FULL PAPER *Anatomy*

Microscopic Structure of the Large Intestinal Mucosa in Piglets during an Antibiotic-Associated Diarrhea

Takamitsu TSUKAHARA[1,2], Yoshie IWASAKI[3], Keizo NAKAYAMA[2,3] and Kazunari USHIDA[1]

[1]*Laboratory of Animal Science, Kyoto Prefectural University, Shimogamo, Kyoto 606–8522,* [2]*KYODOKEN Institute, 585 Shimoitabashi, Kyoto 612–8073 and* [3]*Japan Cytology Research, 577 Omote, Kyoto 612–8219, Japan*

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ABSTRACT. Antibiotic-associated diarrhea (AAD) is caused by the treatments of broad-spectrum antimicrobials that seriously affect the activity and composition of the large intestinal microflora. The pathogenic bacteria or low concentration of short-chain fatty acids (SCFAs) has been repeatedly discussed in relation to AAD. Recently, we reported the detection of a large amount of succinate an d lactate in the diarrheal feces in AAD-induced piglets. In this study, we investigated histologically the large intestinal mucosa in AAD-induced piglets, in which succinate and lactate were accumulated. AAD was induced in the piglets by an oral dose of polymyxin B sulfate (PL) or by an intra-muscular injection of enrofloxacin (ERFX). When the piglets were defecating diarrheal feces with a high concentration of succinate and/or lactate, the large intestine was removed and separated into four segments (cecum, gyri centripetales, gyri centrifugales, and rectum). Healthy piglets were used as the control. In the AAD-induced piglets, the lamina propria was edematous in the gyri centripetales. Piglets treated with ERFX were also edematous in gyri centrifugales. These edematous lamina propria co ntained larger amounts of inflammatory cells than observed in control tissues. ERFX-treated piglets had a more shallow crypt than PL-treated and control piglets. The mucosal tissue of the large intestine was more seriously damaged in the ERFX- than in the PL-treated piglets, which might have been caused by the high succinate and low SCFAs concentration in the digesta.

KEY WORDS: antibiotic-associated diarrhea, edema, large intestinal mucosa, piglet, succinate.

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Short-chain fatty acids (SCFAs) are produced by the resident microflora from the breakdown of dietary carbohydrate in the large intestine of many monogastric animals. SCFAs, as the major energy source of the epithelial cells of the large intestine, stimulate epithelial cell proliferation as well as mineral and water absorption from the lumen [16].

Abnormal fermentation has often been associated with the damaged large intestinal mucosa in ulcerative colitis (UC) [27] and in inflammatory bowel disease (IBD) [11, 28]. In some studies, abnormal fermentation was such that a large amount of succinate and/or lactate was accumulated to compensate for the SCFAs in UC and IBD [9, 28]. Antibiotic-associated diarrhea (AAD) is associated with treatments using broad-spectrum antimicrobials [10] that seriously affect the activity and composition of the large intestinal microflora. The pathogenic role of *Clostridium difficile* has been discussed in relation to pseudomembraneous colitis in AAD [2, 5, 7]. On the other hand, large intestinal fermentation in AAD has been reported; some of the findings have dealt with a considerably low concentration of SCFAs [8, 10, 14] and a predominance of succinate and/or lactate in the feces of AAD animals [15, 20, 21]. Abnormal fermentation, such as UC and IBD, may cause abnormality of the large intestinal mucosa [9, 28]. Thus, we investigated the macroscopic and microscopic structure of the large intestinal mucosa in AAD-piglets with succinate and lactate accumulation. The organic acid profiles of digesta and the results from a bacteriological survey on the piglets used in this study have been published elsewhere [22].

MATERIALS AND METHODS

Animals and diet: Six 25-day-old crossbred (Landrace x Large white x Duroc) castrated male piglets weighing 5–7 kg were obtained from a commercial pig farm. The piglets were individually housed in metabolic cages placed in a temperature-conditioned room (25°C) and adapted for the commercial diet for weaned piglets (Koromeal GS; Nippon Formula Feed Co., Ltd., Yokohama, Japan) free from intestinal flora modifiers, such as antibiotics, prebiotics, and probiotics, which are similar in composition to those in a previous analysis [23]. The piglets received this diet, and water was given *ad libitum*. The animals were handled in accordance with the guidelines for research with laboratory animals of the Kyoto Prefectural University Experimental Animal Committee.

