Increased Butyrate Formation in the Pig Colon by Feeding Raw Potato Starch Leads to a Reduction of Colonocyte Apoptosis and a Shift to the Stem Cell Compartment

J. Mentschel and R. Claus

Whereas butyrate is well known to induce apoptosis in transformed colon cells in vitro, evidence exists that it inhibits apoptosis of colon crypt cells in vivo. In this study, pigs were fed with resistant potato starch to increase microbial butyrate formation in the colon and to investigate its effects on mitosis and apoptosis. In addition, apoptosis regulating proteins were determined by immunocytochemistry, such as proapoptotic Bak, antiapoptotic Bcl-2, and the epidermal growth factor (EGF), which is synthesized by goblet cells and functions as a survival factor. Two groups of 6 barrows were both supplied with 381 g crude protein and 31 MJ metabolizable energy (ME) daily over a 19-day experimental period. The rations differed in the carbohydrate composition. The controls received gelatinized starch as the main carbohydrate, whereas the experimental group (butyrate group) received a ration with raw potato starch (low ileal digestibility). In the feces, butyrate concentration and pH were monitored daily. After killing the pigs, colon tissue was obtained for histologic and immunocytochemical evaluation, which was performed separately in the luminal, middle, and stem cell compartment of the crypts. In the butyrate group, the total number of apoptotic cells was reduced by 34% ($P \leq .001$) compared with controls, whereas the mitotic rate was not altered. The crypt depth was only moderately increased by 15%. Apoptosis in the luminal compartment of the butyrate group was reduced by 18.8%, but was increased by 21.7% in the stem cell compartment. The effect of butyrate on apoptosis was paralleled by an increased number of Bcl-2 positive cells mainly in the luminal compartment (butyrate: 2.6 cells; controls: 1.2 cells, $P \leq .001$), which was more pronounced compared with the number of Bak positive cells in the same compartment. Bak activity in the stem cell compartment was 3.4-fold increased compared with controls ($P \leq .001$). The size of EGF-positive stained mucus-droplets from the goblet cells was increased in the butyrate group ($P \leq .001$). We conclude that butyrate inhibits apoptosis of colonocytes in vivo. An excessive proliferation of crypts is counteracted by a shift of the remaining apoptosis towards the stem cell compartment.

IT IS WELL KNOWN that luminal energy availability stimulates mucosa proliferation in the small intestine.[1,2] In the pig, it was demonstrated that this effect is primarily explained by an elevated mitotic rate due to a glucose-dependent increase of insulin-like growth factor-I (IGF-I) in peripheral blood plasma. An excessive proliferation of villi is counteracted by a concomitant moderate increase of apoptosis in the villi tips.[3]

In the colon, glucose is usually not available. Instead, carbohydrates with a low ileal digestibility, such as resistant starch, are fermented to short chain fatty acids (SCFA), which are regarded as regulators of proliferation of the colonic mucosa.[4-7] Among the SCFA, butyrate plays a specific role, because it is easily metabolized by β-oxidation in the mitochondria and thus provides 60% to 70% of the total energy demand of colonocytes.[8-10] Whereas it is known that butyrate may exert trophic effects on the colon epithelium in vivo,[5] the underlying mechanisms have not been investigated. In contrast, butyrate may induce cell cycle arrest or even apoptosis of colonocytes in vitro by triggering apoptotic pathways originating in the mitochondria.[11,12] The Bcl-2 family of proteins includes antiapoptotic and proapoptotic members, which are involved in these pathways, because they regulate the release of apoptogenic factors, such as cytochrome C and the apoptosis-inducing factor (AIF), from the mitochondrial membranes into the cytoplasma.[13-15] Whereas the interaction of butyrate and members of the Bcl-2 family of apoptosis regulation proteins is well established, the details of the signal transduction cascade remain to be clarified. It was suggested that such proteins regulate membrane channels in the mitochondria, which in turn, determine the release of apoptotic signals.[16-20]

Many in vitro studies with transformed cells clearly indicate that butyrate stimulates apoptosis.[21] In contrast, in guinea pigs, the absence of butyrate in colonic mucosa samples in Ussing chambers increased apoptosis.[10] Similarly, a lack of butyrate was associated with the induction of the proapoptotic regulator, Bax, and led to mass apoptosis in the colon of the guinea pig.[22]

Studies under in vivo situations are still scanty. It was demonstrated previously that feeding of butyrate to calves stimulated ruminal mucosa development mainly by inhibition of apoptosis.[23] Feeding of pigs with resistant potato starch favored the microbial fermentation to butyrate in the colon and reduced apoptosis.[24] More detailed reactions of colonic crypts and the involvement of members of the Bcl-2 family have not been described as yet. Therefore, this study further investigates the consequences of feeding resistant potato starch on the colon mucosa by determination of the compartmental distribution of mitosis, apoptosis, and the regulators, Bcl-2 and Bak, which were shown to play a pivotal role for apoptosis regulation in colonic epithelial cells.[16] As the epidermal growth factor (EGF) serves as a enterocyte survival factor, this protein was included in the immunocytochemical evaluation.

