Original Communications

Stimulation of Intestinal Mucosal Growth with Intracolonic Infusion of Short-Chain Fatty Acids

SCOTT A. KRIPKE, M.D.,* ANDREW D. FOX, M.D.,* JEFFREY M. BERMAN, B.A.,*
R. GREGG SETTLE, PH.D.,† AND JOHN L. ROMBEAU, M.D.*

From the * Departments of Surgery and † Otorhinolaryngology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

ABSTRACT. Dietary fiber, which stimulates intestinal mucosal growth, is fermented by anaerobic bacteria in the rat hindgut to the short-chain fatty acids (SCFA) acetate, propionate, and butyrate. Butyrate is the preferred oxidative fuel of the colonocyte in vitro, and the provision of preferred intestinal fuels has been shown to stimulate mucosal proliferation in vivo. This study determined whether chronic colonic infusion of butyrate or a combination of SCFA would stimulate intestinal mucosal growth in an animal deprived of its normal source of SCFA, fiber fermentation in the cecum. Adult male Sprague-Dawley rats were fed a fat- and fiber-free elemental liquid diet and underwent cecectomy, ileocolic anastomosis, and insertion of a proximal colonic infusion catheter. Rats were then assigned to receive either a continuous infusion of butyrate (20 mM, 40 mM, or 150 mM), SCFA (70 mM acetate + 35 mM propionate + 20 mM butyrate), or saline, or to receive no infusion. A seventh group underwent proximal colonic transection and reanastomosis. After 7 days, jejunal, ileal, and proximal colonic segments were analyzed for mucosal weight, protein, RNA, and DNA. In the colon, the 40-mM butyrate infusion resulted in significant elevations in all mucosal parameters relative to all three control groups, saline infusion, no infusion, and transection. Both the 20-mM butyrate and the SCFA groups showed increased colonic mucosal DNA compared to controls. In the jejunum and ileum, mucosal DNA content was significantly greater in the SCFA group than in the control groups. These data indicate that SCFA infused into the rat colon has a trophic effect throughout the intestinal tract, whereas butyrate appears to have its primary trophic effect locally in the colon. (Journal of Parenteral and Enteral Nutrition 13:109–116, 1989)

Removal of fiber from the diet results in small intestinal and colonic mucosal atrophy,[1-3] which is reversed by the addition of fiber to the diet.[1,3-7] This phenomenon has been used to enhance mucosal adaptation in experimental animals undergoing massive small intestinal resection by supplementing elemental diets with pectin, a fermentable fiber.[8]

Fiber polysaccharides escape digestion in the upper gastrointestinal tract and are metabolized by anaerobic bacteria in the hindgut.[9] The major end products of this fermentative process are the short-chain fatty acids (SCFA), of which acetate, propionate, and butyrate account for 83%.[10,11] These are produced in a nearly constant molar ratio 1:0.3:0.25, respectively.[9] The removal of fiber from the diet results in a 10- to 12-fold reduction in the cecal content of SCFA in the rat.[11]

The SCFA are readily absorbed and metabolized by the cecal and colonic mucosa,[11-15] and, in combination, they have been shown to increase the mucosal mitotic index in both small and large intestine when briefly instilled into a short segment of colon.[16] In vitro studies have demonstrated that butyrate is the preferred oxidative fuel of the colonocyte when compared to other common fuels (ketone bodies, amino acids, and glucose),[15,17] and previous work in this and other laboratories has shown that the enteral or parenteral provision of preferred fuels can stimulate intestinal mucosal growth.[18-20] Butyrate alone may therefore be responsible for the colonotrophic effect attributed to the SCFA.

Thus, the hindgut production of SCFA, butyrate in particular, may mediate the trophic effect of fiber in the colon, and may play an important role in the normal maintenance of the small intestinal mucosa as well. The purpose of this study was to examine the effect of chronic intracolonic infusion of butyrate or the SCFA on intestinal mucosal growth in the absence of normal endogenous SCFA production.

MATERIALS AND METHODS

Forty-two adult male Sprague-Dawley rats (250–300 g) were housed in individual metabolic cages and fed a fat- and fiber-free elemental liquid diet (Polycose, Ross, Columbus, OH; Aminosyn, Abbott, North Chicago, IL; multivitamins and minerals, Nutrisource, Sandoz, Minneapolis, MN) for 3 days. This diet, given to minimize the effects of residual fiber in the gastrointestinal tract, was also used in the postoperative period and provided 225

kcal/kg body weight (BW)/day and 1.2 g N/kg BW/day. Animals were allowed free access to water. On the fourth day, all rats were anesthetized (Ketamine 87 mg/kg and Xylazine 13 mg/kg by intraperitoneal injection), weighed, and underwent laparotomy. Thirty-six rats underwent cecectomy (including the first centimeter of proximal colon), end-to-side ileocolic anastomosis (6-0 silk, interrupted, single-layer anastomosis), and placement of a proximal colonic Silastic infusion catheter (Dow Corning Laboratories, Corning, NY) (Fig. 1). The colonic catheter was secured with a 6-0 silk pursestring suture through the transected end of the colon and was tunnelled through the subcutaneous tissue to exit in the midscapular area within a spring connected to a swivel device (Instech, Horsham, PA). The remaining six rats (TRANSX) underwent transection and reanastomosis of the proximal colon 1 cm distal to the cecum.

