Differential Modulation of Human (Caco-2) Colon Cancer Cell Line Phenotype by Short Chain Fatty Acids (44261)

MARC D. BASSON,[1] NANCY J. EMENAKER, AND FU HONG

Departments of Surgery, Yale University, New Haven, Connecticut 06520-8062 and the CT VA Health Care System, West Haven, Connecticut 06516

Abstract. **Fermentation of dietary fiber within the colonic lumen yields short chain fatty acids (SCFA) such as butyrate, which may modulate colonic mucosal biology and inhibit the development of a malignant phenotype. However, different fibers yield varying proportions of various SCFA. We studied the effects of the three most common SCFA, acetate, butyrate, and propionate, on the proliferation, adhesion, and motiility of the human intestinal Caco-2 cell line, as well as the effects of these SCFA on alkaline phosphatase and dipeptidyl dipeptidase specific activity (common laboratory markers of differentiation). In addition, we examined the modulation of c-*myc* protein and the tyrosine phosphorylation of cellular proteins by these SCFA in order to determine whether the variations in the potency of these three SCFA for phenotypic change extended to variations in effects on intracellular signaling and protooncogene expression. All three SCFA tended to slow proliferation, promote brush border enzyme activity, and inhibit both adhesion to and motility across a type I collagen matrix substrate. However, we observed substantial differences in the potency of these three SCFA with regard to these effects. In particular, butyrate was uniformly more potent than an equimolar concentration of acetate whereas equimolar propionate achieved comparable effects with regard to proliferation and brush border enzyme activity but was intermediate between butyrate and acetate with regard to modulation of cell-matrix interactions. Similarly, the SCFA downregulated c-*myc* protein levels and modulated the phosphorylation of several intracellular tyrosine phosphoproteins, but the effects of the three SCFA varied substantially for these parameters. These results suggest that the common short chain fatty acids are not equipotent in their effects on human Caco-2 colon cancer cell biology. Such differences in potency could contribute to the observed differences in effects of different dietary fibers *in vivo.***

[P.S.E.B.M. 1998, Vol 217]

Epidemiologic data suggest that increased intake of dietary fiber may inhibit colorectal carcinogenesis. Competing (or complementary) explanations for this effect include direct antineoplastic activity by fiber components, dilutional reduction of luminal carcinogen concentrations, fiber-associated alterations in colonic transit that decrease colonic enterocyte exposure to luminal carcinogens, and production of short chain fatty acids by bacterial fermentation of dietary fiber (1). In particular, the short chain fatty acids have been hypothesized to act directly on colonic mucosal cells to oppose neoplastic transformation (2). However, *in vivo* studies have demonstrated differing effects of various dietary fibers on colonocyte proliferation (3, 4). Furthermore, different dietary fibers appear to be metabolized to yield different ratios of the common short chain fatty acids (1).

Therefore, we hypothesized that each of the short chain fatty acids abundant in the colonic lumen might exert different effects on colonic cells. We sought to test this hypothesis using the human Caco-2 cell line. Originally derived from a well-differentiated adenocarcinoma, the

Supported in part by Donaghue Foundation New Investigator Award #041 and the American Institute for Cancer Research #94A55

[1] To whom requests for reprints should be addressed at Dept. of Surgery, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520-8062. Email basson.marc_d+@west-haven.va.gov

Received May 5, 1997. [P.S.E.B.M. 1998, Vol 217]
Accepted November 23, 1997.

0037-9727/98/2174-0476$10.50/0

Caco-2 cell is almost uniquely well differentiated among established human colonic lines (5–7) and may therefore serve as a useful model in which to study the regulation of colonocyte phenotype and proliferation.

We cultured a differentiated subclone of the Caco-2 cell (8) in media supplemented with equimolar concentrations of the three most common short chain fatty acids (butyrate, acetate, and propionate), and evaluated the effect of these short chain fatty acids on Caco-2 brush border enzyme expression (alkaline phosphatase and dipeptidyl peptidase), adhesion to type I collagen, and cell motility across a type I collagen matrix. In addition, we also evaluated the effects of short chain fatty acid supplementation on c-*myc* protein levels and the tyrosine phosphorylation of cellular proteins by Western blotting in order to determine whether the variations in the potency of these three SCFA for phenotypic change extended to variations in potency with regard to suppression of this proto-oncogene, which has previously been demonstrated to be modulated by butyrate treatment (9–13), and of tyrosine phosphorylation signaling events that may also be important for the pathobiology of colon cancer (14–19).