From the FG Tierhaltung und Leistungsphysiologie, Institut für Tierhaltung und Tierzüchtung, Universität Hohenheim, Stuttgart, Germany.

Submitted December 26, 2002; accepted June 17, 2003.

Address reprint requests to Dr. R. Claus, FG Tierhaltung und Leistungsphysiologie (470A), Institut für Tierhaltung und Tierzüchtung, Universität Hohenheim, Garbenstr 17, 70599 Stuttgart, Germany. Email thsekret@uni-hohenheim.de.

0026-0495/03/5211-0009$30.00/0
doi:10.1016/S0026-0495(03)00318-4

Table 1. Composition of the Diet With Gelatinized Starch and With Resistant Potato Starch, Respectively

	Ration With Gelatinized Starch (controls)		Ration With Resistant Starch (butyric acid group)	
Component	g/kg DM	g DM/d	g/kg DM	g DM/d
Gelatinized starch	523	1,146	—	—
Raw potato starch	—	—	615.5	1,690
Potato protein	54.8	120	43.6	120
Wheat gluten	91.4	200	72.8	200
Wheat bran	306.3	670	243.9	670
Minerals and vitamins	24.2	53	24.2	66
CP	173.5	380.5	138.7	381
MJ ME/ kg DM	14.13	31	11.28	31

Abbreviation: DM, dry matter.

MATERIALS AND METHODS

Animals, Experimental Design, and Sampling

The experiments were performed with 12 castrated male pigs (German Landrace x Pietrain) with an average weight of 60 kg (range, 55 to 65 kg) at the beginning of the experiments. To avoid uncontrolled feed intake, the pigs were kept without straw in individual crates (3 × 2.5 m). They were randomly assigned to a control group and a butyrate group. The control group received a ration, which contained gelatinized starch (high ileal digestibility) as the main energy source over the whole 19-day treatment period. The energy content was 14.13 (MJ metabolizable energy (ME) per kilogram dry matter (DM) and the crude protein (CP) content 173.5 g/kg DM. The butyric acid group was instead fed for 19 days with raw potato starch. Raw potato starch has a low ileal digestibility and preferentially leads to microbial butyrate formation. The ration contained 11.28 MJ ME/kg DM and 138.7g CP/kg DM. To compensate for the difference of the energy content, the butyric acid group was fed 2,748 g/d and the controls 2,190 g/d. Details of the rations are given in Table 1. The isocaloric supply was supported by the average daily gain, which was 580 g for the controls and 550 g for the butyrate group. Before the 19-day feeding period in the butyrate group, the pigs were first fed for 10 days with the gelatinized starch ration ("preperiod") and were then slowly adapted to the potato starch diet (5 days of adaptation).

Representative amounts of fresh feces were collected every morning from every pig. They were stored deep frozen (−20°C) for later determination of butyrate and pH. At the end of the experiments, the pigs were killed by intravenous infusion of Narcodorm–n (Alvetra, Neumünster, Germany). Tissue samples from the proximal colon were obtained within 5 minutes postmortem. They were rinsed with cold physiologic saline and were then fixed in 4% paraformaldehyde for 1.5 days.

Immunocytochemical Techniques

For immunocytochemistry, the fixed tissue specimens were dehydrated in a graded series of ethanol and embedded in paraffin. The thickness of the sections was 4 μm. Before immunostaining they were treated with 0.03% hydrogen peroxide in methanol to inhibit peroxidase activity. They were then boiled in a microwave oven (800 W) in 10 mmol/L sodium citrate buffer (pH 6) for 30 minutes. During that time water was added when necessary. To avoid background staining, blocking serum was derived from the same species in which the secondary antibody had been raised. Thereafter the sections were incubated overnight with the individual primary antibodies. After incubation with the secondary antibodies and the streptavidin-peroxidase complex (Dako, Hamburg, Germany), staining was initiated by adding the substrate DAB (3,3′-Diaminobenzidin- tetrahydrochloride; Fluka, Neu Ulm, Germany). Finally the sections were counterstained with hematoxilin. The following primary antibodies were applied.