The 36 cecectomized rats received intracolonic infusions of normal saline at 1.5 ml/hr over the first postoperative night. On postoperative day (POD) 1 rats were assigned to receive a continuous infusion of one of three butyrate solutions (20 mM, 40 mM, or 150 mM), SCFA (70 mM acetate + 35 mM propionate + 20 mM butyrate), or saline (PSS), or to receive no infusion (NO INF). Sodium salts of the SCFA were used (Sigma Chemical Co., St. Louis, MO). All infusion solutions were made isosmolar (300 mosm/liter, measured by freezing point depression, Osmette A 2012, Precision Systems, Sudbury, MA) with the addition of NaCl and were adjusted to a pH of 6.4 with either concentrated NaOH or HCl. Solutions were prepared daily. Infusions were delivered continuously at 1.5 ml/hr (peristaltic pump, Holter #903, Critikon, Tampa, FL) for 7 days.

The SCFA solution was designed to approximate the intracecal concentrations of the primary SCFA in a rat consuming a regular chow diet.[11,21] The butyrate solutions spanned a range of concentrations from that equal to the butyrate component of the SCFA mixture (20 mM or "physiologic") to 7.5 times the physiologic concentration.
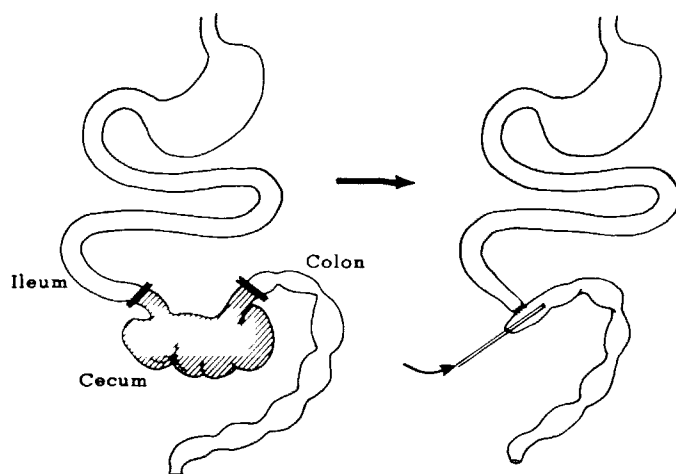

FIG. 1. Schematic diagram of cecectomy procedure including end-to-side ileocolic anastomosis and placement of colonic infusion catheter.

In terms of the absolute amount of fatty acid infused, the 20, 40, and 150 mM butyrate solutions provided 0.5, 1.0, and 3.75 μmol/min, respectively. The SCFA infusion provided 1.75 μmol/min of acetate, 0.875 μmol/min of propionate, and 0.5 μmol/min of butyrate. These amounts are slightly lower than SCFA fluxes measured across the rat cecum in animals consuming a diet containing about 50% fiber (a very high-fiber diet).[11] If entirely absorbed, the SCFA infusion provided 1.12 kcals/day, or roughly 2% of caloric intake.[22] Similarly, butyrate infusions of 20, 40, and 150 mM provided 0.25 kcal/day (0.4%), 0.5 kcal/day (0.8%), and 1.875 kcals/day (3.1%), respectively. Total caloric intake, including the caloric content of the colonic infusion, was equivalent among groups.

On POD 8 the rats were anesthetized, weighed, and euthanized by cardiac puncture and exsanguination. The intestine from the ligament of Treitz to the anus was rapidly excised, freed of its mesenteric fat, and rinsed in ice-cold saline. Ten-centimeter segments (measured under 10 gm tension) of proximal jejunum (just distal to the ligament of Treitz), distal ileum (ending 5 cm proximal to the anastomosis or the cecum in the TRANSX group), and proximal colon (beginning 2 cm distal to the anastomosis) were obtained. All segments were cleaned, weighed, opened longitudinally, rinsed in ice-cold saline solution, blotted dry, and scraped with a glass slide for complete removal of mucosa. The mucosal scrapings were weighed, homogenized in water, sonicated, and frozen at $-25°C$ until assayed for DNA[23,24] RNA,[25] and protein.[26]

Statistical Analysis

All data are presented as the mean and standard error of the mean. A one-way analysis of variance was used to determine whether there were significant differences among groups for each of the variables measured. Post-hoc comparisons between groups were made using Duncan's multiple range test.[27] The degree of correlation between mucosal parameters was determined by calculating the Spearman rank correlation coefficient and the degree of correlation among parameters was determined by calculating Kendall's coefficient of concordance.[28] A p value less than 0.05 was considered significant.

RESULTS

Seven animals died postoperatively, a mortality of 16.6%. Death rates did not differ significantly among groups. Two additional animals were eliminated from the study: one animal in the 150 mM butyrate group had severe diarrhea and anorexia and one TRANSX animal was found at sacrifice to have an obstruction at the colo-colonic anastomosis. Thus the data from the remaining 33 animals were analyzed. There was no significant change in body weight in any of the groups over the study period, and body weight change did not differ significantly among groups.

