.

We used the following three kinds of viable bacterial preparations: *B. breve* alone; two species, *B. breve* and *L. casei*; and *B. breve*, *B. bifidum* and *L. acidophilus*. Because *B. breve* was a component common to the three preparations and because it predominated among the administered bacteria in all of our patients, *B. breve* was thought to play the most important role in the cure of diarrhea.

We have so far discussed the mechanisms involved in the recovery of diarrhea from the viewpoint of normalization of abnormal enteric flora by viable bacterial preparations. However, clinical symptoms were improved before the completion of enteric flora normalization in some of our patients. In other words, the normalization of enteric flora was not always correlated with improvement in clinical symptoms. There were very interesting clinical findings such as that, prior to the improvement of fecal states, borborygmus was decreased, abdominal pain was reduced, and systemic status was markedly improved, and that fecal consistency was improved preceding the normalization of enteric flora in some patients. If fecal state improved after enteric flora was normalized in the recovery course of diarrhea treated with viable bacterial preparations, it could be said that physiological changes in the intestinal tract, which were induced by the normalization of enteric flora, were useful in the treatment of diarrhea. However, since some patients showed improvement of clinical symptoms before the completion of enteric flora normalization, other factors may be participating in the recovery of diarrhea besides those normalizing enteric flora. This point remains to be further investigated.

Enteric flora partially serves the physiological functions of its host through its metabolic activities. With this in mind, we hope that clinicians keep a watchful eye on dangerous aspects of chemotherapy and on their countermeasures and treatments (including the use of viable bacterial preparations).

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