Induction of AAD and sampling procedure: After three days of adaptation, two piglets (#s 1 and 2) were given polymyxin B sulfate (PL; Pfizer, Tokyo, Japan; 3,000,000 units/day orally), and the other two (#s 3 and 4) were given enrofloxacin (ERFX; Bayer, Tokyo, Japan; 0.6 g ERFX/day injected intramuscularly). The remaining two piglets (#s 5 and 6) were used as the untreated control. Antimicrobials were divided into two equal portions and dosed at 9:00 a.m. and 6:00 p.m. Feces were collected, and induction of AAD was determined by fecal water content higher than 70%. One gram of feces was also analyzed for organic acid by ion-exclusion HPLC, as described elsewhere [26]. The animals were slaughtered under general anesthesia with ketamine HCl (Ketalar 50; Sankyo, Tokyo, Japan) when succinate or lactate was detected as one of the major organic

acids in the feces (after five doses in the ERFX-treated piglets and after three doses in the PL-treated piglets). The control piglets (#s 5 and 6) received a basal diet. They were slaughtered at seven days after the adaptation period.

After a midline incision, the whole large intestine was immediately removed and separated into the cecum, gyri centripetales, gyri centrifugales, and rectum with string ligations. Each part of the intestine was incised, and the digesta was carefully removed so as not to injure the mucosa. The luminal contents in the large intestine were used for another study [22]. Each portion of the intestine was flushed with 10% (v/v) neutralized formalin solution to remove the residual digesta and fixed in 10% neutralized formalin solution.

Histological and morphometrical examination: The fixed intestine was further cut into cross-sections of an approximate length of 10 mm. The intestinal segments were divided as follows; the middle portion for the cecum; 200 mm below the ceco-colonic junction for the gyri centripetales; 100 mm below the flexura centralis for the gyri centrifugales; and 100 mm below the portion adjacent to the right kidney for the rectum. These cross-sectioned tissue samples were embedded in paraffin wax. Three-μm-thick cross-sections were prepared and stained with hematoxylin and eosin (HE) or alcian-green counter-stained with hematoxylin (AG) for light microscopic examination. AG staining was easy and successful to show both mucin-containing cells and nuclei [24].

Twenty well-oriented crypts were randomly selected, and the absolute depths of axial crypts were measured with an eyepiece micrometer on HE-stained preparations at 100 × magnification. The numbers of columnar epithelial cells, mucin-containing cells, and mitotic cells per longitudinal section of the left side of the crypt column were also counted on AG-stained preparations at 400 × magnification. The mitotic zone was estimated as described by Ichikawa and Sakata [12]. The mucin containing cell index was estimated as the same manner as mitotic index. The number of crypts per unit of length (mm) of luminal circumference (crypt density) was counted according to Ichikawa and Sakata [12].

Chemicals: Chemicals were obtained from Wako Pure Chemical (Osaka, Japan) or Nacalai Tesque (Kyoto, Japan) unless otherwise stated.

RESULTS

Histological observation of the large intestinal mucosa in AAD-induced piglets (Figs. 1 and 2): The pseudomembrane was not seen in the large intestine of the AAD-induced piglets. The pathogenic *C. difficile* was not detected in any cases of AAD-piglets [22]. In the control piglets (piglets #5 and 6), focal and mild inflammatory infiltration consisting of lymphocytes and plasma cells in the cecum, and consisting of neutrophils in the rectum was observed in the lamina propria (Fig. 2). However, lamina propria was not edematous (Fig. 1). The colon (gyri centripetales and gyri centrifugales) tissue did not have any edema and inflammatory infiltration (Figs. 1 and 2).

In the PL-treated piglets (piglets #1 and 2), the lamina propria of their gyri centripetales was swollen and edematous (Fig. 1). Surface of the epithelia further appeared sloughing in the gyri centripetales (Fig. 1). Inflammatory infiltration was observed in the lamina propria. There were considerable numbers of inflammatory cells such as lymphocytes and plasma cells in the edematous lamina propria (Fig. 2). In the rectum, focal inflammatory infiltration consisting of neutrophils was more seriously observed than control piglets in the lamina propria (Fig. 2). These were unclear in the cecum and the gyri centrifugales.