Methods

Cells. The Caco-2 cells used for these studies represent a clonal subpopulation of this established cell line selected for enterocytic differentiation (8). Cells were maintained at 37°C in 8% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum, 10 μg/ml transferrin (Boehringer Mannheim, Indianapolis, IN), 2 m*M* glutamine, 1 m*M* pyruvate, 10 m*M* HEPES, 100 μ/ml penicillin G and 0.1 mg/ml streptomycin. The medium was supplemented with 10 m*M* butyrate, acetate, or propionate depending on the condition studied. The 10 m*M* concentration represents a physiologically relevant one since alterations in dietary fiber might be expected to alter butyrate or propionate concentrations within the colonic lumen by this order of magnitude.

Digestive Enzyme Activity. The digestive enzyme activity was assessed in confluent Caco-2 monolayers after 24 hr supplementation of the culture medium with 10 m*M* acetate, butyrate, or propionate by spectrophotometric assays using cell lysates and synthetic substrates in conventional manner (20, 21, 22). In brief, cells were lysed in Dulbecco's PBS with 0.5% Triton X-100 and 0.35 *M* NaCl, and cell lysates were assayed in triplicate for protein by BCA technique (Pierce, Rockford, IL). Lysates normalized for equal protein concentrations were then assayed in triplicate for alkaline phosphatase and dipeptidyl peptidase by hydrolysis of p-nitrophenylphosphate and alanine-p-nitroaniline respectively (23). In each case, standard solutions of known concentrations of purified enzymes (Sigma Chemical Co., St. Louis, MO) were assayed simultaneously so that unknowns could be interpolated against the standard curve thus produced.

Motility. The motility was quantitated as previously described (24, 25). Briefly, cells were plated to confluence within stainless steel "fences" on bacteriologic plastic dishes pre-coated with type I collagen. After the cells had achieved confluence the fences were removed, permitting outward migration, and the cells were cultured in control medium or medium supplemented with short-chain fatty acids. After 6 days, cells were fixed *in situ,* and the area of the confining fence was subtracted from the area covered by the cells.

Adhesion. Adhesion was assessed by plating equal numbers of cells pretreated for 24 hr with 10 m*M* short-chain fatty acids onto dishes pre-coated with saturating concentrations of type I collagen. After 1 hr, nonadherent cells were gently washed away with phosphate buffered saline, and adherent cells were fixed with 10% formalin, stained with hematoxylin, and counted by light microscopy. At least 10 random high-power fields were counted per dish studied.

Proliferation. Proliferation was assessed by plating equal numbers of Caco-2 cells onto dishes pre-coated with saturating concentrations of type I collagen. After 12 hr, nonadherent cells were gently washed away with phosphate buffered saline and Time 0 dishes were trypsinized and counted by Coulter Counter ZM (Coulter Corporation, Miami, FL). In the remaining dishes, adherent cells were treated every other day in control media or control media supplemented with 10 m*M* acetate, propionate, or butyrate. Nonadherent cells were gently washed away with phosphate buffered saline, and adherent cells were trypsinized and counted by Coulter Counter on Days 1, 3, 5, and 7.

Dose Response Proliferation. The dose response proliferation was assessed again by plating equal numbers of Caco-2 cells onto dishes pre-coated with saturating concentrations of type I collagen. After 12 hr, nonadherent cells were gently washed away with phosphate buffered saline and Time 0 dishes were trypsinized and counted by Coulter Counter ZM (Coulter Corporation, Miami, FL). Adherent cells were treated every other day in control media or control media supplemented with 1, 3, 7, 10, 30, and 70 m*M* acetate, propionate, or butyrate. After 72 hr, nonadherent cells were gently washed away with phosphate buffered saline, and adherent cells were trypsinized and counted by Coulter Counter.

C-*myc* Protein. The c-*myc* protein was assessed by Western blotting of protein-matched aliquots of cell lysates (50 μg/lane), resolved by 10% SDS-PAGE with a 4% stacking gel, and transferred to Hybond ECL (Amersham, Arlington Hts, IL) by tank transfer using a Hoeffer TE Transphor Electrophoresis Unit (Pharmacia, San Francisco, CA). All samples for Western blotting for c-*myc* had been preliminarily assayed for protein (BCA, Pierce, Rockford, IL) and diluted with buffer to equal protein concentrations prior to loading. Equivalency of loading across the lanes of the gel was also routinely verified by Coomassie blue staining. After blocking overnight in 10 m*M* Tris, 100 m*M* NaCl, and 0.1% Tween-20 containing 5% nonfat milk, the membrane