Intestinal Parameters

Colon. Proximal colonic mucosal DNA was significantly elevated in the 20- and 40-mM butyrate groups

and the SCFA group compared to all three control groups (Figs. 2 and 3). The 150-mM butyrate infusion resulted in a significant increase in colonic mucosal DNA compared to No INF and TRANSX, but not compared to PSS. Mucosal RNA was significantly elevated in the 40-mM butyrate group, and mucosal weight and protein content were significantly elevated in both the 40- and 150-mM butyrate groups compared to all three control groups (Table I). SCFA infusion resulted in elevations in mucosal weight and protein compared to TRANSX, and the 20-mM butyrate infusion significantly increased mucosal protein vs TRANSX. Colonic segment weight
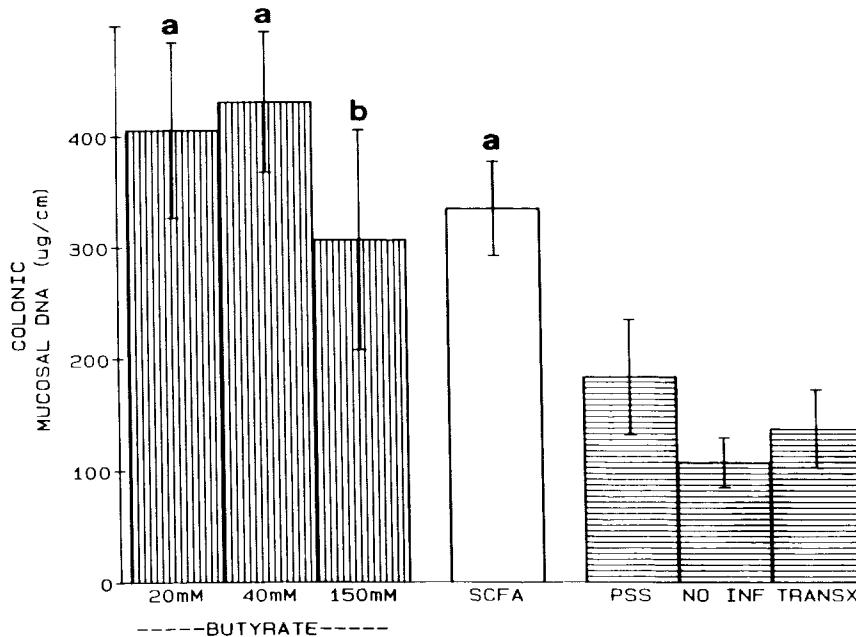

FIG. 2. Effect of 7 days of intracolonic butyrate or SCFA infusion on colonic mucosal DNA (μg/cm). 20 mM, 40 mM, 150 mM = butyrate infusions; SCFA = infusion of 70 mM acetate + 35 mM propionate + 20 mM butyrate; PSS = saline infusion; NO INF = no infusion; TRANSX = transection and reanastomosis of proximal colon without cecectomy or infusion. a, $p < 0.05$ vs PSS, NO INF, TRANSX; b, $p < 0.05$ vs NO INF, TRANSX.
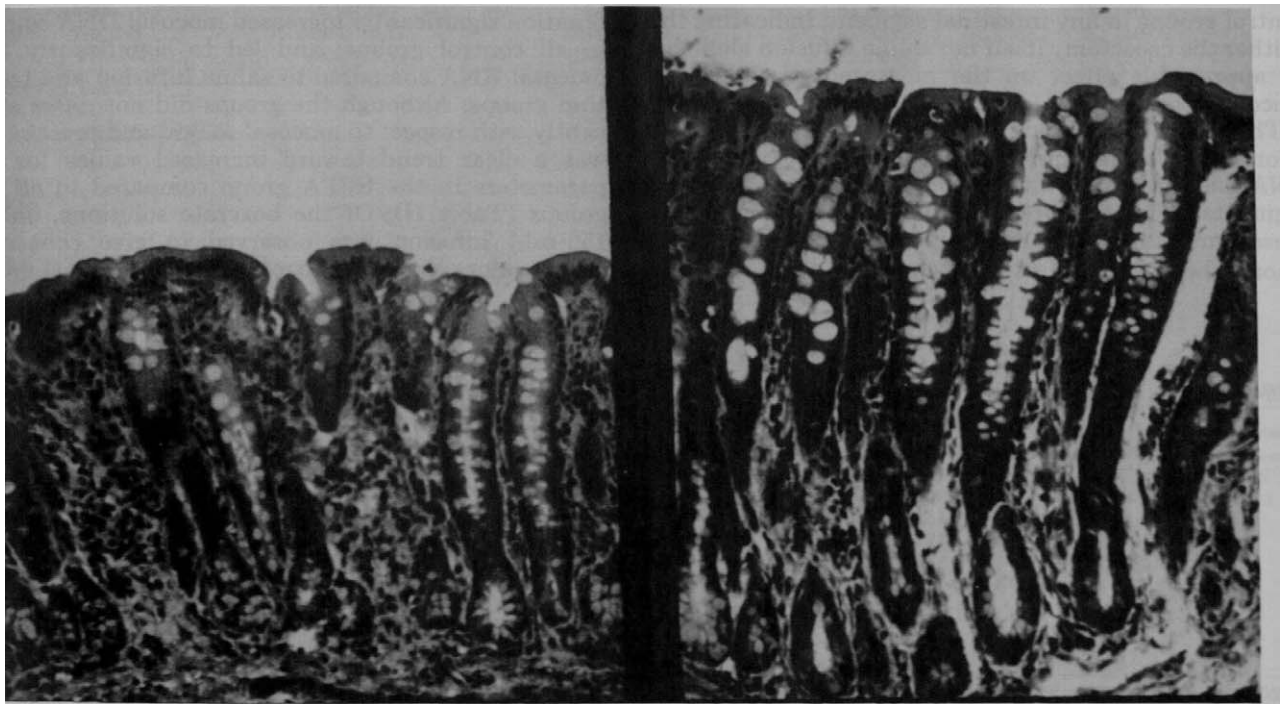

