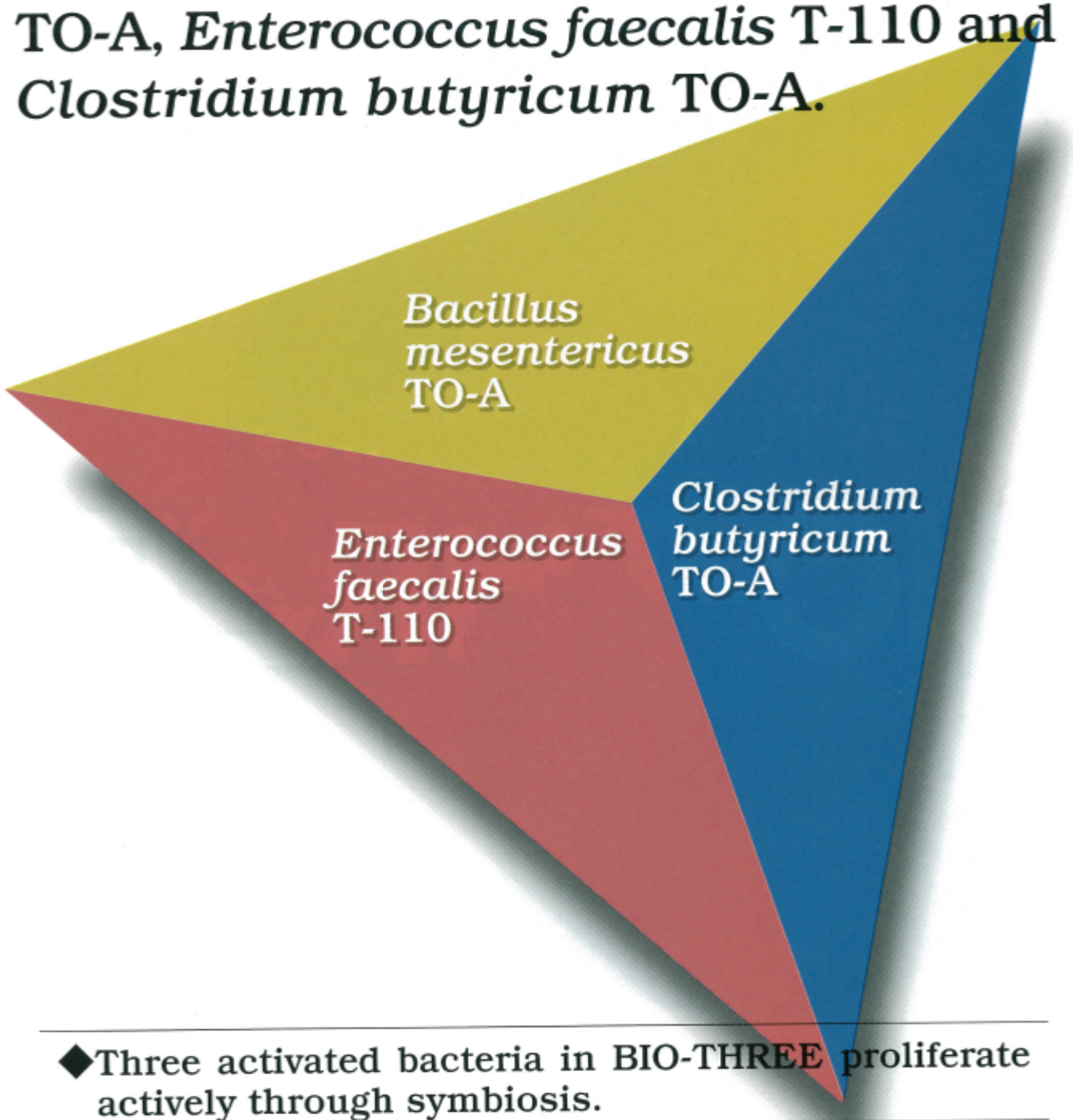
TOA Probiotics

BIO-THREE®

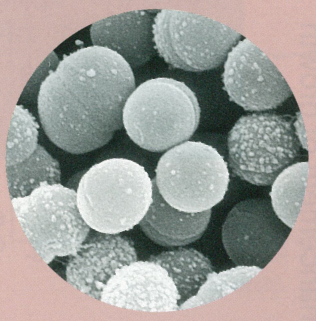
TOA PHARMACEUTICAL CO.,LTD

Symbiosis of *Bacillus mesentericus* TO-A, *Enterococcus faecalis* T-110 and *Clostridium butyricum* TO-A.



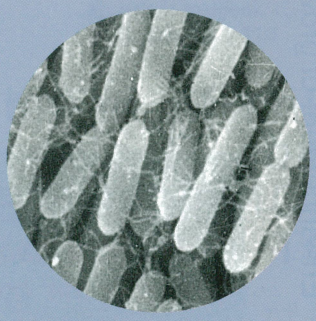
- ◆Three activated bacteria in BIO-THREE proliferate actively through symbiosis.
- ◆BIO-THREE inhibits strongly the growth of harmful bacteria by three activated bacteria.
- ◆BIO-THREE facilitates proliferation of *Bifidobacterium*.
- ◆BIO-THREE normalizes intestinal flora to show excellent control of intestinal condition.

BIO-THREEis a medicine for intestinal disorder. Containing three useful activated bacteria such as *B.mesentericus* TO-A, *E.faecalis* T-110 and *C.butyricum* TO-A,which act on various sites of the intestinal tract.