was blotted with a rabbit affinity-purified polyclonal antibody to human c-*myc* (Upstate Biotechnology, Lake Placid, NY) and a goat anti-rabbit IgG secondary antibody conjugated to horseradish peroxidase (Biorad, Hercules, CA) prior to development by the enhanced chemiluminescence (ECL) technique (Amersham, Arlington Hts, IL) and visualization on Hyperfilm MP (Amersham, Arlington Hts, IL). Images were then quantified densitometrically using a Microtek IIXE scanner, a PC, and SigmaScan/Image software (Jandel Scientific, Anaheim, CA).

Tyrosine Phosphorylation. Tyrosine phosphorylation of intracellular proteins was also assessed by Western blotting. Caco-2 cells were treated 24 hr prior to lysing. Protein-matched aliquots of cell lysates (100 μg/lane) resolved by 7.5% SDS-PAGE with a 4% stacking gel, and transferred to Hybond ECL (Amersham, Arlington Hts, IL) by tank transfer using a Hoeffer TE Transphor Electrophoresis Unit (Pharmacia, San Francisco, CA). After blocking overnight in 10 m*M* Tris, 100 m*M* NaCl, and 0.1% Tween-20 containing 5% nonfat milk, the membrane was blotted with a mouse affinity-purified polyclonal antibody to human phosphotyrosine (PY20, Transduction Laboratories, Lexington, KY) and a sheep anti-mouse anti-IgG secondary antibody conjugated to horseradish peroxidase (Amersham, Arlington Hts, IL) prior to development by the ECL technique (Amersham, Arlington Hts, IL) and visualization on Hyperfilm MP (Amersham, Arlington Hts, IL). Images were then quantified densitometrically using a Microtek IIXE scanner, a PC, and SigmaScan/Image software (Jandel Scientific, Anaheim, CA). All samples for Western blotting for tyrosine phosphoproteins had been preliminarily assayed for protein (BCA, Pierce, Rockford, IL) and diluted with buffer to equal protein concentrations prior to loading. Equivalency of loading across the lanes of the gel was also routinely verified by Coomassie blue staining.

Colonocyte Isolation. Colonocytes were isolated from pathologically confirmed nonmalignant human surgical specimens from patients undergoing resection for benign disease. Briefly, mucosa was stripped from the colon and rinsed in phosphate buffered saline (PBS) until clean. The mucosa was rinsed in PBS supplemented with 0.3 m*M* EDTA, and the resulting colonocytes were rinsed in cell culture media to remove traces of EDTA prior to seeding onto collagen-coated dishes and treatment with either control media or control media supplemented with either 10 m*M* acetate, propionate, or butyrate.

Statistics. Individual results from each experiment were normalized to the mean control value from each experiment. The results were first analyzed by ANOVA with $P < 0.05$ taken as the level of statistical significance, and then relevant comparisons of SCFA treatments to values in control (untreated) cells were performed using a modified Bonferroni's *t* test.

Results

Proliferation. Preliminary studies demonstrated that proliferation was slowed by all three SCFA but with a substantially greater effect for butyrate. For instance, in one study, the calculated length of the cell cycle based on logarithmic transformation of serial cell counts was essentially doubled by 10 m*M* butyrate and propionate with a substantially lesser effect by 10 m*M* acetate. ($n = 20$ from one of three similar studies, $P < 0.001$, data not shown). Figure 1 demonstrates that this effect became statistically significant at 1 m*M* propionate or butyrate and 10 m*M* acetate treatments ($n = 10$, $P < 0.005$). Figure 2 demonstrates that the differences in SCFA effects on proliferation were sustained over a 7-day period. The 10 m*M* butyrate significantly inhibited Caco-2 cell growth after 1 day whereas similar effects were not observed in