FIG. 3. Light micrographs of representative cross sections of proximal colons from rats receiving intracolonic saline infusion (*left*) and 20 mM butyrate infusion (*right*). Crypt depth per specimen (in microns) was determined by measuring 10 well-oriented crypts per sample with an eyepiece micrometer and calculating the mean value. Compared to the control groups, the 20 and 40 mM butyrate and SCFA infusions gave rise to a significant increase in crypt depth in the proximal colon: 20 mM, 236(5); 40 mM, 242(14); 150 mM, _____ FA. 240(8); PSS, 190(16); NO INF, 183(8); and TRANSX, 179(5). (Hematoxylin & eosin, ×50).

per unit length was increased in the 40-mM group compared to all three controls, and in the 150-mM group compared to No INF and TRANSX. The butyrate and SCFA groups did not differ significantly in any of the measured parameters. Likewise, there were no significant differences among the three control groups.

Ileum. Ileal mucosal DNA content was significantly elevated in the SCFA group compared to PSS, No INF, TRANSX, and 20-mM butyrate (Fig. 4). DNA was also significantly increased in the 40-mM and 150-mM butyrate groups *vs* the TRANSX group. Mucosal RNA was significantly elevated in the 150-mM and SCFA groups compared to PSS and TRANSX. No significant differences among groups were found in segment weight, mucosal weight, or protein content (Table II), although the SCFA and 150-mM butyrate infusions produced values for these parameters that were always greater than all controls.

Jejunum. Jejunal mucosal DNA was significantly elevated in the SCFA group when compared to PSS, No INF, and TRANSX (Fig. 5). The 20-mM butyrate group showed a significant increase in mucosal DNA content when compared to the NO INF and TRANSX groups. The 40-m and 150-mM butyrate groups showed significant increases in DNA compared only to TRANSX. The three butyrate and the SCFA groups did not differ significantly with respect to mucosal DNA, nor did the three control groups differ among themselves. There were no significant differences among any of the groups in segment weight or mucosal weight, protein, or RNA (Table III).

There were no significant differences among the three control groups in any intestinal segment, indicating that neither the cecectomy itself nor saline infusion alone had an appreciable effect on the measured parameters of mucosal growth.

The data summarized in Tables I, II, and III suggest a strong correlation among mucosal weight, protein, and RNA measurements within each of the three intestinal segments. The rank ordering of the groups for each of these parameters showed a high degree of correlation among these parameters in each of the intestinal segments [Kendall coefficient of concordance (W), $p < 0.01$]: colon, $W = 0.93$; ileum, $W = 0.96$; and jejunum, $W = 0.80$. However, the degree of correlation between these three mucosal parameters and mucosal DNA deteriorates from distal to proximal intestine (Spearman rank correlation coefficient, *rho*): colon, *rho* = 0.79 ($p < 0.05$); ileum, *rho* = 0.68 ($p < 0.1$); jejunum, *rho* = 0.11 ($p > 0.1$). This indicates that there is no relationship between mucosal DNA and the other parameters of mucosal growth in the jejunum, whereas this relationship is present in the ileum and most strongly in the colon.

DISCUSSION

This study demonstrated that the chronic intracolonic infusion of a 40-mM butyrate solution in a cecectomized rat fed with a fiber-free elemental diet significantly stimulated colonic mucosal growth, as measured by increased mucosal mass, protein, RNA, and DNA, compared to all three control groups. Furthermore, the 20-mM butyrate infusion stimulated increases in mucosal parameters as effectively as the SCFA combination, and increasing the concentration of the butyrate infusion up to 7.5 times normal intracecal levels did not significantly enhance colonic mucosal growth compared to the 20-mM butyrate infusion. Taken together, these results strongly suggest that butyrate is the primary colonotrophic factor among the SCFA, and that normal intraluminal levels are near optimal with respect to colonic epithelial proliferation.

Infusion of SCFA into the rat hindgut influenced mucosal growth in the small intestine as well as the colon. In the ileum, colonic infusion of the SCFA combination significantly increased mucosal DNA compared to all control groups, and led to significantly higher mucosal RNA compared to saline infusion and transection groups. Although the groups did not differ significantly with respect to mucosal weight and protein, there was a clear trend toward increased values for these parameters in the SCFA group compared to all other groups (Table II). Of the butyrate solutions, only the 150-mM infusion was observed to give consistently higher values than all control groups for all ileal mucosal

TABLE I
Effect of 7 days of intracolonic butyrate or SCFA infusion (1.5 ml/hr) on the proximal colon of cecectomized rats fed a fiber-free elemental diet

	Segment weight (mg/cm)	Mucosal weight (mg/cm)	Mucosal protein (mg/cm)	Mucosal RNA (µg/cm)
Group*				
20 mM	105.5 (8.0)†	27.8 (3.4)	2.57 (0.37)¶	344 (60)
40 mM	136.1 (15.5)‡	38.8 (5.8)‡	3.28 (0.64)‡	523 (97)‡
150 mM	124.2 (4.9)§	34.0 (2.6)‡	2.88 (0.07)‡	359 (114)
SCFA	113.3 (6.1)	30.4 (2.9)¶	2.58 (0.11)¶	365 (49)
Controls				
PSS	105.7 (4.9)	21.4 (1.8)	1.80 (0.14)	190 (10)
No INF	90.5 (6.5)	18.3 (1.4)	1.78 (0.14)	217 (23)
TRANSX	93.9 (10.4)	17.4 (1.8)	1.46 (0.19)	195 (26)