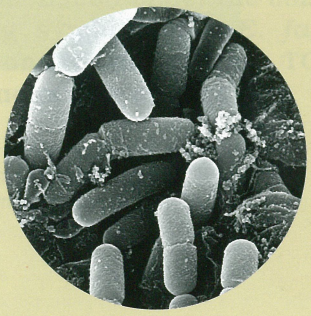
E.faecalis T-110

This bacterium proliferates actively through the symbiotic action with *B.mesentericus* TO-A and *C.butyricum* TO-A to yield lactic acid with inhibition of growth of harmful bacteria. Additionally, this bacterium has high resistance against bile acid,and even in raw bile it shows a higher residual rate than any other bacteria.



C.butyricum TO-A

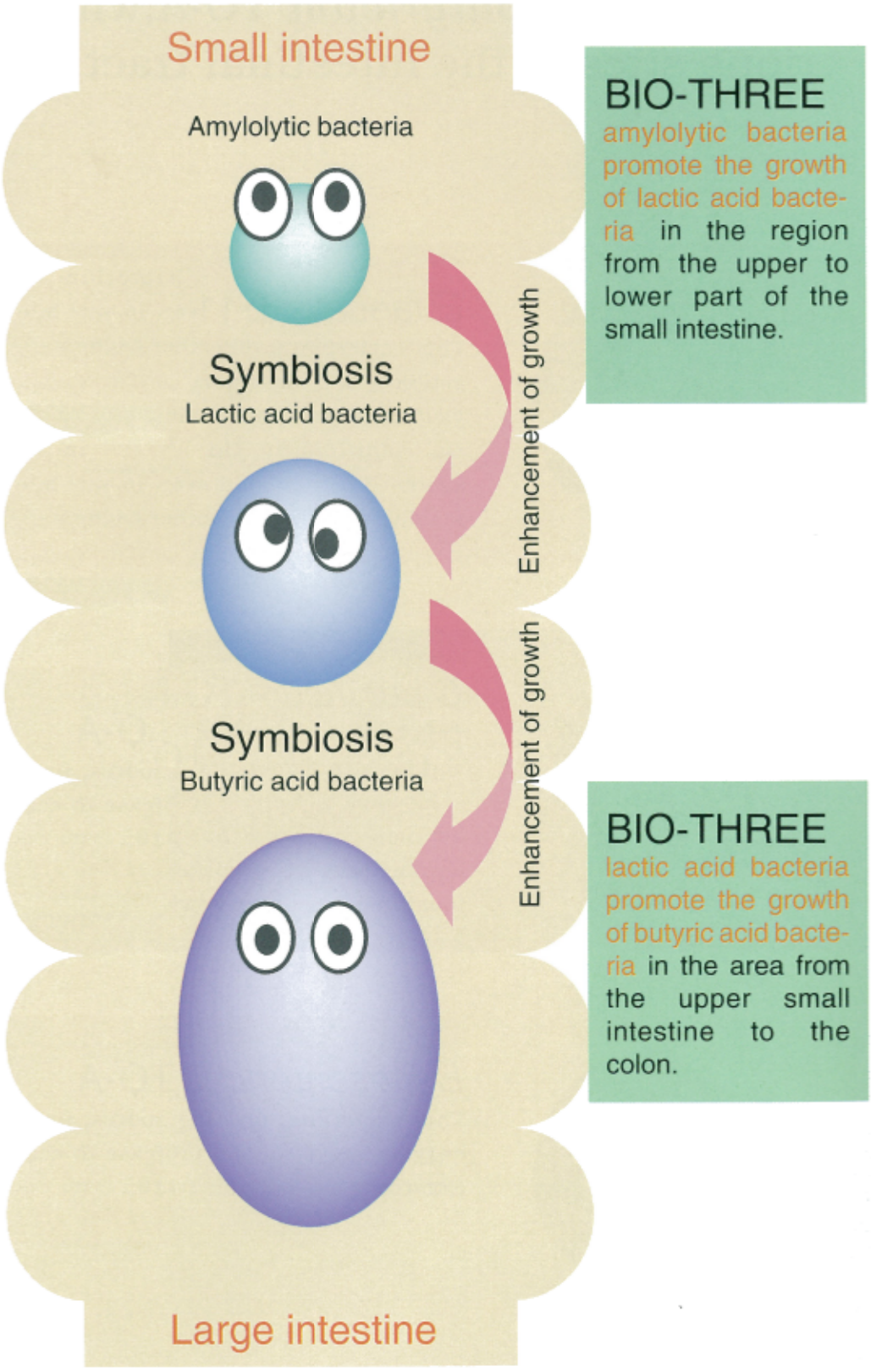
This bacterium proliferates actively through the symbiotic action with *E.faecalis* T-110 to yield short-chain fatty acids such as butyric acid and acetic acid with a resultant decrease in intestinal pH and inhibition of growth of harmful bacteria. Additionally, it acts on the intestinal tract to improve abnormal bowel activity.



B.mesentericus TO-A

This bacterium, forming spores, produces an amylolytic enzyme (amylase) and protease to activate proliferation of *Enterococcus faecalis* T-110.

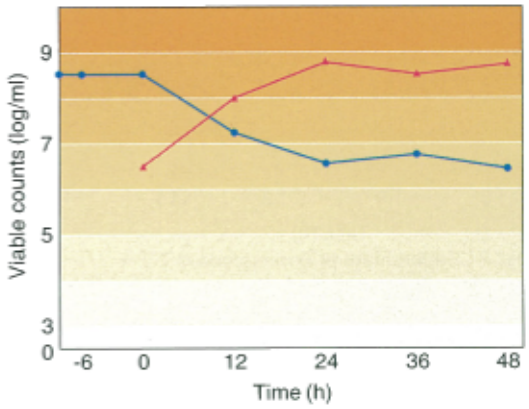
How do the three BIO-THREE bacteria work in the intestine? (Reasons for their combination)



Inhibition of pathogenic bacteria through symbiosis

Through symbiosis of *E. faecalis* T-110 and *C. butyricum* TO-A, inhibition of pathogenic bacteria in the intestinal tract is enhanced.