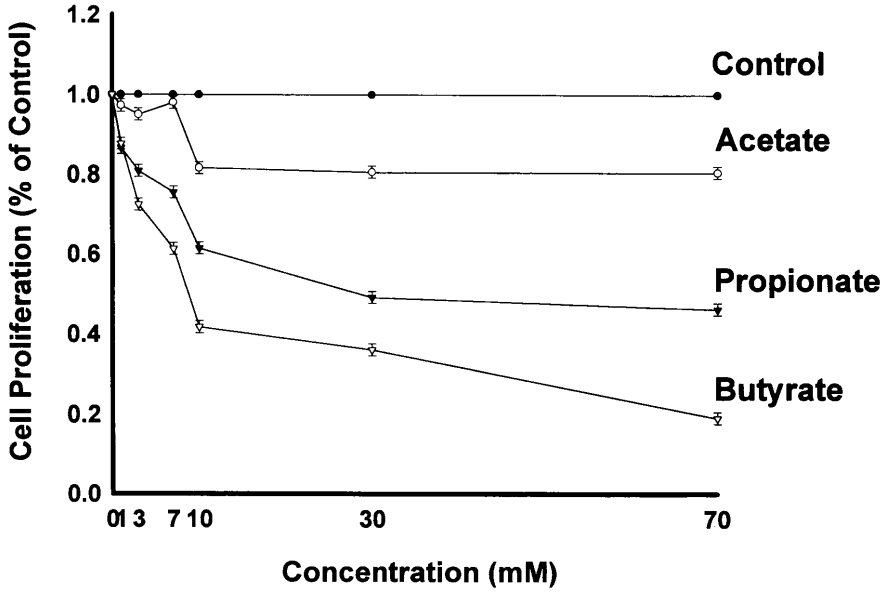

Figure 1. Effect on Caco-2 cell proliferation of 24-hr treatment with 0—70 m*M* short chain fatty acids acetate, propionate, and butyrate normalized to control untreated cells. (Mean ± SE, $n = 10$, $^*P < 0.005$)

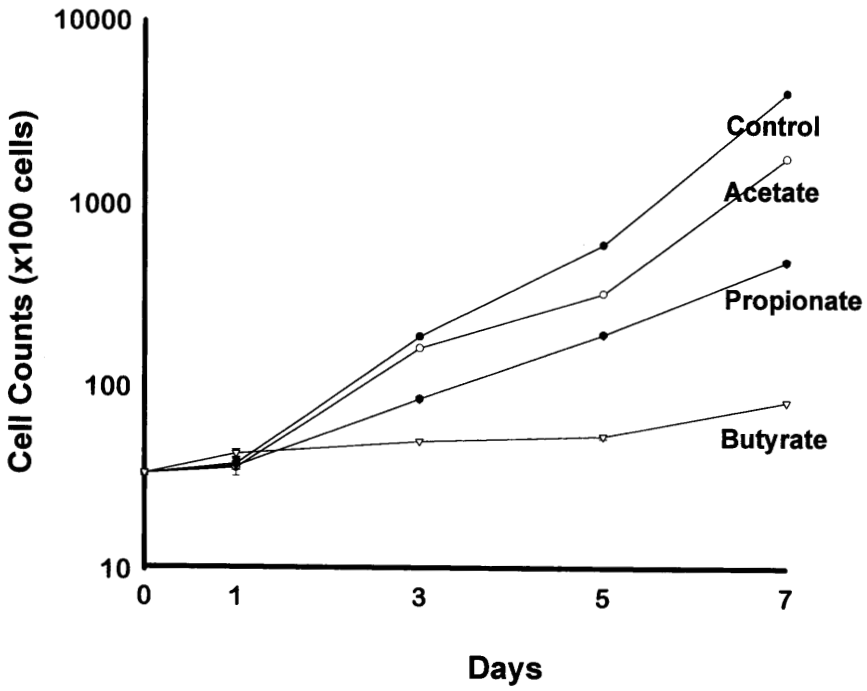

Figure 2. Effect on Caco-2 cell growth of 0–7-day treatment with 10 m*M* short chain fatty acids acetate, propionate, and butyrate as compared to control untreated cells. (Mean ± SE, $n = 10$, $^*P < 0.05$)

acetate and propionate treated cells until Day 3 ($n = 10$, $P < 0.05$).

Cell Motility. The cell motility was also altered by treatment with short-chain fatty acids. Caco-2 migration across a type I collagen matrix was inhibited by each of the three short-chain fatty acids studied, but once again differences between the effects of the three agents studied were observed. Caco-2 monolayer expansion was reduced to 49.6 ± 0.3% of control values by 10 m*M* butyrate whereas inhibition by propionate was less pronounced and that by acetate even less substantial (Table I, $n = 12$ pooled from three similar studies, $P < 0.001$ for each). Cell adhesion was affected in parallel to cell motility (Table I). For instance, in one study, 10 m*M* butyrate reduced the number of adherent cells/high-power field from 56.9 ± 0.6 to 2.6 ± 0.2 ($P < 0.001$ from one of five similar studies) whereas the comparable values for propionate and acetate were 18.1 ± 0.4 and 27.3 ± 0.5 respectively ($P < 0.001$ from one of five similar studies).