Mitosis was characterized by using the histoprime monoclonal antibody (Ki 67, MIB-1; Canon, E059, Wiesbaden, Germany). Details of the staining procedure have been described elsewhere.[25]

EGF was stained with polyclonal primary antibodies, which had been raised against recombinant pEGF in rabbits. Details of the staining procedure have been published previously.[27]

The antiapoptotic protein, Bcl-2, was detected with a monoclonal antibody (Dako, M0887, Hamburg, Germany). The sections were incubated overnight at 4°C with the first antibody diluted 1:80. The secondary antibody had been raised in rabbits (Dako, E0413) and was used at a dilution of 1:400.

For the proapoptotic protein, Bak, a polyclonal antibody was used, which had been raised in sheep (Dako, A3538). This substance was selected because it had been shown earlier to respond specifically to butyrate.[16] The staining procedure was identical to Bcl-2, except that blocking was performed with normal sheep serum (1:10) and the first antibody was used at a dilution of 1:100. The secondary antibody (rabbit antisheep, Biozol, 2AB02B, Eching, Germany) was diluted 1:400.

Apoptosis was determined by a modified terminal deoxy-nucleotidyl transferase mediated 2′-deoxyuridine 5′-triphosphate nick-end labeling (TUNEL) assay, which leads to a staining of apoptosis-specific DNA fragments. Details have been described previously.[23,26] Negative controls were treated identically to the normal slides, but the enzyme solution in the TUNEL reaction mixture was omitted.

Immunocytochemical Evaluation

All slides for the same parameter were evaluated by only one person. The evaluation was confined to those crypts in which the entire length could be visualized. From each pig at least 30 to 40 crypts were examined. The depth of a crypt was defined as the number of colonocytes from the crypt bottom until the luminal ending at one side (hemicrypt). The stained cells for each parameter were referred to the corresponding crypt depth and are thus given as cells per hemicrypt.

Because some of the parameters (apoptosis, Bcl-2, Bak) revealed a clear compartmental distribution along the longitudinal axis of the crypts, an additional evaluation was performed in which the stained cells were attributed either to the basal compartment, the middle compartment, or the luminal compartment. Each of these compartments comprised one third of the whole depth (cell number) of the relevant crypt. For mitosis evaluation, the Ki-67–positive cells were related to the total number of epithelial cells along the hemicrypts. The ratio between the stained and the total cells is given in percent. The apoptotic activity, as well as the expression of Bcl-2 and Bak, is given as the total number of positive cells per crypt and additionally according to their location in the 3 compartments.

EGF activity was only associated with goblet cells and their mucus secretion. Because the mucus bubbles revealed uniform staining, the size of the bubbles was determined as a criterion for different secretory activities as described previously.[27]

Analytical Procedures

Whereas immunocytochemical parameters were determined in 1 tissue sample per animal, pH and butyrate measurements could be compared on a daily basis during the whole feeding period. These parameters do not directly represent butyrate concentrations and pH in the proximal colon, but they indicate relative changes along the feeding period and their differences between the 2 groups.

The pH was determined by diluting an aliquot of homogenized feces (0.5 g) with 4 wt/vol water. The samples were centrifuged (3,000*g*, 15 minutes, 20°C) and the pH was measured.[28]

Butyrate in feces was determined by gas chromatography as described previously.[24] Sample preparation was based on dilution of feces, addition of 0.25 mol/L sulfuric acid and centrifugation. Chromatography was performed after addition of 2-methylvaleric acid as internal standard on a 30 m × 0.25 mm BP21 column with 0.25 μm film thickness. The injection port temperature was 170°C and the flame ionization detector temperature was 20°C. The temperature program was 100°C (1 minute), 200°C (20°C/min), 210°C (2 minutes).

Statistical Analysis

The data are presented as group means ± SEM and were tested for normal distribution by the Kolmogorov-Smirnov test. For butyrate, pH, and crypt depth, the differences between groups were analyzed by independent-sample *t* test. Differences between treatment periods within 1 group were evaluated by paired-samples *t* test. Both tests were evaluated using daily values of each individual. Immunocytochemical data were processed as follows: for each animal and parameter, the mean value was calculated in each compartment from at least 30 crypts. These data were analyzed as a split-plot design using the mixed model analysis of the Statistical Package for the Social Sciences, version 11 (SPSS, Chicago, IL). The following model was used:

$Y_{ijk} = \mu + \alpha_i + f_{ik} + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$ where Y_{ijk} = mean count at j-th position on k-th animal within i-th group; μ = general effect; α_i = main effect of i-th group(treatment); β_j = main effect of j-th compartment; f_{ik} = effect of k-th animal within the i-th group; $(\alpha\beta)_{ij}$ = group × compartment interaction; e_{ijk} = residual error.