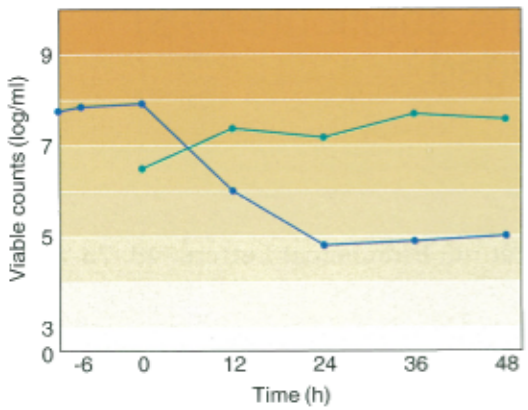
Inhibition of entero toxigenic *Escherichia coli* by *E.faecalis* T-110 alone



Time-course change in viable counts in a mixed culture of *E. faecalis* T-110 and entero toxigenic *E.coli* in continuous flow culture

● entero toxigenic *E. coli*
▲ *E. faecalis* T-110

Inhibition of entero toxigenic *E. coli* by *C. butyricum* TO-A alone



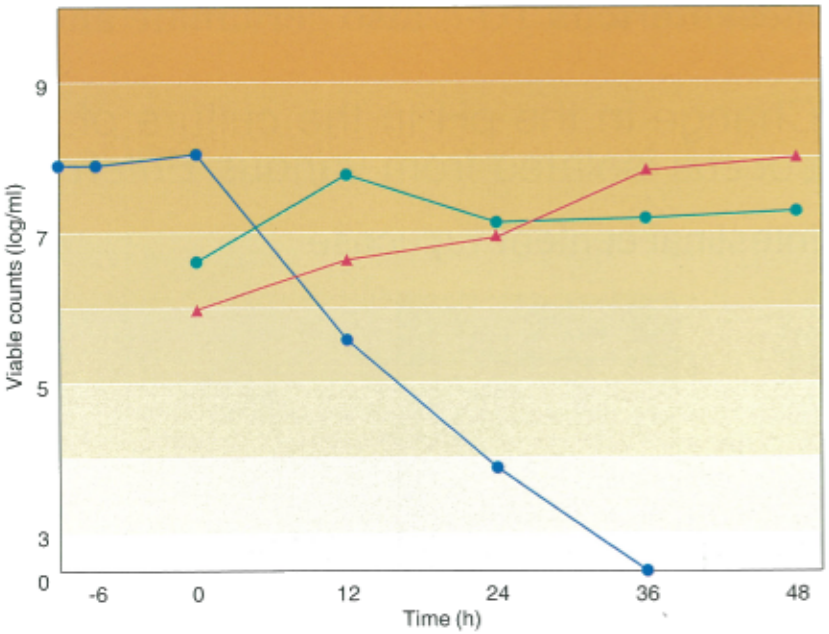
Time-course change in viable counts in a mixed culture of *C. butyricum* TO-A and entero toxigenic *E. coli* in continuous flow culture

● entero toxigenic *E. coli*
● *C.butyricum* TO-A

Inhibition of entero toxigenic *E. coli* by *E .faecalis* T-110 and *C. butyricum* TO-A alone

Time-course change in viable counts in a mixed culture of *E. faecalis* T-110, *C. butyricum* TO-A and entero toxigenic *E. coli* under the condition keeping symbiosis of *E. faecalis* T-110 and *C. butyricum* TO-A in continuous flow culture

● entero toxigenic *E. coli*
▲ *E. faecalis* T-110
● *C.butyricum* TO-A

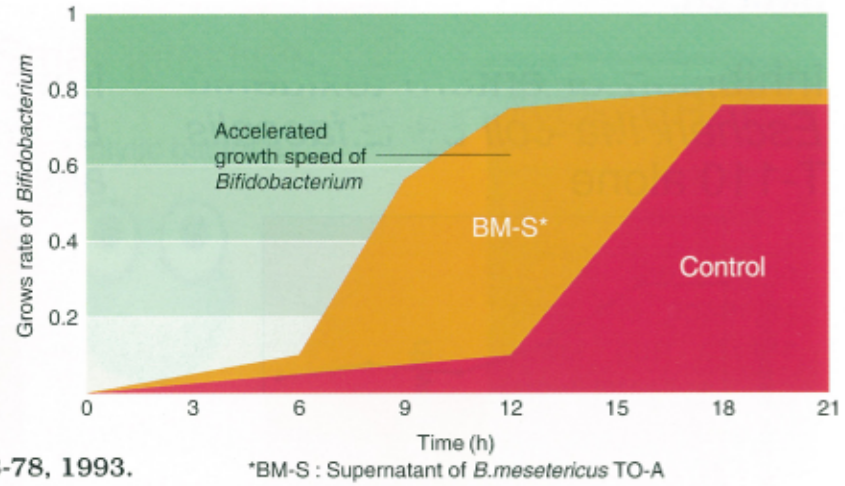


G. Seo et al.: Microbios Letters. 40, 151-160, 1989.

Proliferation of useful bacteria by *B. mesentericus* TO-A

B.mesentericus TO-A facilitates proliferation of useful bacteria such as _Bifidobacterium_.