Western Blotting. Western blotting for myc also demonstrated differences in SCFA potency. Western blotting of Caco-2 lysates with antibody to c-*myc* revealed two reproducible bands. The higher had an approximate apparent molecular weight of 60 kD, and the lower exhibited an approximate apparent molecular weight of 49 kD. These bands correspond in apparent molecular weight to previously described c-*myc* 1 and c-*myc* 2 proteins (Ref. 26, and personal communication Liisa Eisenlohr, UBI, Lake Placid, NY). All three SCFA downregulated c-*myc* 1 protein levels. However, marked differences in potency were observed between the three SCFA studied, with butyrate the most potent (decreasing band intensity by 39 ± 4%) and acetate the least potent (decreasing band intensity by 28 ± 3%) ($n = 10$, $P < 0.001$ for all, Fig. 3 and Table I); c-*myc* 2 was also significantly downregulated by butyrate and propionate, but not by acetate (Fig. 3 and Table I).

Tyrosine Phosphorylation. Tyrosine phosphorylation of intracellular proteins was also affected by the three SCFA studied. Numerous tyrosine phosphoprotein bands could be identified upon SDS-PAGE resolution of Caco-2 cell lysates and Western blotting, with different bands being of markedly different intensities. Upon densitometric analysis of several exposures of each blot, chosen to achieve an appropriate densitometric range for each relevant band, it

Table I. Effects of Short Chain Fatty Acids on Caco-2 Phenotype, Proliferation, and c-*myc* Protein Levels

Assay	Control	Acetate	Propionate	Butyrate
Alk Phos (U/ng/protein)	14.3 ± 1.1	48.3 ± 2.5**	359 ± 7.3**	321 ± 11**
DPDD (U/ng/protein)	1.00 ± .09	5.78 ± 0.11**	6.97 ± 0.10**	8.53 ± 0.18**
Motility (mm^2/day)	144.8 ± 3.3	103.5 ± 1.7**	127.5 ± 0.2**	71.8 ± 0.4**
Adhesion (cells/HPF)	56.9 ± 6.6	27.3 ± 0.5**	18.1 ± 0.4**	2.6 ± .02**
Doubling time (hr)	23.7 ± 0.7	34.9 ± 1.0**	44.1 ± 1.0**	46.2 ± 1.1**
c-*myc* 1 band intensity (tx/control)	1.00	0.72 ± 0.003**	0.61 ± 0.004**	0.51 ± 0.004**
c-*myc* 2 band intensity (tx/control)	1.00	0.93 ± 0.057	0.55 ± 0.045**	0.47 ± 0.053**

Note. Mean ± SE, ANOVA $^{**}P < 0.005$ compared to control

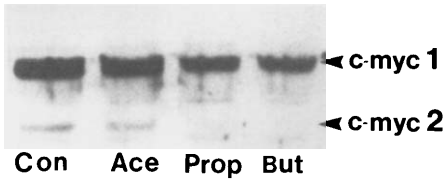

Figure 3. Effect on Caco-2 c-*myc* 1 and c-*myc* 2 protein levels of 24-hr treatment with 10 m*M* short chain fatty acids acetate (Ace), propionate (Prop), and butyrate (But) as compared to control (Con) untreated cells. Protein-matched lysates from untreated and treated cells were resolved by SDS-PAGE and Western blotted for c-*myc*. Results shown represent a typical blot from 1 of 10 separate studies. The upper (dominant) band of molecular weight 60 kD represents c-*myc* 1 whereas the lower molecular weight (49 kD) fainter band represents c-*myc* 2.

became apparent that not only were substantially different effects observed between the three SCFA studied, but also the tyrosine phosphorylation of the various bands visualized by Western blotting varied significantly in differential susceptibility to the SCFA (Table II).

In preliminary observations, we have recently observed effects of 24-hr short-chain fatty acid treatment on alkaline phosphatase expression in primary human colonocytes isolated from three separate nonmalignant surgical specimens (Fig. 4) similar to those described above in Caco-2 cells.