In this design, the animal effect was considered as random. Treatment effects were significant for all parameters except for mitosis. Compartment effects were significant for all parameters except for area of mucus bubbles stained positive for EGF.

RESULTS

Butyrate Concentrations and pH

The effects of feeding either gelatinized starch (controls) or resistant starch (butyric acid group) on butyrate concentrations in feces are given in Fig 1, and on pH in feces in Fig 2.

In the controls butyrate remained at an average level of 65 ± 4 μmol/g DM. A similar level was determined in the preperiod of the butyrate group when they were fed with the same ration. During the adaptation period, the butyrate concentrations increased continuously up to a maximum of 220 μmol/g DM. The mean butyrate concentration in the following 19-day period was 136 ± 8 μmol/g DM compared with 65 ± 4 μmol/g DM in the controls ($P \leq .001$). Even if butyrate in the treatment group revealed a decreasing tendency, the difference on the day of tissue sampling (101 *v* 52 μmol/g DM) still was significant ($P \leq .002$).

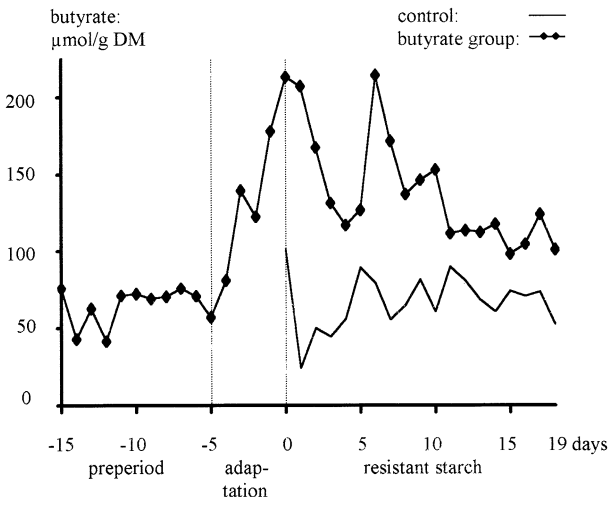

Fig 1. Mean concentrations of butyrate in feces of the control and butyrate groups. Days −15 to −5 correspond to the preperiod in which the butyrate group received the same diet as the controls between days 0 to 19. Between day −5 and 0, the butyrate group was adapted to the experimental ration containing the resistant starch.

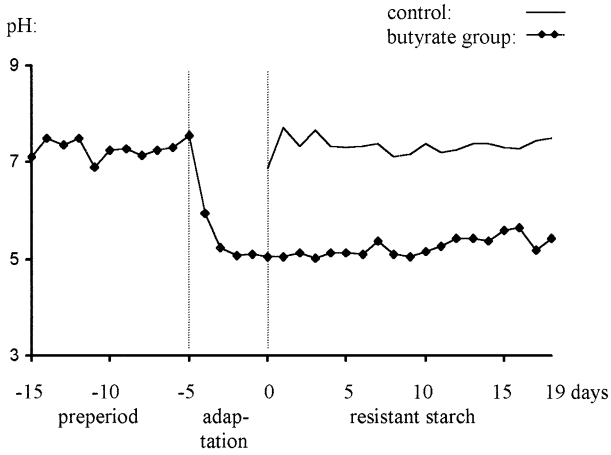

Fig 2. Mean pH in feces of the control and butyrate groups.

The mean pH (Fig 2) in the preperiod of the butyrate group was 7.35 ± 0.06 and did not differ with the pH in the 19-day period of the controls (7.3 ± 0.09). In the adaptation period of the butyrate group, the pH dropped to 5.2 ± 0.05.; the same level was maintained during the resistant starch period, so that the mean pH in the butyrate group (5.2 ± 0.05) was significantly ($P \leq .001$) different from the controls (7.3 ± 0.09).

Morphology and Average Data From Immunocytochemistry

The average data on the morphology and histocytochemical results are summarized in Table 2. The crypt depth was significantly increased in the butyric acid group ($P \leq .001$) by about 15%. This difference, however, cannot be explained by an increased mitotic rate because the minor difference of only 0.4 Ki-67–positive cells per 100 counted cells was not significant. Thus, under the same mitotic activity, the morphologic difference has to be attributed to the differences in the apoptotic activity, which was significantly lower ($P \leq .01$) in the butyric acid group. The difference between the 2 feeding groups was 34%.