Facilitation of division of *Bifidobacterium* when the filtrate of *B. mesentericus* TO-A culture solution(BM-S) is added



H. Iino *et al.*: Biomedical Letters. 48, 73-78, 1993.

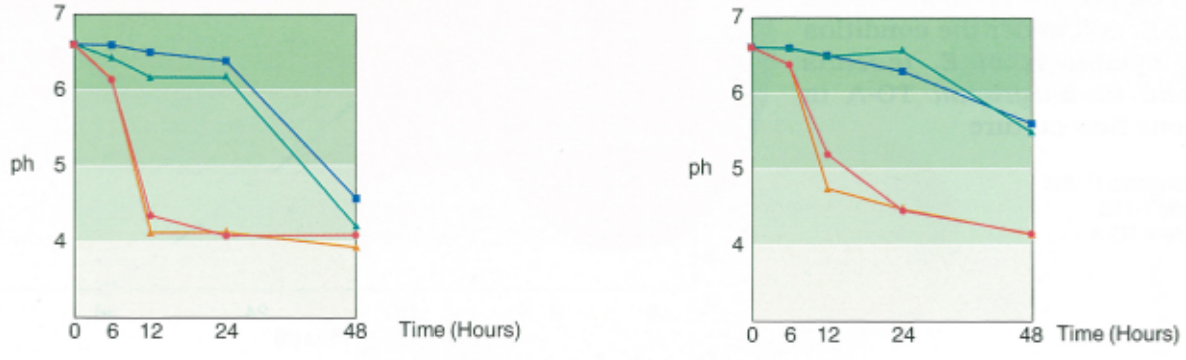
*BM-S : Supernatant of *B.mesetericus* TO-A

Proliferation in intestinal content

It was confirmed that _E. faecalis_ T-110 in BIO-THREE may show high proliferation in the culture using intestinal content. And it was observed in clinical trials that _C. butyricum_ TO-A and _B. mesentericus_ TO-A also germinate and proliferate in the intestine.

Change in the pH in the culture of intestinal content of *Enterococcus* isolated from various *Enterococcus* preparations

Intestinal content+glucose

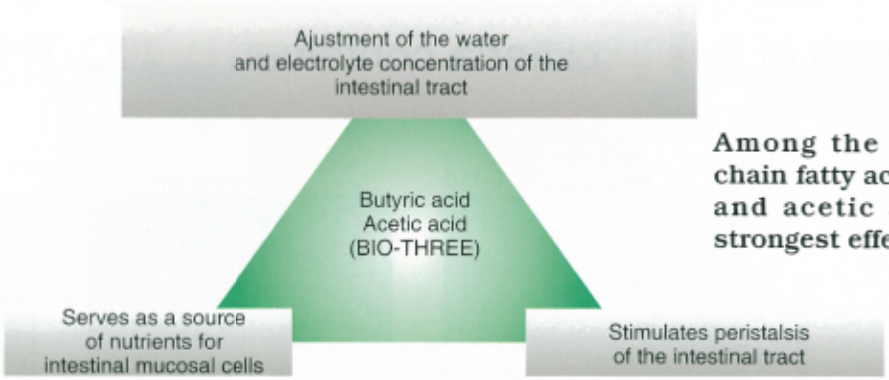


Preparations B and C are those containing resistant *Enterococcus*

Yukinari Shimazaki et al.: Hospital Pharmacy. 12, 295-302, 1993.

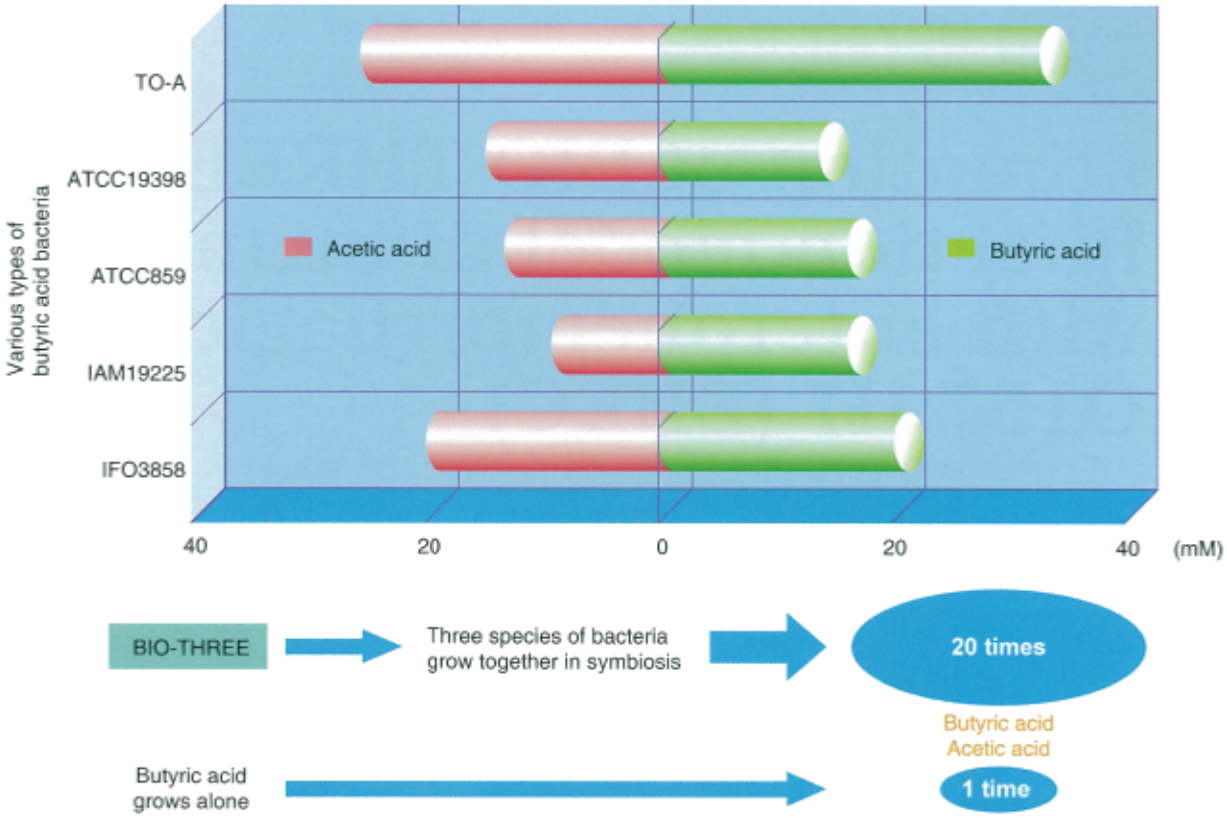
Effects of short chain fatty acids produced by BIO-THREE bacteria

Effect of short chain fatty acids



Among the various short chain fatty acids, butyric acid and acetic acid have the strongest effects.