In the ERFX-treated piglets (piglets #3 and #4), the edema of the lamina propria of the large intestine was more serious than in the PL-treated piglets (Fig. 1). Inflammatory infiltration was also observed in the edematous lamina propria (Fig. 2). Abnormality in the colonic mucosa was significant in the ERFX-treated piglets. Neither edema nor inflammatory infiltration was clear in the cecum or the rectum.

Morphometry of the large intestinal mucosa in the AAD-induced piglets (Table 1): The number of epithelial cells and mucin-containing cells per crypt column of the four parts of the large intestine were smaller in the AAD-induced piglets (#1-#4) than in the control (#5 and #6). The numbers of mitotic cells per crypt column in the colon and rectum were smaller in the AAD-induced piglets than in the control. However, the cecal crypts contained more mitotic cells in the AAD-induced piglets than in the control. The mitotic zone of the crypt column was smaller in the AAD-induced piglets than in the control. The differences were large in these three parameters between the ERFX-treated piglets and the control. The mucin-containing cell index was large in the cecum and the colon of the ERFX-treated piglets, while there was no clear difference between the PL-treated piglets and the control. In the colon and the rectum, the crypt depth was the largest in the control piglets, followed by PL-treated piglets and ERFX-treated piglets. The difference was unclear in the cecum. Crypt density and mucin-containing cell index were not affected by AAD induction.

DISCUSSION

AAD is a frequent and mild side effect of antibiotic therapy [5, 10]. Antimicrobials may disturb the bacterial ecology in the large intestine. Ecologic alteration, and, therefore, disturbance microflora, will cause an abnormal fermentation in which succinate and/or lactate accumulate. Succinate and lactate are not detected in normal fermentation because these acids are rapidly metabolized to SCFAs by acid-utilizing bacteria [19]. In this study, succinate and lactate accumulated in the large intestinal contents up to 15.3 to 54.3 mmol/kg and 0.8 to 29.0 mmol/kg in the PL-treated piglets and 18.8 to 56.3 mmol/kg and 0.0 to 5.9 mmol/kg in the ERFX-treated piglets, respectively [22]. Such concentrations of succinate and lactate in the AAD-induced piglets were much higher than those in the control