* 20 mM, 40 mM, 150 mM = butyrate infusions; SCFA = infusion of 70 mM acetate + 35 mM propionate + 20 mM butyrate; PSS = saline infusion; No INF = no infusion; TRANSX = transection and reanastomosis of proximal colon without cecectomy or infusion.
† Mean (SEM).
‡ $p < 0.05$ *vs* PSS, NO INF, TRANSX.
§ $p < 0.05$ *vs* NO INF, TRANSX.
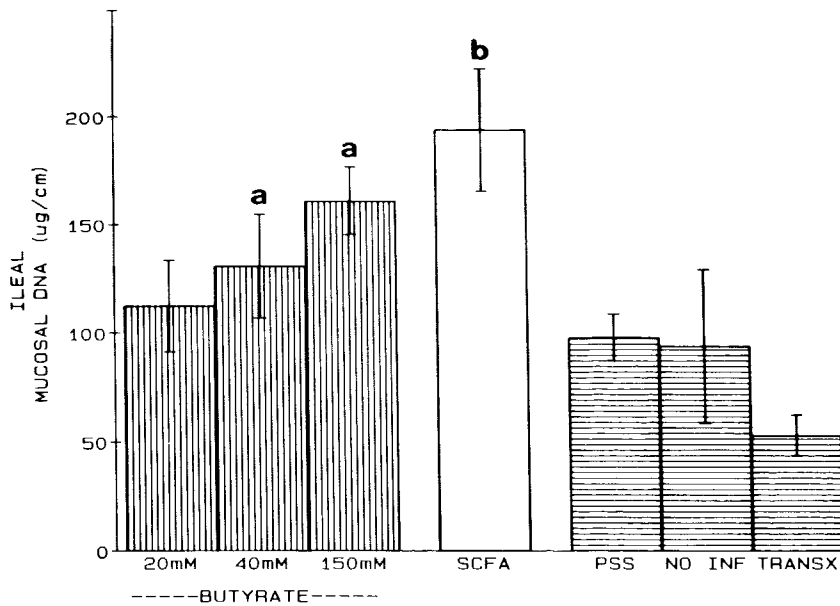¶ $p < 0.05$ *vs* TRANSX.

FIG. 4. Effect of 7 days of intracolonic butyrate or SCFA infusion on ileal mucosal DNA (μg/cm). a, p < 0.05 vs TRANSX; b, p < 0.05 vs PSS, NO INF, TRANSX, 20 mM. (see Fig. 2 for group definitions.)

TABLE II

Effect of 7 days of intracolonic butyrate or SCFA infusion (1.5 ml/hr) on the ileum of cecectomized rats fed a fiber-free elemental diet

	Segment weight (mg/cm)	Mucosal weight (mg/cm)	Mucosal protein (mg/cm)	Mucosal RNA (μg/cm)
Group*				
20 mM	49.8 (4.9)†	18.5 (2.0)	1.71 (0.25)	166 (19)
40 mM	49.3 (5.5)	17.2 (1.7)	1.61 (0.18)	167 (24)
150 mM	57.3 (13.3)	21.3 (4.2)	1.92 (0.29)	246 (46)‡
SCFA	55.4 (5.4)	23.3 (2.6)	2.04 (0.23)	244 (26)‡
Controls				
PSS	45.1 (2.2)	14.8 (2.6)	1.31 (0.18)	136 (20)
No INF	49.7 (6.1)	19.2 (2.3)	1.77 (0.29)	211 (40)
TRANSX	44.8 (3.1)	16.2 (1.4)	1.39 (.016)	153 (25)

* Defined in Table I.
† Mean (SEM).
‡ p < 0.05 vs PSS, TRANSX.
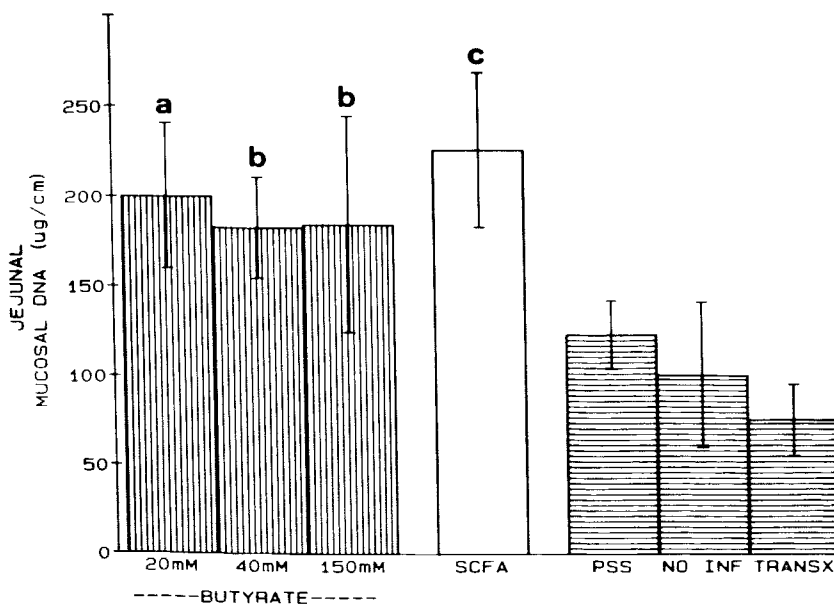

FIG. 5. Effect of 7 days of intracolonic butyrate or SCFA infusion or jejunal mucosal DNA (μg/cm). a, p < 0.05 vs NO INF, TRANSX; b, p < 0.05 vs TRANSX; c, p < 0.05 vs PSS, NO INF, TRANSX. (See Fig. 2 for group definitions.)