Amounts of butyric acid and acetic acid produced by BIO-THREE



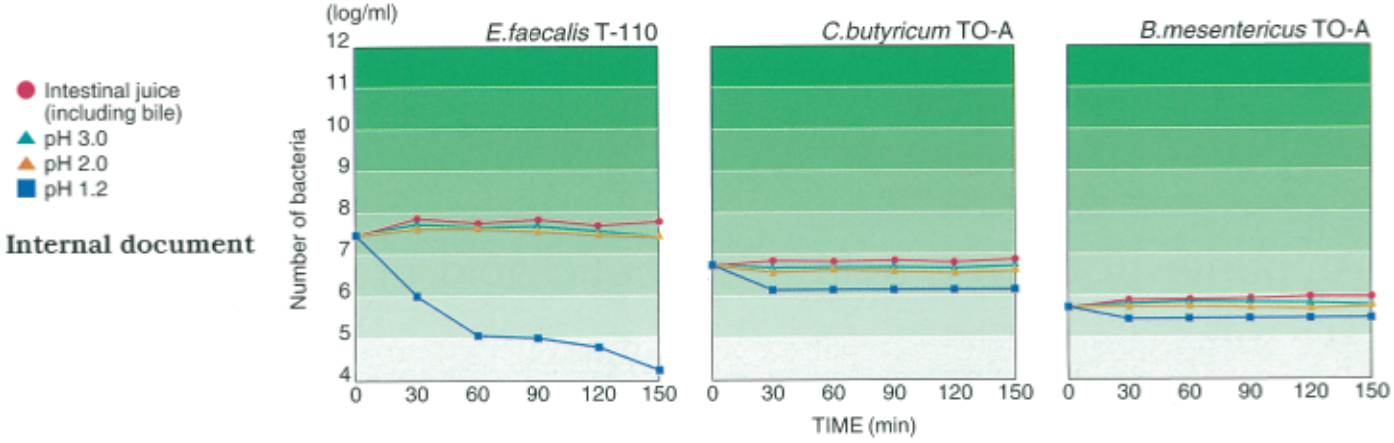
Sensitivity to drug

It is observed that *E. faecalis* T-110 in BIO-THREE shows low sensitivity to cephalosporins, aminoglycosides and new quinolones.

Residual rate in gastric and intestinal juices

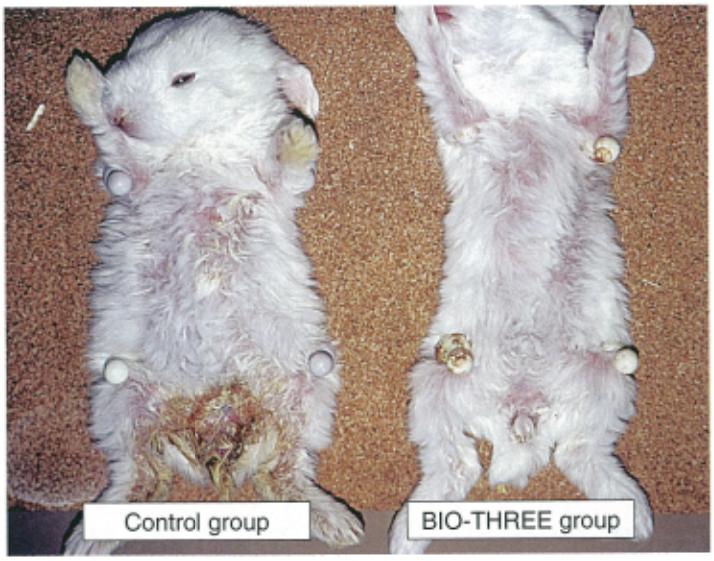
It was confirmed that *E. faecalis* T-110 in BIO-THREE is not inactivated in artificial gastric juice of more than *B. mesentericus* TO-A are not affected at pH 1.2 at all.

Residual rate of three bacteria contained in BIO-THREE at various pH



Internal document

Estimation of BIO-THREE by infection model of infant rabbit with enterohemorrhagic *E. coli* O157:H7 (EHEC)



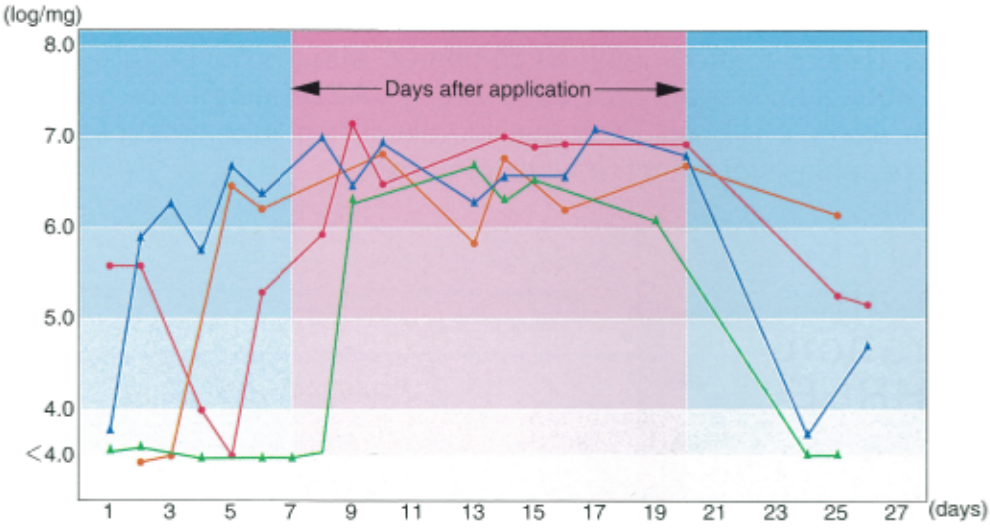
Three days after EHEC inoculation, diarrhea was observed in approximately 80% of the control group (left rabbit). While, in Bio-Three group, diarrhea was only 15% (right rabbit).