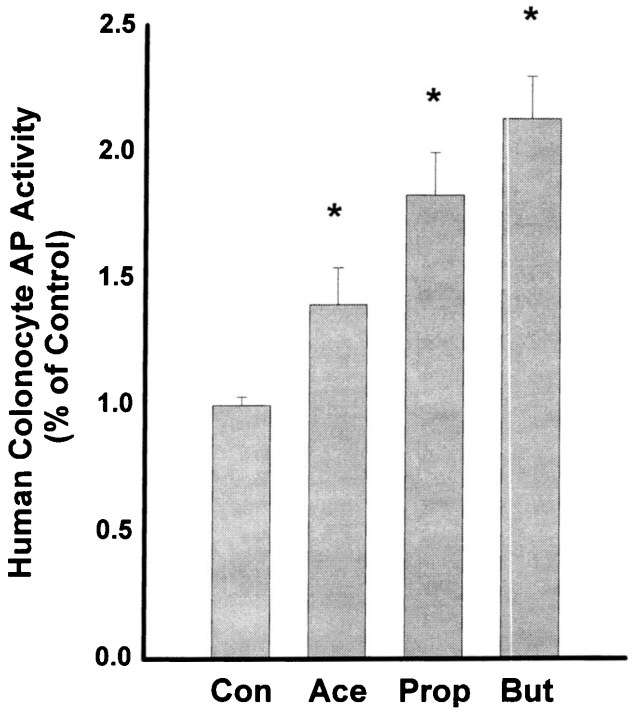

Figure 4. Effect on primary human colonocytes isolated from three separate surgical specimens treated for 24 hr with either 10 m*M* short chain fatty acids acetate (Ace), propionate (Prop), or butyrate (But) normalized to control (Con) untreated primary human colonocytes. (Mean ± SE, $n = 3$, $^*P < 0.05$)

Discussion

Short-chain fatty acid concentrations within the colonic lumen exceed those at any other physiologic site in humans. In addition to acting as a metabolic fuel for the mucosa, these fermentation products of dietary fiber have been implicated in the regulation of colonic epithelial proliferation and phenotype and the prevention of malignant transformation to colon cancer. Recently, this subject has been reviewed extensively (27). Observations such as those described here and previously by others (28–36) suggest that butyrate may be sufficient to promote the differentiation and inhibit the proliferation of some colonic neoplastic cells, and observations are consistent with the hypothesis that butyrate induces colonic epithelial cells to differentiate toward an absorptive phenotype and away from a rapidly reproducing, motile, and poorly differentiated phenotype resembling colonic malignancy. We present here data in a Caco-2 cell culture model to demonstrate that different short-chain fatty acids may have radically different potencies in this regard.

The Caco-2 cell line is an established human colon carcinoma cell line that differentiates spontaneously and reproducibly during culture. Indeed, the Caco-2 cell is almost uniquely differentiated among extant human intestinal epithelial cell lines (5–7). These cells express electrical and morphological tight junctions and a highly specialized brush border with regulatable brush border enzymes. Peterson and

Table II. Effects of short chain fatty acids on intracellular Caco-2 tyrosine phosphoprotein band intensity

Band	~Mr (kD)	Control	Acetate	Propionate	Butyrate
1	181	1.00 ± .10	0.77 ± .05**	0.78 ± .10*	0.80 ± .05**
2	170	1.00 ± .17	0.93 ± .11	0.83 ± .21	0.91 ± .11
3	150	1.00 ± .09	0.81 ± .04**	0.91 ± .05*	0.90 ± .06
4	130	1.00 ± .11	1.02 ± .06	0.92 ± .05	079 ± .09**
5	120	1.00 ± .12	0.95 ± .04	0.97 ± .05	0.79 ± .05**
6	111	1.00 ± .13	0.92 ± .04	0.86 ± .07	0.89 ± .06
7	90	1.00 ± .12	0.85 ± .09	1.11 ± .06	0.86 ± .07*
8	72	1.00 ± .07	0.75 ± .05**	.081 ± .07*	.075 ± .05**
9	60	1.00 ± .12	0.91 ± .05*	1.24 ± .09*	0.99 ± .09
10	52	1.00 ± .10	1.06 ± .05	1.11 ± .06*	1.07 ± .07
11	47	1.00 ± .09	0.79 ± .05**	0.79 ± .05**	0.67 ± .07**

Note. Effect on the tyrosine phosphorylation of intracellular Caco-2 proteins of 24-hr treatment with 10 m*M* short chain fatty acids acetate, propionate, and butyrate as compared to control untreated cells. *Protein-matched lysates from untreated and treated cells were resolved by SDS-PAGE and Western blotted for phosphotyrosine. Results shown represent normalized and pooled data from densitometric analysis of seven separate studies. (Mean ± SE, ANOVA $^*P < 0.05$, $^{**}P < 0.005$ compared to control)