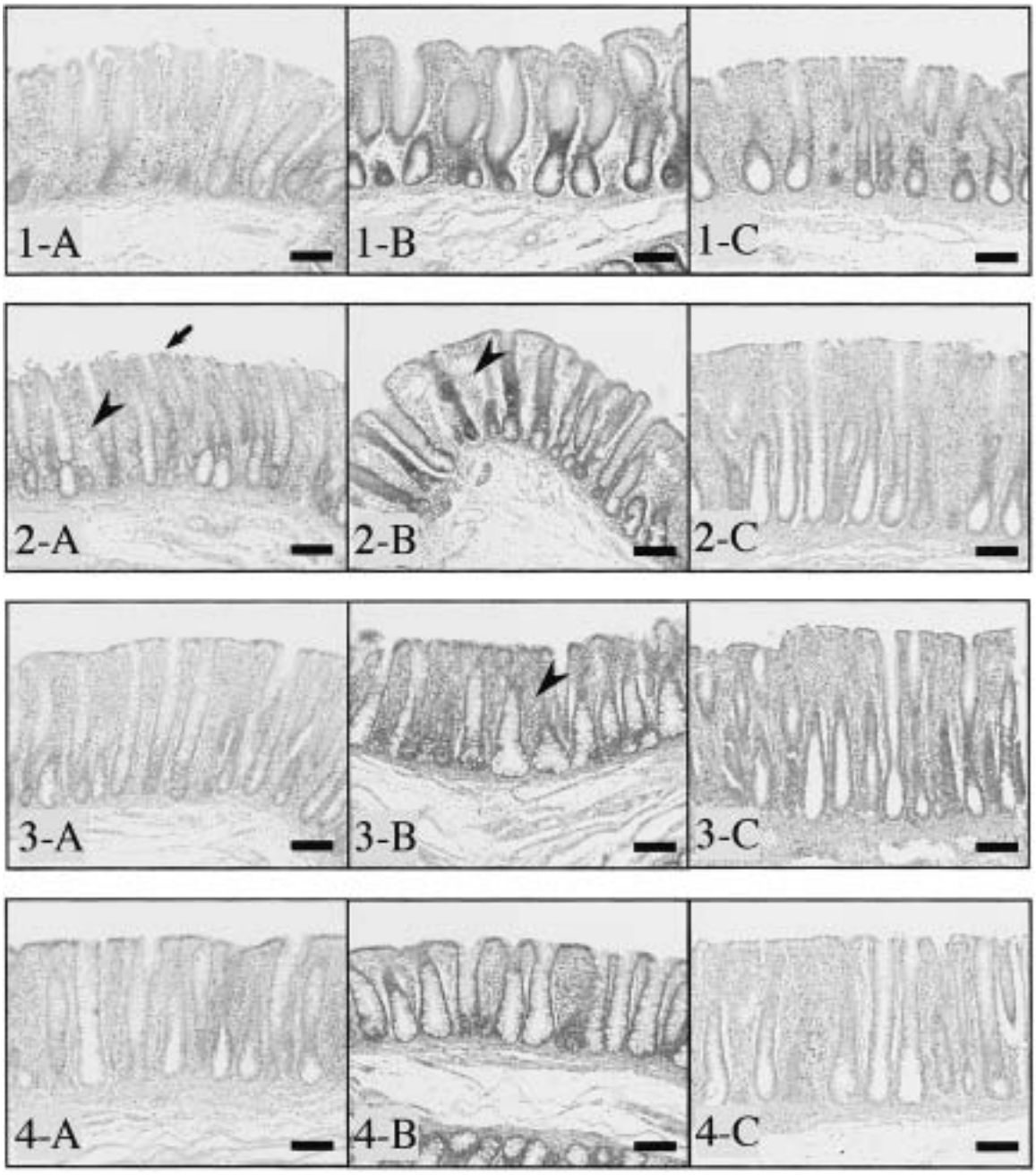

Fig. 1. Photomicrographs of large intestinal thin sections of piglets. An AAD-induced piglet by PL (A), an AAD-induced piglet by ERFX (B), and a control piglet (C). Stained with HE. Bars represent 100 μm ($\times$ 100). 1, Cecum; 2, Gyri centripetales; 3, Gyri centrifugales; and 4, Rectum. Arrowheads indicate edematous lamina propria. A black arrow indicates mucosal erosion.

[22]. Succinate and lactate are very slowly absorbed by the epithelial cells [25] and do not promote water and electrolyte absorption [3, 4]. Furthermore, succinate rather stimulates water secretion from the epithelium to the lumen [18]. Therefore, the luminal accumulation of these acids may increase the moisture content of the digesta and induce diarrhea [21, 22].

In this study, morphometrical data, such as the number of epithelial cells, were smaller in the AAD-induced piglets (Table 1). The number of mitotic cells was also smaller in the colon of ERFX-treated piglets (Table 1). The lamina propria was edematous, and inflammatory cell infiltration was observed in the colon of the AAD-induced piglets, although erosion was not seen (Figs. 1 and 2). Succinate is