TABLE III
Effect of 7 days of intracolonic butyrate or SCFA infusion (1.5 ml/hr) on the jejunum of cecectomized rats fed a fiber-free elemental diet

	Segment weight (mg/cm)	Mucosal weight (mg/cm)	Mucosal protein (mg/cm)	Mucosal RNA (μg/cm)
Group*				
20 mM	48.8 (2.7)†	21.1 (2.2)	1.81 (0.25)	185 (45)
40 mM	53.3 (4.8)	25.2 (3.5)	2.59 (0.34)	251 (31)
150 mM	52.4 (3.0)	22.7 (2.1)	2.13 (0.03)	221 (18)
SCFA	47.7 (2.5)	20.7 (2.8)	1.99 (0.21)	239 (19)
Controls				
PSS	48.8 (3.3)	20.8 (2.9)	2.04 (0.32)	188 (28)
No INF	53.5 (6.9)	23.2 (4.0)	2.27 (0.47)	198 (34)
TRANSX	51.0 (1.6)	18.5 (1.4)	1.70 (0.18)	180 (18)

* Defined in Table I.
† Mean (SEM).

parameters (Fig. 4 and Table II). The changes in mucosal weight, protein, and RNA correlated with mucosal DNA in the ileum. In the jejunum, SCFA infusion resulted in a significant increase in mucosal DNA compared to all control groups, but this was not accompanied by parallel increases in RNA, protein, and weight. Further, there was no correlation between DNA and the other parameters of mucosal growth. Thus, the SCFA infusion, and to a lesser extent the 150-mM butyrate infusion, produced a consistent trophic effect in the ileum compared to controls. However, in the proximal small intestine, the SCFA infusion, and to a lesser extent the butyrate infusions, resulted in an increase in mucosal DNA which was unrelated to the other mucosal parameters, suggesting that the rate of crypt cell replication is increased without an increase in villus mass.

The SCFA are normally produced by the anaerobic bacterial fermentation of fiber polysaccharides in the rat cecum.[10] They are readily absorbed by the colonic mucosa,[12,13] and it has recently been shown that the SCFA can increase intestinal crypt mitotic rate when injected intraperitoneally or briefly instilled into the colon.[29,16] Thus, although the mucosal trophic effects of dietary fiber have been largely attributed to the presence of luminal bulk,[1,30] it has been recently postulated that these effects may be mediated by the SCFA.[16]

The cecal content of the SCFA is reduced 10- to 12-fold after removal of fiber from a high-fiber diet,[11] and cecectomy itself reduces the hindgut generation of SCFA by 60 to 70%.[21] In the present experimental model, therefore, the hindgut infusion of SCFA or butyrate in quantities approximating those generated by anaerobic fermentation in the cecum of a rat consuming a moderately high-fiber diet[11,22] represented the replacement of these missing byproducts of fiber fermentation and permitted the examination of mucosal trophism in the absence of bulk effects.

It is well known that animals maintained on a fiber-free diet develop intestinal mucosal atrophy which is most pronounced in the distal small intestine and colon[1-3] and is reversible by the addition of fiber to the diet.[3-7] Although most types of fiber exert a strong trophic effect on distal intestinal mucosa, only the most fermentable types of dietary fiber produce any trophic effect on the proximal jejunal mucosa, and these effects are small.[31] Studies with [3H]thymidine have shown that animals on fiber-supplemented diets had increased jejunal crypt cell turnover compared to animals consuming a fiber-free diet without a net increase in the total number of cells per villus.[32]

Interestingly, the pattern of small and large intestinal mucosal trophism resulting from the hindgut infusion of the SCFA described in this study closely resembles that which results from the addition of fiber to an elemental liquid diet. That is, the greatest stimulus to mucosal growth occurs in the colon and distal small bowel and is dissipated in the jejunum. The jejunal effect of intracolonic SCFA infusion in this study was limited to an increase in DNA without a similar increase in mucosal mass, which parallels the reported increase in crypt cell turnover without a net increase in total number of cells per villus found when fiber was added to the diet.[32] In addition, the colonic mucosal parameters in the three control groups are similar to values reported for rats ingesting a fiber-free diet,[2] and the colonic mucosal values in the SCFA and butyrate infusion groups are similar to those reported for rats consuming a regular chow diet or fiber-supplemented elemental diet.[2,8,20,33] These findings indicate that the hindgut production of SCFA may play an important role in the mucosal effects of fiber in both the small and large intestine.