Tachikawa et al. J Jap Infect. Dis.: 72.1300-1305.1998.

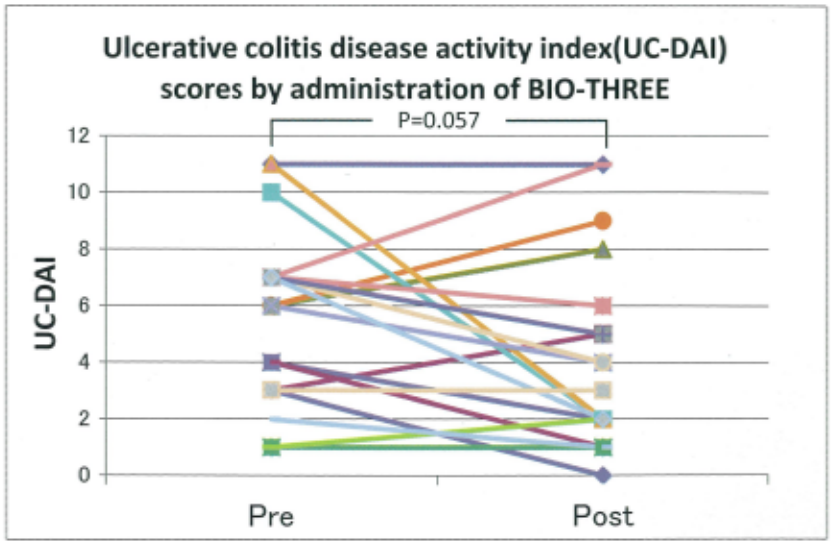
Influence on intestinal flora

Bifidobacterium were increased significantly when "BIO-THREE" was administered to bottle-fed infants.

Time course of change of fecal *Bifidobacterium* of bottlefed infants during the administration of "BIO-THREE".



Clinical effectiveness of probiotics therapy (BIO-THREE) in patients with ulcerative colitis refractory to conventional therapy

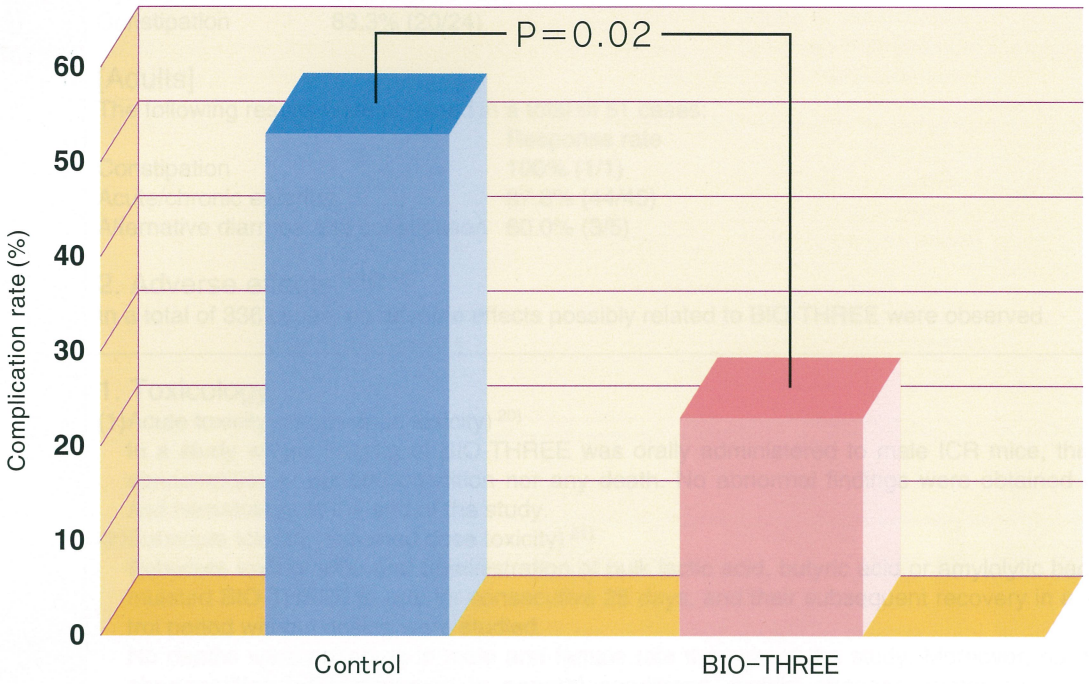


Total 20 outpatients were given daily 9 tablets for 4weeks. Patients age ranged from 19 to 71. With the exception of 6 patients who were intolerant to mesalamine, other 14 patients received medication for UC.

Y Tsuda et al. Scand. J. Gastroent: 42.1306-1311.2007.