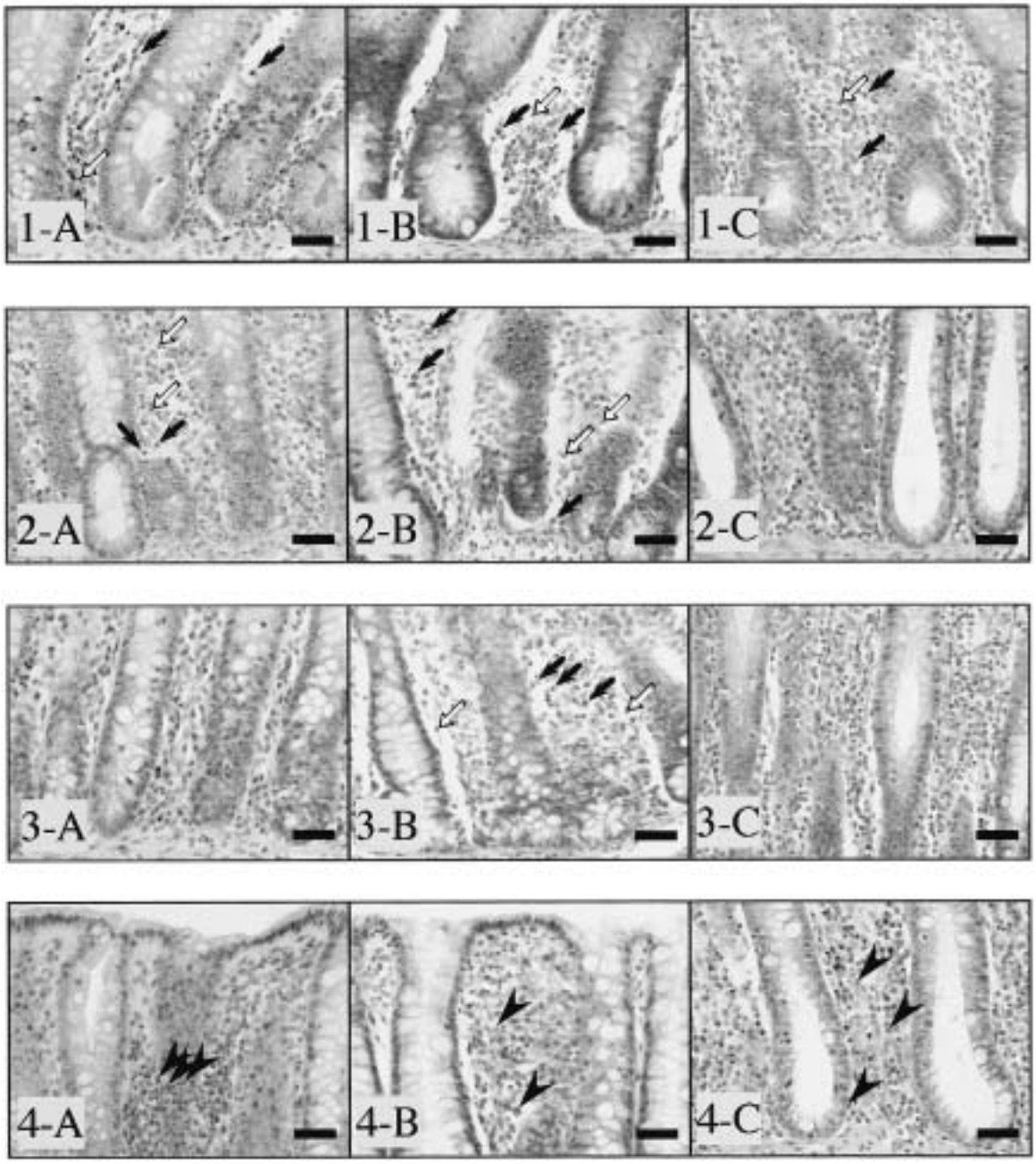

Fig. 2. Photomicrographs in high magnification of Fig. 1. Inflammatory infiltration was observed in the lamina propria. An AAD-induced piglet by PL (A), an AAD-induced piglet by ERFX (B), and a control piglet (C). Stained with HE. Bars represent 30μm ($\times$ 400). 1, Cecum; 2, Gyri centripetales; 3, Gyri centrifugales; and 4, Rectum. White arrows indicate lymphocytes. Black arrows indicate plasma cells. Arrowheads indicate neutrophils.

known to have cytotoxicity to the tissue cultured cell line [18], probably due to the decrease in the mucosal blood flow [6]. Succinate caused mucosal erosion, submucosal edema, and inflammatory cell infiltration in the rat colon *in vivo* [1, 6, 18]. Lactate also damaged the large intestinal mucosa by inducing mucosal erosion, edema in the lamina propria, and inflammatory cell infiltration [12, 17]. Succinate and lactate further induced sloughing of the surface epithelium [1, 12], and lactate decreased the mitotic activity [12].