Provision of a preferred oxidative fuel source into a colon denied its normal intraluminal nutrient load may account for the colonic effects described in this study. The presence of luminal nutrients in general is a major stimulus to intestinal mucosal growth,[34-37] and fuels preferred by the intestine are especially effective in this regard.[18,38] Butyrate has been shown to be a preferred oxidative substrate for colonocytes *in vitro*,[15] and the results of this study support the idea the provision of such substrates is stimulatory to mucosal growth *in vivo* even beyond simple caloric effects, since the 40-mM butyrate infusion provided only 45% of the calories of the SCFA infusion, but resulted in consistently higher values for all colonic mucosal parameters. Additionally, the trophic effect described here in the colon is comparable to that which occurs in the small intestine after the infusion of small amounts of luminal nutrients into the atrophic upper-intestinal tract of parenterally-nourished animals.[34-37]

The stimulation of colonic mucosal growth with the 40-mM butyrate infusion was independent of infusion pH, osmolarity, volume, or sodium content since 40 mM butyrate significantly increased all mucosal parameters

compared to saline infusion, which controlled for all of these variables. Similarly, the mere presence of an infusion had no stimulatory effect since the saline infusion group did not differ significantly from the no infusion or transection groups. Finally, the no infusion group did not differ significantly from the transection group, indicating that cecectomy itself had no effect on mucosal growth, a finding in agreement with previous work.[39]

The mechanism by which SCFA modulate small intestinal mucosal proliferation is unknown. Many peptide hormones influence mucosal growth, most prominent among these is enteroglucagon.[40] Immunohistochemical studies have located the enteroglucagon-producing cells in the distal ileum, cecum, and proximal colon,[41] and intracecal infusion of carbohydrate or fat has been shown to stimulate enteroglucagon secretion.[42,40] The SCFA, normally generated in the cecum, may be important physiologic stimulators of enteroglucagon release, and this hypothesis warrants further study.

Host metabolism of the SCFA provides another possible mechanism for the small-bowel effects. SCFA not metabolized by the cecal and colonic mucosa are transported to the liver via the portal blood. Portal blood concentrations of the SCFA are four to 10 times higher than systemic levels, indicating a substantial clearance function for the liver.[43,44] Hepatic metabolism of butyrate and acetate results in the production of glutamine and the ketone bodies acetoacetate and B-hydroxybutyrate[45,47] which are the preferred oxidative fuels of enterocytes.[48] The enteral or parenteral provision of glutamine and acetoacetate has been shown to be trophic to both small and large intestinal mucosa.[18,20,38]

In summary, this study has shown that in the rat the intracolonic infusion of butyrate in physiologic concentrations significantly stimulated colonic mucosal growth, and that of the three principal SCFA, butyrate was the primary trophic factor in the colon. Furthermore, the hindgut infusion of a SCFA solution comprising acetate, propionate, and butyrate at normal intracecal levels stimulated mucosal growth in the ileum and increased mucosal DNA in the jejunum in a manner which closely approximates the effect of fiber in the intestinal tract. Thus, intracolonic SCFA replicate the trophic effects of dietary fiber in the absence of mechanical bulk effects and may therefore be the principal mediators of the intestinal effects of fiber in the diet.

REFERENCES

1. Ryan GP, Dudrick SJ, Copeland EM, et al: Effects of various diets on colonic growth in rats. Gastroenterology 77:658–663, 1979
2. Morin CL, Ling V, Bourassa D: Small intestinal and colonic changes induced by a chemically defined diet. Dig Dis Sci 25:123–128, 1980
3. Ecknauer R, Sircar B, Johnson LR: Effect of dietary bulk on small intestinal morphology and cell renewal in the rat. Gastroenterology, 81:781–786, 1981
4. Gordon DT, Besch-Williford C, Ellersieck MR: The action of cellulose on the intestinal mucosa and elemental absorption by the rat. J Nutr 113:2545–2556, 1983
5. Jacobs LR, Schneeman BO: Effects of dietary wheat bran on rat colonic structure and mucosal cell growth. J Nutr 111:798–803, 1981
6. Jacobs LR, White FA: Modulation of mucosal cell proliferation in the intestine of rats fed a wheat bran diet. Am J Clin Nutr 37:945–953, 1983
7. Jacobs LR, Lupton JR: Effect of dietary fibers on rat large bowel mucosal growth and cell proliferation. Am J Physiol 246:G378–G385, 1984
8. Koruda MJ, Rolandelli RH, Settle RG, et al: The effect of a pectin-supplemented elemental diet on intestinal adaptation to massive small bowel resection. JPEN 10:343–350, 1986
9. Cummings JH, Branch WJ: Fermentation and the production of short chain fatty acids in the human large intestine. IN Dietary Fiber: Basic and Clinical Aspects. Vahouny GB, and Kritchevsky D, (eds). Plenum Press, New York, 1986, pp 131–152
10. Nyman M, Aso NG: Fermentation of dietary fiber components in rat intestinal tract. Br J Nutr 47:357–366, 1982
11. Demigne C, Remesy C: Stimulation of absorption of volatile fatty acids and minerals in the cecum of rats adapted to a very high fiber diet. J Nutr 115:53–60, 1985
12. McNeil NI, Cummings JH, James WPT: Short chain fatty acid absorption by the human large intestine. Gut 19:919–922, 1978
13. Ruppin H, Bar-Meir S, Soergel KH: Absorption of short chain fatty acids by the colon. Gastroenterology 78:1500–1507, 1980
14. Roediger WEW, Moore P: Effect of short chain fatty acids on sodium absorption in isolated human colon perfused through the vascular bed. Dig Dis Sci 26:100–106, 1981
15. Roediger WEW: Utilization of nutrients by isolated epithelial cells of the rat colon. Gastroenterology 83:424–429, 1982
16. Sakata T, Yajima T: Influence of short chain fatty acids on the epithelial cell division of the gastrointestinal tract. Q J Exp Physiol 69:639–648, 1984
17. Ardawi MSM, Newsholme EA: Fuel utilization in colonocytes of the rat. Biochem J 231:713–719, 1985
18. Fox AD, Kripke SA, Berman JM, et al: Reduction of the severity of enterocolitis by glutamine-supplemented enteral diets. Surg Forum 38:43–44, 1987
19. Hwang TL, O'Dwyer ST, Smith RJ, et al: Preservation of small bowel mucosa using glutamine-enriched parenteral nutrition. Surg Forum 37:56–58, 1986
20. Kripke SA, Fox AD, Berman JM, et al: Inhibition of TPN-associated intestinal mucosal atrophy with monoacetoacetin. J Surg Res 44:436–444, 1988
21. Remesy C, Demigne C: Partition and absorption of volatile fatty acids in the alimentary canal of the rat. Ann Rech Vet 7:39–55, 1976
22. Yang MG, Manoharan K, Mickelsen O: Nutritional contribution of volatile fatty acids from the cecum of rats. J Nutr 100:545–550, 1970
23. Burton K: A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J 62:315–323, 1965
24. Giles KW, Myers A: An improved diphenylamine method for the estimation of deoxyribonucleic acid. Nature 206:93, 1965
25. Fleck A, Begg D: The estimation of ribonucleic acid using ultraviolet absorption measures. Biochim Biophys Acta 108:333–339, 1965
26. Lowry OH, Rosebrough NJ, Farr AL, et al: Prot   rement with the folin phenol reagent. J Biol Chem 193:2   71
27. Winer BJ: Statistical Princi   rimental Design. McGraw-Hill, New York, 1971, pp 19
28. Siegel S: Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill, New York, 1956, pp 195–244
29. Tutton PJM, Barkla DH: Further studies on the effect of adenosine cyclic monophosphate derivatives on cell proliferation in jejunal crypts of rat. Clin Exp Pharmacol Physiol 9:671–674, 1982