Mooseker have subcloned from the parent Caco-2 line a cell line that exhibits an even more differentiated brush border (8), suggesting that this subclone may be useful to model the regulation of colonocytic differentiation. We have previously characterized the differentiation and motility of this subclone and demonstrated that it may serve as a model for the regulation of intestinal epithelial differentiation and motility by extracellular matrix proteins, soluble bioactive peptides, luminal nutrients, and growth factors (25, 27, 37–40). The concentrations of short-chain fatty acids used in this study are of a similar order of magnitude to and actually lower than those observed within the colonic lumen. Substantially higher concentrations inhibited the adhesion of the cells to their substrate sufficiently that anchorage-dependent growth could not be maintained in culture (data not shown).

Although the Caco-2 cell line was derived from a human adenocarcinoma, the Caco-2 cell is uniquely well-differentiated and is extensively utilized as an *in vitro* model of normal intestinal epithelial biology (41–48). Indeed, experiments in nonmalignant primary human colonocytes reveal short-chain fatty acid effects on alkaline phosphatase similar to those we observed in Caco-2 cells, although these data must clearly be interpreted cautiously and require further investigation.

The 10 m*M* short-chain fatty acid concentration represents a physiologically relevant one since alterations in dietary fiber might be expected to alter butyrate or propionate concentrations within the colonic lumen by this order of magnitude. Although we have shown that short-chain fatty acids inhibit cell proliferation at concentrations ranging from 1–7 m*M* (Fig. 1), stabilization of these inhibitory effects do not occur until the 10 m*M* concentration and remain unaltered as concentrations of acetate and propionate reach 70 m*M*. The effects on butyrate are similar from 10–30 m*M*, but decline again at 70 m*M*, a physiologic concentration rarely achieved by those consuming a "typical" Western diet.

The present study suggests that short-chain fatty acids inhibit Caco-2 motility, adhesion, and proliferation while stimulating the specific activity of the brush border enzymes alkaline phosphatase and dipeptidylpeptidase, which serve as differentiation markers in this cell line (5). These results are consistent with previous observations of the effects of sodium butyrate on colonocyte cell line phenotype and proliferation (9–13). However, there appear to be marked differences in potency among equimolar concentrations of the three most common short-chain fatty acids (butyrate, acetate, and propionate). In particular, although there are differences in relative potency for each of the parameters studied, butyrate appears more potent than equimolar acetate for all parameters studied, and propionate appears generally intermediate in potency. These results contrast with previous studies in the LIM1215 cell line (49) in which propionate was not effective, but are consistent with other observations of the effects of propionate on colorectal cell-line apoptosis (50) and T84 cell-line growth factor modulation of paracellular permeability (51).

The differences in the effects of the three short-chain fatty acids on phenotype and proliferation were also reflected in differences in the effects of these agents on protein levels of c-*myc*, a proto-oncogene involved in the regulation of cell differentiation and proliferation. Butyrate has previously been reported to downregulate c-*myc* expression in several human colon cancer cell lines (9–13), although it remains unclear whether this block occurs by potentiating a transcriptional elongation block and a shift in relative usage of the two major promoters of the c-*myc* gene (11) or at the post-transcriptional level (13). This study confirmed the downregulation of c-*myc* by butyrate, but further demonstrated that propionate and acetate were proportionately less potent in this regard. This investigation also demonstrated that butyrate and propionate each downregulate c-*myc* 2 protein levels; c-*myc* 2 is a second *myc*-related protein of approximate molecular weight 49 kD and is known to cross-react with the c-*myc* antibody (26) although its function is as yet unclear.

Alterations in tyrosine kinase activity or the tyrosine phosphorylation state of a variety of potential oncoproteins are known to occur in colon cancer and premalignant colonic lesions (13–17) whereas butyrate is known to alter intracellular tyrosine kinase activity in human colon cancer cell lines (18). Once again, the tyrosine phosphorylation of several intracellular proteins was also observed to be differentially modulated by the three SCFA studied in these experiments in a complex pattern suggesting that various tyrosine phosphoproteins are likely to be differently sensitive to the three SCFA.