On the other hand, SCFAs may alleviate the cytotoxic effect of succinate and/or lactate because SCFAs are known to stimulate epithelial cell proliferation [16] and effectively prevent or cure ulcerative colitis [10]. The cytotoxicity of succinate and lactate is still unclear, but it has been sug-

Table 1. Morphoimetrical analyses of tissue from the large intestine of piglets with induced AAD or from that of healthy control piglets

		Antimicrobials					
		Polymyxin B sulfate		Enrofloxacin		Control	
Factors[a)]	Large intestine	Piglet#1	Piglet#2	Piglet#3	Piglet#4	Piglet#5	Piglet#6
Epithelial cells (n)	Cecum	78.9	81.0	76.3	71.7	91.1	82.3
	Gyri centripetales	82.9	90.2	67.0	65.5	96.6	95.0
	Gyri centrifugales	83.8	85.8	58.1	51.7	106.1	92.0
	Rectum	80.0	80.0	58.6	48.9	93.3	91.2
Mitotic cells (n)	Cecum	1.8	2.3	2.2	2.1	1.6	1.7
	Gyri centripetales	1.0	2.8	1.0	0.8	2.7	1.4
	Gyri centrifugales	2.7	2.3	0.4	0.1	1.3	1.7
	Rectum	1.1	1.8	0.6	0.3	2.5	1.5
Mitotic zone (n)	Cecum	26.7	24.9	24.4	22.1	35.1	38.1
	Gyri centripetales	29.7	33.7	22.6	19.9	44.2	42.5
	Gyri centrifugales	29.9	31.4	13.4	11.5	45.8	43.8
	Rectum	27.3	34.3	15.0	12.0	39.2	36.2
Mitotic index (%)	Cecum	2.2	2.7	2.9	2.9	1.8	2.0
	Gyri centripetales	1.2	3.1	1.4	1.2	2.8	1.5
	Gyri centrifugales	3.2	2.6	0.6	0.2	1.2	1.9
	Rectum	1.4	2.2	0.9	0.5	2.7	1.7
Mucin-containing cells (n)	Cecum	29.6	26.0	30.9	30.5	34.3	32.7
	Gyri centripetales	27.5	32.0	30.6	30.9	39.7	40.3
	Gyri centrifugales	35.5	37.0	29.1	26.8	41.7	43.4
	Rectum	38.6	36.9	23.6	24.0	45.2	43.8
Mucin-containing cell index (%)	Cecum	37.6	32.3	40.6	42.6	37.8	39.7
	Gyri centripetales	33.3	35.5	46.0	47.4	41.2	42.6
	Gyri centrifugales	42.5	43.0	50.5	51.9	39.7	47.3
	Rectum	48.4	46.4	40.3	49.2	48.7	48.1
Crypt depth (μm)	Cecum	350.8	359.7	345.5	306.3	363.6	322.9
	Gyri centripetales	365.5	356.7	274.9	269.5	445.9	469.4
	Gyri centrifugales	411.1	377.8	269.5	226.4	507.2	429.7
	Rectum	387.6	420.4	270.0	238.6	438.1	404.3
Crypt density (n/mm)	Cecum	11.2	13.8	13.8	11.2	11.7	14.3
	Gyri centripetales	14.8	15.3	16.3	17.3	14.3	11.2
	Gyri centrifugales	12.8	15.3	17.9	20.9	14.8	14.8
	Rectum	15.3	16.8	14.3	17.3	14.3	15.3

a) The number of epithelial, mitotic, and mucin-containing cells on the left side of each crypt was counted. The mitotic zone and mitotic index were estimated according to Ichikawa and Sakata [12]. The mucin-containing cell index was estimated to be the same as the mitotic index.