No effect was found for the number of EGF-secreting goblet cells. Their secretory activity, however, was remarkably increased in the butyric acid group, because the area of the secreted mucus bubbles was nearly 2-fold compared with the controls ($P \leq .01$). Because staining intensity of the mucus did not change due to enlargement, an increased secretion of EGF might be assumed. The 2 apoptosis regulating proteins, Bcl-2 (antiapoptotic) and Bak (proapoptotic), both were increased in the butyric acid group ($P \leq .05$ and $P \leq .01$, respectively).

Table 2. Effects of the Rations on Morphologic Parameters, Mitosis, Apoptosis, and Selected Regulators of Apoptosis (Bcl-2: anti-apoptotic; Bak: pro-apoptotic)

	Controls	Butyric Acid Group	*P*
Crypt depth (no. of cells from bottom to top)	66.0 ± 1.5	78.0 ± 0.9	≤.001
Mitosis (Ki-67–positive cells/100 cells)	16.1 ± 1.1	16.5 ± 0.9	NS
Apoptosis (positive cells/hemicrypt)	0.9 ± 1.1	0.6 ± 0.6	≤.01
EGF activity (area of stained goblet mucus bubbles, μm^2)	13.8 ± 0.8	24.0 ± 2.0	≤.01
Bcl-2 (positive cells/hemicrypt)	3.4 ± 0.3	6.0 ± 0.4	≤.05
Bak (positive cells/hemicrypt)	1.8 ± 0.7	5.4 ± 0.7	≤.01

NOTE. Data are mean ± SEM.
Abbreviation: NS, not significant.

Compartment Distribution

Except for EGF, the parameters revealed a compartmental distribution along the longitudinal axis as shown in Table 3. The statistical analysis within groups confirmed significant effects of the compartment on these parameters. The mitotic activity in the stem cell compartment was significantly lower in the butyric acid group compared with controls ($P \leq .001$). The remarkable decrease of mitosis was instead compensated by an increase both in the middle and luminal compartment in favor of butyric acid pigs so that the overall mitotic rate (Table 2) did not differ significantly between the treatment groups.

The localization of apoptosis differed significantly between the 2 groups ($P < .001$). In the controls, the main apoptotic compartment was located in the luminal region of the crypts. This compartment contained a mean of 0.54 apoptotic cells and thus significantly ($P \leq .001$) more compared both with the middle and the stem cell compartments. As a consequence, the lumen compartment represents 63.2% of all apoptotic cells in this group.

In the butyric acid group, a considerable number of apoptotic cells had shifted from the lumen (0.24 cells) to the stem cell compartment (0.21 cells). Thus, these compartments did not differ significantly. The middle compartment (0.11 cells) did not contribute much to the overall apoptotic activity. Consequently, in the butyrate group, the percentage of apoptotic cells in the luminal compartment had decreased by 18.8% compared with controls, but had increased by 21.7% in the stem cell compartment.

The decrease of apoptosis in the luminal compartment of the butyric acid group was paralleled by increased Bcl-2 activity. Compared with the controls, the Bcl-2 activity was increased significantly ($P \leq .001$) in all compartments of the butyric acid group, but the ratio was maximal (2.16:1) in the luminal ending. When compared with the controls, Bak-positive cells were also elevated both in the stem cell and luminal compartments of the butyric acid group, but the increase tended to be more pronounced in the stem cell region (ratio butyric acid /controls, 3.4:1) compared with the luminal compartment (ratio, 3.2:1).

DISCUSSION

To date, butyrate has been investigated mainly due to its effects on transformed cells in vitro. Such cells include several lines of adenoma cells (AA/C1, RG/C2, BH/C1) and carcinoma cells (S/ KS/FI).[16,29] Alternatively, butyrate withdrawal in guinea pig colon tissue culture led to mass apoptosis.[22] An apoptosis-inhibiting effect of butyrate in vivo had been demonstrated for the bovine rumen and for the colon in the pig.[23,24] It was shown earlier that butyric acid is the main SCFA, which increases after resistant starch feeding, because propionate remained unchanged and acetate revealed an increase which, however, was far less pronounced compared with butyrate.[24] In this report, we show that increased butyrate formation was accompanied by reduced apoptosis, in general, and additionally by a change in the compartmental distribution of apoptosis, mitosis, and factors involved in apoptosis regulation. Such an in vivo approach unavoidably has an impact on the qualitative and quantitative assemblages of bacteria, which again, may influence mucosal function both in a positive or negative direction. Such effects are released either directly by the attachment of the bacteria to the epithelial cells or indirectly by products of bacterial metabolism, which can lead to complex alterations of the physical and chemical features of the gastrointestinal environment and may even influence the expression of genes by the epithelial cells.[30,31]

Such complex interactions require future studies, which characterize changes in the microbial ecosystem of the gut. Nevertheless, butyrate is a candidate metabolite, which has potential to influence gene expression by the colonocytes.