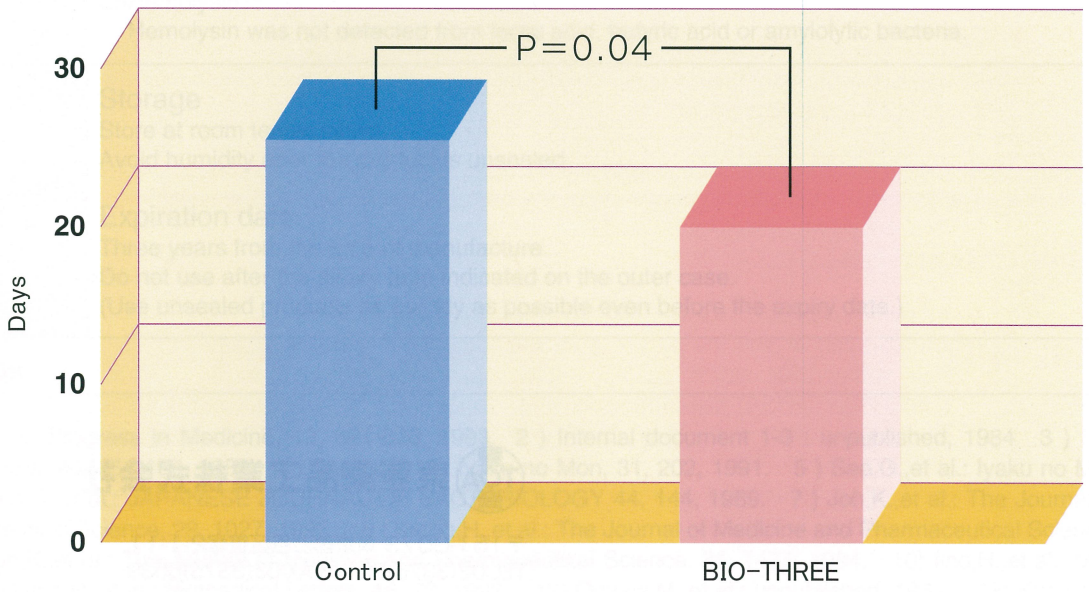
BIO-THREE Reduces Infectious Complications after Pancreaticoduodenectomy.

Incidence of infectious complications



Incidence of infectious complications in BIO-THREE group (23%, 7/30) was significantly lower than in the control group (53%, 18/34). (P=0.02)

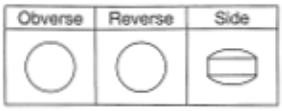
Length of postoperative hospital stay



Median length of postoperative hospital stay was 19 days for patients in BIO-THREE group (range 11-40) and 24 days for those in the control group (range 11-91; P=0.04).

T. Nomura et al., Hepato-Gastroenterology 2007; 54: 661-663

Drug Information

<table>
<tr><td>Trade Name:</td><td>BIO-THREE® POWDER</td><td>BIO-THREE® TABLETS</td></tr>
<tr><td>Approval NO.:</td><td>13800AZZ00418000</td><td>14000AZZ00262000</td></tr>
<tr><td>Date:</td><td>January 29, 1963</td><td>January 28, 1965</td></tr>
<tr><td>Packaging:</td><td>(H.S) 1g× 630packs
1g×1260packs
(bulk) 1kg,5kg</td><td>(PTP) 630 tablets
3150 tablets
(bulk) 3000 tablets</td></tr>
<tr><td>Dosage and administration:</td><td>Usually, administer orally 1.5g to 3g a day in 3 divided doses in adults.
Increase and decrease the dose based on age and symptoms where appropriate.</td><td>Usually, administer orally 3 to 6 tablets a day in 3 divided doses in adults.
Increase and decrease the dose based on age and symptoms where appropriate.</td></tr>
<tr><td>Composition:</td><td>One(1)g of BIO-THREE contains the following components:
Enterococcus faecalis T-110 10mg
Clostridium butyricum TO-A 50mg
Bacillus mesentericus TO-A 50mg</td><td>Two(2) BIO-THREE TABLETS contains the following components:
Enterococcus faecalis T-110 4mg
Clostridium butyricum TO-A 20mg
Bacillus mesentericus TO-A 20mg</td></tr>
<tr><td>Description:</td><td colspan="2">A white or slightly yellow delicate granular powder, with no odor or slightly characteristic odor and somewhat sweet taste.
White tablets and have a slightly sweet taste.
Diameter : 8mm
Thickness : 4.6mm
Weight : 200mg
 
<table><tr><th>Description \ Bacterial species</th><th>Clostridium butyricum TO-A</th><th>Enterococcus faecalis T-110</th><th>Bacillus mesentericus TO-A</th></tr><tr><td>Form</td><td>Bacilli</td><td>Cocci</td><td>Bacilli</td></tr><tr><td>Gram-staining</td><td>Positive</td><td>Positive</td><td>Positive</td></tr><tr><td>Acid resistance</td><td>pH1.2</td><td>pH2−3</td><td>pH1.2</td></tr></table></td></tr>
<tr><td>Indication:</td><td colspan="2">Improvement of various symptoms caused by aberrations in the enterobacterial flora.</td></tr>
<tr><td>Precaution for use:</td><td colspan="2">Precaution for formulation
Preferably avoidable combinations: Combination with aminophylline or isoniazid should be avoided as far as possible because these compounds may color the formulation.</td></tr>
<tr><td>Efficacy and pharmacology:</td><td colspan="2">1. Normalization of enterobacterial flora [1)]
Studies on fecal bacterial flora in infants with bacterial diarrhea, to whom BIO-THREE was administered, showed increases in the number of Bifidobacteria and significant improvement in enterobacterial flora, as indicated by an increase in the ratio of the number of aerobic bacteria to that of anaerobic bacteria.