gested that succinate decreases the mucosal blood flow of the large intestinal mucosa [6]. The decrease in blood flow might impair epithelial cell proliferation and mitotic activity by decreasing the delivery of oxygen and nutrients. On the other hand, SCFAs increase the blood flow and oxygen supply [16]. SCFAs were detected in the contents of AAD-induced piglets by as much as 50.1 to 120.8 mmol/kg in the PL-treated piglets and 9.6 to 50.6 mmol/kg in the ERFX-treated piglets [22]. SCFAs stimulated the proliferation of epithelial cells in a dose-dependent manner [16]. The higher succinate and the lower SCFAs concentrations in the ERFX-treated piglets were compared with those in the PL-treated piglets. It seemed reasonable that the abnormality of the colonic mucosa was much more serious in the ERFX-induced piglets. Indeed, in the ERFX-treated piglets, fewer epithelial and mitotic cells were observed than in the PL-treated piglets. Effect of ERFX and PL on the development of mucosal inflammation apparently showed site-dependency. The mitotic cell number per crypt and mitotic index in the cecum of ERFX-treated piglets were somewhat larger than the control. The metachromatic reaction was affected among the different site of the large intestine in the murine dextran sulfate sodium-induced colitis [13]. However, the reasons for these site dependencies have not been clear yet. Succinate was probably an only organic acid involved in the development of the mucosal inflammation in ERFX-treated piglets. While, lactate in addition to succinate might be involved in mucosal inflammation in PL-treated piglets. Chemical stimuli themselves were therefore different between these two treatments. The response of large intestinal mucosa to luminal chemical stimuli showed site-dependency in rats [29]. In particular, enhanced SCFAs production stimulated epithelial cell proliferation in the cecum, but not in the distal colon. Site-dependency in the response of large intestinal mucosa to luminal stimuli may be plausible. However, this idea has not been well estab-

lished yet [16], and needs further investigations.