30. Stragand JJ, Hageman CF: Effect of luminal contents on colonic cell replacement. Am J Physiol 233:E208–211, 1977

31. Jacobs LR: Effects of dietary fiber on mucosal growth and cell proliferation in the small intestine of the rat: A comparison of oat bran, pectin, and guar with total fiber deprivation. Am J Clin Nutr 37:954–960, 1983

32. Vahouny GV, Cassidy MM: Dietary fiber and intestinal adaptation. IN Dietary Fiber: Basic and Clinical Aspects. Vahouny GB, Kritchevsky D, (eds). Plenum Press, New York, 1986, pp 181–209

33. Koruda MJ, Rolandelli RH, Settle RG, et al: The effect of short chain fatty acids on the small bowel mucosa (abstr). JPEN 11(suppl):86, 1987

34. Levine GM, Deren JJ, Steiger E, Zinno R: Role of oral intake in maintenance of gut mass and disaccharidase activity. Gastroenterology 67:975–982, 1974

35. Weser E, Vandeventer A, Tawil T: Stimulation of small bowel mucosal growth by midgut infusion of different sugars in rats maintained by total parenteral nutrition. J Pediatr Gastroenterol Nutr 1:411–416, 1982

36. Spector MH, Traylor J, Young EA, et al: Stimulation of mucosal growth by gastric and ileal infusion of single amino acids in parenterally nourished rats. Digestion 21:33–40, 1981

37. Weser E, Babbitt J, Hoban M, et al: Intestinal adaptation: Different growth responses to disaccharides compared with monosaccharides in rat small bowel. Gastroenterology 91:1521–1527, 1986

38. Jacobs DO, Evans DA, O'Dwyer ST, et al: Disparate effects of 5-fluorouracil on the ileum and colon of enterally fed rats with protection by dietary glutamine. Surg Forum 38:45–47, 1987

39. Scarpello JHB, Cary BA, Sladen GE: Effects of ileal and cecal resection on the colon of the rat. Clin Sci Molec Med 54:241–249, 1978

40. Bloom SR, Polak JM: The hormonal pattern of intestinal adaptation. Scand J Gastroenterol, 17(Suppl 74):93–103, 1982

41. Bloom SR: Gut and brain—endocrine connections. The Gaulstonian Lecture, 1979. J R Coll Physicians Lond 14:51–57, 1980

42. Miazza BM, Al-Mukhtar MYT, Salmeron M, et al: Hyperenteroglucagonemia and small intestinal mucosal growth after colonic perfusion of glucose in rats. Gut 26:518–524, 1985

43. Hoverstad T: Studies of short chain fatty acid absorption in man. Scand J Gastroent 21:257–60, 1986

44. Dankert J, Zijlstra JB, Wolthers BG: Volatile fatty acids in human peripheral and portal blood: Quantitative determination by vacuum distillation and gas chromatography. Clin Chim Acta 110:301–307, 1981

45. Whitelaw E, Williamson DH: Effects of lactation on ketogenesis from oleate or butyrate in rat hepatocytes. Biochem J 164:521–528, 1977

46. Desmoulin F, Canioni P, Cozzone PJ: Glutamate-glutamine metabolism in the perfused rat liver: 13-C NMR study using 2-113C-enriched acetate. Febs Lett 185:29–32, 1985

47. Cross TA, Pahl C, Oberhansli, R, et al: Ketogenesis in the living rat followed by 13C NMR spectroscopy. Biochemistry, 23:6398–6402, 1984

48. Windmueller HG, Spaeth AE: Identification of ketone bodies and glutamine as the major respiratory fuels *in vivo* for postabsorptive rat small intestine. J Biol Chem 253:69–76, 1978