2. Improvement of growing abilities of live bacteria in the drug through symbiosis [2)]
The number of the butyric acid bacteria cultured with Lactomin was about 11 times greater than that of its monoculture. Also, their number almost increased by a factor of nine when the filtrate of a culture of amylolytic bacillus was added.

3. Control of intestinal symptoms through symbiosis [2),3)]
The three bacterial strains contained in the drug grow in the human intestine through symbiosis to control the intestinal symptoms by inhibiting the growth of pathogenic bacteria and normalizing the enterobacterial flora.

4. Inhibition of pathogenic bacteria through symbiosis
(1)When Lactomin and the butyric acid bacterium were cultured together by continuous liquid culture, their antagonistic action against pathogenic bacteria (E.coli, Vibrio parahaemolyticus, C.difficile, C.botulinum, MRSA) was enhanced, when compared with monocultures of these bacteria.
The mixed culture showed symbiotic relationship with Bifidobacterium and Lactobacillus without any inhibitory effects. [3)-6)]
(2)In cases of Salmonella diarrhea in children, the bacterial strains in BIO-THREE inhibited the pathogens through symbiosis. [7)]
(3)In cases of endocrine or rheumatic diseases, the drug improved their symptoms of abnormal bowel movement through its effect of normalizing the enterobacterial flora by increasing the Bifidobacterium and decreasing C.perfringens. [8)]

5. Promotion of the growth of beneficial bacteria [1)-9), 11)]
It has been shown that BIO-THREE promotes the growth of Bifidobacterium and that metabolites of B.mesentericus have mitogenic effects on Bifidobacterium.</td></tr>
</table>

Clinical application:

1. Clinical effects [1), 12)-19)]

The following results have been reported from general clinical studies in 336 subjects:

[Children]

The following results were obtained in a total of 285 cases:

	Response rate
Enterogastritis	100% (13/13)
Diarrhea	93.2% (82/88)
Dyspeptic diarrhea	88.8% (142/160)
Constipation	83.3% (20/24)

[Adults]

The following results were obtained in a total of 51 cases:

	Response rate
Constipation	100% (1/1)
Acute/chronic enteritis	97.8% (44/45)
Alternative diarrhea and constipation	60.0% (3/5)

2. Adverse effects [1),12)-19)]

In a total of 336 cases, no adverse effects possibly related to BIO-THREE were observed.

Nonclinica studies:

1. Toxicology

(1)Acute toxicity (single dose toxicity) [20)]

In a study where 3.3g/kg of BIO-THREE was orally administered to male ICR mice, there were no abnormalities in general condition nor any death. No abnormal findings were obtained in necropsy and hematology at the end of the study.

(2)Subacute toxicity (repeated dose toxicity) [21)]

Subacute toxicity after oral administration of bulk lactic acid, butyric acid or amylolytic bacteria or formulated BIO-THREE to rats for consecutive 28 days, and their subsequent recovery in a 14-day control period without dosing were studied.

No deaths were observed in male and female rats throughout the study. Moreover, no dose-related abnormalities were observed in general conditions, weight changes, water consumption, food consumption, urinalysis, hematology, biochemistry, organ weight, necropsy and histopathology. The administered bacteria were not present in the vein. The no observable effect level of the test substance for this study was estimated to be 3,000mg/kg.

2. Other studies

(1)Plasmid [22)]

Plasmid was not detected from lactic acid bacteria by agarose gel electrophoresis.

(2)Hemolysin [23)]

Hemolysin was not detected from lactic acid, butyric acid or amylolytic bacteria.

Storage and handling:

Storage

Store at room temperature.
Avoid humidity after the product is unsealed.

Expiration date

Three years from the date of manufacture.
Do not use after the expiry date indicated on the outer case.
(Use unsealed products as quickly as possible even before the expiry date.)

References

1) Joh,K.,et al.: Progress in Medicine, 13, 621-626, 1993. 2) Internal document 1-3 : unpublished, 1984 3) Seo,G.,et al.: Microbios Letters, 40, 151-160, 1989 4) Seo,G.,et al.: Iyaku no Mon, 31, 202, 1991. 5) Seo,G.,et al.: Iyaku no Mon, 33, 155, 1993. 6) Seo,G.,et al.: JAPANESE JOURNAL OF BACTERIOLOGY 44, 144, 1989. 7) Joh,K.,et al.: The Journal of Medicine and Pharmaceutical Science, 29, 1027, 1993. 8) Katoh,H.,et al.: The Journal of Medicine and Pharmaceutical Science, 31, 1483, 1994. 9) Joh,K.,et al.: The Journal of Medicine and Pharmaceutical Science, 31, 1475, 1994. 10) Iino,H.,et al.: Microbios, 80, 49, 1994. 11) Iino,H.,et al.: Biomedical Letters, 48, 73, 1993. 12) Ogawa,M.,et al.: unpublished, 1964. 13) Kimura,T.: Practical Pediatrics, 17, 723, 1964. 14) Kanegae,S.,et al.: Practical Pediatrics, 17, 1339, 1964. 15) Yamada,I.,et al.: Practical Internal Medicine and Pediatrics, 18, 1479, 1963. 16) Yamanaka,D.,et al.: New Drugs and Practice, 15, 695, 1966. 17) Kouno,G.,et al.: unpublished, 1965. 18) Aritaki,S.,et al.: Practical Pediatrics, 17, 1228, 1964. 19) Okamoto,K.,et al.: Practical Pediatrics, 17, 571, 1964. 20) Internal document,4: unpublished, 1982. 21) Sakamaki,S.,et al.: Japanese Pharmacology & Therapeutics, 17, 157, 1989. 22) Seo,G.,et al.: Clinical Microbiology, 13, 359, 1986. 23) Internal document,6 : unpublished, 1987.

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01-05