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REFERENCES

1. Ariake, K., Ohkusa, T., Sakurazawa, T., Kumagai, J., Eishi, Y., Hoshi, S. and Yajima, T. 2000. Roles of mucosal bacteria and succinic acid in colitis caused by dextran sulfate sodium in mice. *J. Med. Dent. Sci.* **47**: 233–241.
2. Aronsson, B., Mollby, R. and Nord, C. E. 1981. Occurrence of toxin -producing *Clostridium difficile* in antibiotic-associated diarrhea in Sweden. *Med. Microbiol. Immunol.* **170**: 27–35.
3. Bugaut, M. 1987. Occurrence, absorption, and metabolism of short-chain fatty acids in the digestive tract of mammals. *Comp. Biochem. Physiol.* **86B**: 439–472.
4. Engelhardt, W. V. 1995. Absorption of short-chain fatty acids from the large intestine, pp. 149–170. *In*: Physiological and Clinical Aspects of Short-Chain Fatty Acids (Cummings, J. H., Rombeau, J. L. and Sakata, T. eds.), Cambridge University Press, Cambridge.
5. Fekety, R. 2001. Antibiotic-associated diarrhea. pp. 293–317. *In*: Diarrheal Disease (Field, M. ed.), Elsevier Science, New York.
6. Fukui, S., Shimoyama, T., Tamura, K., Yamamura, M. and Satomi, M. 1997. Mucosal blood flow and generation of superoxide in rat experimental colitis induced by succinic acid. *J. Gastroenterol.* **32**: 464–471.
7. George, W. L., Rofle, R. D. and Finegold, S. M. 1982. *Clostridium difficile* and its cytotoxin in feces of patients with antimicrobial agent-associated diarrhea and miscellaneous conditions. *J. Clin. Microbiol.* **15**: 1049–1053.
8. Högenauer, C., Hammer, H. F., Krejs, G. J. and Reisinger, E. C. 1998. Mechanisms and management of antibiotic-associated diarrhea. *Clin. Infect. Dis.* **27**: 702–710.
9. Hori, S. and Shimoyama, T. 1991. Faecal bacterial flora and organic acids in patients with ulcerative colitis. *J. Germfree Life Genotobiol.* **21**: 19–21 (in Japanese).
10. Hove, H. 1998. Lactate and short-chain fatty acid production in the human colon: implications for D-lactic acidosis, short-bowel syndrome, antibiotic-associated diarrhea, colonic cancer, and inflammatory bowel disease. *Dan. Med. Bull.* **45**: 15–33.
11. Hove, H. and Mortensen, P. B. 1995. Influence of intestinal inflammation (IBD) and small and large bowel length on fecal short-chain fatty acids and lactate. *Dig. Dis. Sci.* **40**: 1372–1380.
12. Ichikawa, H. and Sakata, T. 1997. Effect of L-lactic acid, short-chain fatty acids, and pH in cecal infusate on morphometric and cell kinetic parameters of rat cecum. *Dig. Dis. Sci.* **42**: 1598–1610.
13. Kitajima, S., Takuma, S. and Morimoto, M. 1999. Tissue distribution of dextran sulfate sodium (DSS) in the acute phase of murine DSS-induced colitis. *J. Vet. Med. Sci.* **61**: 67–70.
14. Mørtensen, P. B. and Clausen, M. R. 1995. Short-chain fatty acids, antibiotic-associated diarrhea, colonic adenomas, and cancer. pp. 373–389. *In*: Physiological and Clinical Aspects of Short-Chain Fatty Acids (Cummings, J. H., Rombeau, J. L. and Sakata, T. eds.), Cambridge University Press, Cambridge.
15. Rubinstein, R., Howard, A. V. and Wrong, O. M. 1969. *In vivo* dialysis of feces as a method of stool analysis. The organic anion component. *Clin. Sci.* **37**: 549–564.
16. Sakata, T. 1997. Influence of short-chain fatty acids on intestinal growth and functions. pp. 191–199. *In*: Dietary Fiber in Health and Disease (Kritchevsky, D. and Bonfield, C. eds.), Plenum Press, New York.
17. Saunders, D. R. and Sillery, J. 1982. Effect of lactate and H+ on structure and function of rat intestine. Implications for the pathogenesis of fermentative diarrhea. *Dig. Dis. Sci.* **27**: 33–41.
18. Shimazaki, T. 1992. Detection of cytotoxic substances from *Bacteroides vulgatus* isolated from ulcerative colitis. *Nihon Daicyo-Koumonbyo Kaishi* **45**: 282–291 (in Japanese).
19. Stewart, C. S. and Bryant, M. P. 1988. The rumen bacteria. pp. 21–75. *In*: The Rumen Microbial Ecosystem (Hobson, P. N. ed.), Elsevier Applied Science, New York.
20. Tsukahara, T. and Ushida, K. 2000. Succinate accumulation in pig large intestine during the antibiotic-associated diarrhea (AAD) and isolation of succinate-producing bacteria. *Reprod. Nutr. Dev.* **40**: 213.
21. Tsukahara, T. and Ushida, K. 2001. Organic acid profiles in feces of pigs with pathogenic or non-pathogenic diarrhea. *J. Vet. Med. Sci.* **63**: 1351–1354.
22. Tsukahara, T. and Ushida, K. 2002. Succinate accumulation in pig large intestine during antibiotic-associated diarrhea and the constitution of succinate-producing flora. *J. Gen. Appl. Microbiol.* **48**: 143–154.
23. Tsukahara, T., Azuma, Y. and Ushida, K. 2001. The effect of a mixture of live lactic acid bacteria on intestinal gas production in pigs. *Microb. Ecol. Health Dis.* **13**: 105–110.
24. Tsukahara, T., Iwasaki, Y., Nakayama, K. and Ushida, K. 2002. An improved technique for the histological evaluation of the mucus-secreting status in rat cecum. *J. Nutr. Sci. Vitaminol.* **48**: 311–314.
25. Umesaki, Y., Yajima, T., Yokokuwa, T. and Mutai, M. 1979. Effect of organic acid absorption or bicarbonate transport in rat colon. *Pflügers Arch.* **379**: 43–47.
26. Ushida, K. and Sakata, T. 1998. Effect of pH on oligosaccharide fermentation by porcine cecal digesta. *Anim. Sci. Technol. (Jpn.)* **69**: 100–107.
27. Vernia, P., Caprilli, R., Latella, G., Barbetti, F., Magliocca, F. M. and Cittadini, M. 1988. Fecal lactate and ulcerative colitis. *Gastroenterology* **95**: 1564–1568.
28. Vernia, P., Gnaedinger, A., Hauck, W. and Breuer, R. I. 1988. Organic anions and the diarrhea of inflammatory bowel disease. *Dig. Dis. Sci.* **33**: 1353–1358.
29. Zhang, J. and Lupton, J. 1994. Dietary fibers stimulate colonic cell proliferation by different mechanisms at different site. *Nutr. Cancer* **22**: 267–276.