The reason for apoptosis inhibition in vivo may be an unspecific effect, because butyrate is the preferred fuel for colono-

Table 3. Compartmental Distribution of Ki-67, Apoptosis, Bcl-2, and Bak Depending on Treatment

	Ki-67		Apoptosis		Bcl-2		Bak	
Compartment	C	B	C	B	C	B	C	B
Luminal	0.39 ± 0.06	1.15 ± 0.16	0.54 ± 0.03	0.24 ± 0.03	1.20 ± 0.25	2.60 ± 0.33	0.91 ± 0.1	2.90 ± 0.55
Middle	1.63 ± 0.11	5.14 ± 0.27	0.15 ± 0.12	0.11 ± 0.02	0.81 ± 0.15	1.12 ± 0.20	0.25 ± 0.05	0.45 ± 0.18
Stem cell	8.59 ± 0.10	6.56 ± 0.29	0.16 ± 0.02	0.21 ± 0.03	1.35 ± 0.15	2.30 ± 0.35	0.61 ± 0.1	2.05 ± 0.25

NOTE. All values are given as the number of positive cells/compartment (mean ± SEM).
Abbreviations: C, control group; B, butyrate group.

cytes.[9,32] Energy availability seems to be essential to maintain the membrane integrity of mitochondria and thus may inhibit the release of apoptosis-inducing factors, such as AIF and cytochrome C, from the mitochondria.[33] Moreover, the overall number of Bcl-2–positive cells was increased. A direct effect of butyrate on apoptosis-regulating proteins, including Bcl-2, has already been described and supports the assumption of similar mechanisms in this study.[16,22]

In the present study, the proapoptotic signal, Bak, was also increased in the butyric acid group, as described previously to be the consequence of butyrate in the culture medium for adenoma and carcinoma cells.[16] This increase seems to contradict the above-mentioned antiapoptotic mechanisms, but may be explained by the finding that Bcl-2 and Bak are expressed in different compartments of the crypt.

Apart from an overall reduction of apoptosis, but not mitosis, the main butyrate effect was a shift of apoptosis towards the middle and basal compartments.

An increased apoptotic activity in the basal stem cell compartment of the crypts has been observed earlier and was assumed to be a protective mechanism against hyperplasia of colonic crypts.[34,35] This assumption was substantiated by a comparative analysis of normal human colon tissue and tissue with adenomatous polyposis. The latter tissue revealed a pronounced increase of apoptosis in the proliferative compartment.[36] The same effect in the butyric acid group in our study might similarly be explained by a shortening of the average life span of colonocytes so that the degree of crypt elongation (15%) was moderate compared with the overall reduction of apoptosis (34%). The mitotic activity in the basal compartment decreased so that the maintenance of the overall number of mitotic cells in the 2 groups was explained by a shift towards the middle and luminal compartments. Similarly, long-term feeding of a diet, which led to an increase of SCFA in rats, led to a shift of proliferating cells to a higher region in each crypt.[37] The butyrate-dependent shift in the mitotic and apoptotic compartments is partly paralleled by a shift in the Bak and Bcl-2 proteins.

Thus, the increased apoptosis in the stem cell compartment is accompanied by a higher Bak activity. A concomitant slight increase of Bcl-2 was less pronounced (1.7-fold compared with controls) than the Bak increase (3.4-fold compared with controls). It appears that the proapoptotic signal overrides the antiapoptotic effect. Furthermore, it was found previously that an overexertion of Bcl-2 does not protect against Bak-mediated apoptosis.[16] In the luminal compartment the reduction of apoptosis by 66% was related to an increase in Bcl-2 activity. An even more pronounced increase of Bak, however, should have led to a higher increase of apoptosis, suggesting that additional survival factors are active. It is known that several growth factors, such as IGF-I and EGF, function as mitogens, which stimulate tissue regeneration by stem cell mitosis.[27] Additionally, they act as survival factors, which interact with the apoptosis-regulating system in mature cells, so that overexpression of the EGF receptor is characteristic for many tumor cells.[41] An increased availability of the EGF protein in the normal gut thus may be part of a general survival strategy counteracting the Bak effects.[27,38-40] The increased mucus production of goblet cells in the butyrate group, in turn, may be regarded as a reaction due to the decreased pH in this group.

The detailed mechanisms, which lead to the compartmental redistribution of mitosis and apoptosis, however, are completely unknown. Components of the mechanisms may include (1) an improved availability of butyrate in the luminal compartment for colonocytes. (2) An AIF-dependent increase of stem cell sensitivity against apoptotic signals.[2,4] Such effects would also explain the different reaction of transformed and normal cells. (3) Signal mechanisms provided by mobile monocytes in the lamina propria as it was also shown in the small intestine of the pig .[25]

ACKNOWLEDGMENT

We thank Drs H.J. Schlüsener and M.H. Deininger, University of Tuebingen, for assistance in the immunocytochemical procedures; H. Hägele for technical assistance; C. Fischinger, M. Mecellem, and B. Dunne for their excellent care of the animals. We also thank B. Deininger for typing the manuscript.

REFERENCES

1. Butzner JD, Gall DG: Impact of refeeding on intestinal development and function in infant rabbits subjected to protein-energy malnutrition. Pediatr Res 27:245-251, 1990

2. Fleming SE, Youngman LD, Ames BN: Intestinal cell proliferation is influenced by intakes of protein and energy, aflatoxin, and whole-body radiation. Nutr Cancer 22:11-30, 1994

3. Raab S, Leiser R, Kemmer H, et al: Effects of energy and purines in the diet on proliferation, differentiation, and apoptosis in the small intestine of the pig. Metabolism 47:1105-1111, 1998

4. Sakata T: Stimulatory effect of short-chain fatty acids on epithelial cell proliferation in the rat intestine: A possible explanation for trophic effects of fermentable fiber, gut microbes and luminal trophic factors. Br J Nutr 58:95-103, 1987

5. Kripke SA, Fox AD, Berman JM, et al: Stimulation of intestinal mucosal growth with intracolonic infusion of short chain fatty acids. JPEN 13:109-116, 1989

6. Scheppach W, Bartram P, Richter A, et al: Effect of short-chain fatty acids on the human colonic mucosa in vitro. JPEN 16:43-48, 1992

7. Augenlicht LH, Anthony GM, Church TL, et al: Short-chain fatty acid metabolism, apoptosis, and Apc-initiated tumorigenesis in the mouse gastrointestinal mucosa. Cancer Res 59:6005-6009, 1999

8. Heerdt BG, Houston MA, Anthony GM, et al: Initiation of growth arrest and apoptosis of MCF-7 mammary carcinoma cells by tributyrin, a triglyceride analogue of the short-chain fatty acid butyrate, is associated with mitochondrial activity. Cancer Res 59:1584-1591, 1999

9. Roediger WEW: Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. Gut 21:793-798, 1980

10. Luciano L, Gross S, Busche R, et al: Massive apoptosis of colonocytes induced by butyrate deprivation overloads resident macrophages and promotes the recruitment of circulating monocytes. Cell Tissue Res 309:393-407, 2002

11. Sakata T, Yajima T: Influence of short chain fatty acids on the epithelial cell division of digestive tract. Q J Exp Physiol 69:639-648, 1984

12. Heerdt BG, Houston MA, Augenlicht LH: Short-chain fatty acid-initiated cell cycle arrest and apoptosis of colonic epithelial cells is linked to mitochondrial function. Cell Growth Differ 8:523-532, 1997

13. Tsujimoto Y, Shimizu S: Bcl-2 family: Life-or-death switch. FEBS Lett 466:6-10, 2000

14. Susin SA, Zamzami N, Castedo M, et al: Bcl-2 inhibits the mitochondrial release of an apoptogenic protease. J Exp Med 184: 1331-1341, 1996

15. Cai J, Yang J, Jones DP: Mitochondrial control of apoptosis: The role of cytochrome c. Biochim Biophys Acta 1366:139-149, 1998

16. Hague A, Diaz GD, Hicks DJ, et al: Bcl-2 and bak may play a pivotal role in sodium butyrate-induced apoptosis in colonic epithelial cells: however overexpression of bcl-2 does not protect against bak-mediated apoptosis. Int J Cancer 72:898-905, 1997

17. Motyl T: Regulation of apoptosis: Involvement of Bcl-2-related proteins. Reprod Nutr Dev 39:49-59, 1999

18. Minn AJ, Velez P, Schendel SL, et al: Bcl-x(L) forms an ion channel in synthetic lipid membranes. Nature 385:353-357, 1997

19. Schendel SL, Xie Z, Montal MO, et al: Channel formation by antiapoptotic protein Bcl-2. Proc Natl Acad Sci USA 94:5113-5138, 1997

20. Antonsson B, Conti F, Ciavatta A, et al: Inhibition of Bax channel-forming activity by Bcl-2. Science 277:370-372, 1997

21. Heerdt BG, Houston MA, Augenlicht LH: Potentiation by specific short chain fatty acids of differentiation and apoptosis in human colonic carcinoma cell lines. Cancer Res 54:3288-3294, 1994

22. Hass R, Busche R, Luciano L, et al: Lack of butyrate is associated with induction of Bax and subsequent apoptosis in the proximal colon of guinea pig. Gastroenterology 112:875-881, 1997

23. Mentschel J, Leiser R, Muelling C, et al: Butyric acid stimulates rumen mucosa development in the calf mainly by a reduction of apoptosis. Arch Anim Nutr 55:85-102, 2002

24. Claus R, Lösel D, Lacorn M, et al: Effects of butyrate on apoptosis in the pig colon and its consequences for skatole formation and tissue accumulation. J Anim Sci 81:239-248, 2003

25. Mentschel J, Deininger MH, Schluesener HJ, et al: Effects of malnutrition on the expression of daintain/AIF-1 in the gut mucosa of pigs. Zentralbl Veterinarmed A 49:184-188, 2002

26. Gavrieli Y, Shermon Y, Ben-Sasson SA: Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 119:493-501, 1992

27. Claus R, Mentschel J, Blazey B, et al: Preliminary studies on epidermal growth factor (EGF) immunoreactivity in goblet cells of the small intestine by a species-specific antiserum in healthy piglets and piglets with diarrhea. J Anim Feed Sci 10:309-315, 2001

28. Topping DL, Illman RJ, Clarke JM, et al: Dietary fat and fiber alter large bowel and portal venous volatile fatty acids and plasma cholesterol but not biliary steroids in pigs. J Nutr 123:133-143, 1993

29. Hague A, Manning AM, Hamlon KA, et al: Sodium butyrate induces apoptosis in human colonic tumor cell lines in a p53 independent pathway: Implications for the possible role of dietary fibre in prevention of large bowel cancer. Int J Cancer 55:498-505, 1993

30. Buddington RK: The use of nondigestible oligosaccharides to manage the gastrointestinal ecosystem, in Piva A, Bach Knudsen KE, Lindberg JE (eds): Gut Environment of Pigs. Nottingham, UK, Nottingham University Press, 2001, pp 133-147

31. Bry L, Falk PG, Midtvedt T, et al: A model of host-microbial interactions in an open mammalian ecosystem. Science 273:1380-1383, 1996

32. Britton R, Krehbiel C: Nutrient metabolism by gut tissues. J Dairy Sci 76:2125-2131, 1993

33. Brunner S, Kramar K, Denhardt DT, et al: Cloning and characterization of murine carnitine acetyltransferase: Evidence for a requirement during cell cycle progression. Biochem J 322:403-410, 1997

34. Loeffler M, Birke A, Winton D, et al: Somatic mutation, monoclonality and stochastic models of stem cell organization in the intestinal crypt. J Theor Biol 160:471-491, 1993

35. Potten CS, Li YQ, O'Connor PJ, et al: A possible explanation for the differential cancer incidence in the intestine, based on distribution of the cytotoxic effects of carcinogens in the murine large bowel. Carcinogenesis 13:2305-2312, 1992

36. Strater J, Koretz K, Gunthert AR, et al: In situ detection of enterocytic apoptosis in normal colonic mucosa and in familial adenomatous polyposis. Gut 37:819-825, 1995

37. Edwards CA, Wilson RG, Hanlon L, et al: Effect of the dietary fibre content of lifelong diet on colonic cellular proliferation in the rat. Gut 33:1076-1079, 1992

38. Hague A, Hicks DJ, Bracey TS, et al: Cell-cell contact and specific cytokines inhibit apoptosis of colonic epithelial cells: Growth factors protect against c-myc-independent apoptosis. Br J Cancer 75: 960-968, 1997

39. Petschow BW, Carter IDL, Hutton GD: Influence of orally administered epidermal growth factor on normal and damaged intestinal mucosa in rats. J Pediatr Gastroenterol Nutr 17:49-58, 1993

40. Playford RJ, Hauby AM, Gschmeissner S, et al: The epidermal growth factor receptor (EGF-R) is present on the basolateral, but not the apical, surface of enterocytes in the human gastrointestinal tract. Gut 39:262-266, 1996

41. Ritter CA, Arteaga CL: The epidermal growth factor receptor-tyrosine kinase: A promising therapeutic target in solid tumors. Semin Oncol 30:3-11, 2003

42. Cande C, Cohen I, Daugas E, et al: Apoptosis-inducing factor (AIF): A novel caspase-independent death effector released from mitochondria. Biochimie 84:215-222, 2002