The magnitude of some of these tyrosine phosphoprotein changes on densitometry are small, and not all may be biologically significant. This is particularly true since the fact that a given protein is more or less tyrosine phosphorylated does not necessarily mean that there is a biologically significant difference in its function. Nevertheless, many tyrosine phosphoproteins are integral parts of amplifying signal transduction cascades so a relatively small change might be further amplified by downstream events into an effect of biological significance. Elucidation of the biological significance of these changes must await identification of the phosphoproteins themselves, which is a subject for further study, but certainly the present experimental data further suggest that different SCFA may have different potencies on intracellular events as well as cellular phenotype.

The pharmacologic modulation of tyrosine phosphorylation using the nonspecific tyrosine kinase inhibitor genistein and the tyrosine phosphatase inhibitor orthovanadate has also been reported to correlate inversely with c-*myc* expression *via* postranscriptional effects in two colon cancer cell lines (19), but whether this association is causal or epiphenomenal has yet to be established.

The mechanism for the differences in potency between butyrate, propionate, and acetate with regard to cell pheno-

type, proliferation, and intracellular characteristics also awaits further investigation. Although butyrate is more readily metabolized than either acetate or propionate (52), the differences in potency between these short-chain fatty acids do not completely parallel their differences in metabolism. Thus, other factors may also be involved, which await further clarification of the different effects of these compounds at the molecular level. In particular, butyrate has been demonstrated to inhibit histone deacetylase and thus increase histone acetylation states more potently than propionate, whereas acetate has little effect (53–56).

However, the present data are consistent with previous observations that butyrate is more potent than other short-chain fatty acids with regard to proliferation (57), and suggest the potential complexity of the responses to dietary fiber *in vivo,* which may be induced by the short-chain fatty acid products of fiber fermentation. These results may have clinical significance as well as biologic interest since the fermentation of various dietary fibers by colonic bacteria yields varying proportions of the most common short-chain fatty acids (1). Although these results await further investigation and confirmation in primary tissues and *in vivo,* such observations suggest the possibility that dietary supplementation with equal amounts of various dietary fibers may have different effects. It is possible that clarification of which effect(s) of these short-chain fatty acids are clinically relevant may be important for determining which types of dietary fibers may be most protective against colonocyte neoplastic transformation.

1. Fleming SE, Fitch MD, Chansler MW. High-fiber diets: Influence on characteristics of cecal digesta including short-chain fatty acid concentrations and pH. Am J Clin Nutr **50:**93–99, 1989.
2. Dexter DL, Lev R, McKendall GR, Mitchell P, Calabresi P. Sodium butyrate-induced alteration of growth properties and glycogen levels in cultured human colon carcinoma cells. Histochem J **16:**137–149, 1984.
3. Boffa LC, Lupton JR, Mariani MR, Ceppi M, Newmark HL, Scalmati A, Lipkin M. Modulation of colonic epithelial cell proliferation, histone acetylation, and luminal short-chain fatty acids by variation of dietary fiber (wheat bran) in rats. Cancer Res **52:**5906–5912, 1992.
4. Young GP, McIntyre A, Albert V, Folino M, Muir JG, Gibson PR. Wheat brain suppresses potato starch–potentiated colorectal tumorigenesis at the aberrant crypt state in a rat model. Gastroenterology, **110:**508–514, 1996.
5. Jumarie C, Malo C. Caco-2 cells cultured in a serum-free medium as a model for the study of enterocytic differentiation *in vitro.* J Cell Phys **149:**24–33, 1992.
6. Chantret I, Barbat A, Dussaulx E, Brattain MG, Zweibaum A. Epithelial polarity, villin expression, and enterocytic differentiation of cultured human colon carcinoma cells: A survey of twenty cell lines. Cancer Res **48:**1936–1942, 1988.
7. Louvard D, Kedinger M, Hauri HP. The differentiating intestinal epithelial cell: Establishment and maintenance of functions through interactions between cellular structures. Ann Rev Cell Biol **8:**157–195, 1992.
8. Peterson MD, Mooseker MS. Characterization of the enterocyte-like brush border cytoskeleton of the $C2_{BBe}$ clones of clones of the human intestinal cell line, Caco-2. J Cell Sci **102:**581–600, 1992.
9. Schroy PC, Rustgi AK, Ikonomu E, Liu XP, Polito J, Andry C, O'Keane JC. Growth and intestinal differentiation are independently regulated in HT29 colon cancer cells. J Cell Physiol **161:**111–123, 1994.
10. Itoh F, Ikuta S, Hinoda Y, Arimura Y, Ohe M, Adachi M, Ariyanma T, Inazawa J, Imai K, Yachi A. Expression and chromosomal assignment of PTPH1 gene encoding a cytosolic protein tyrosine phosphatase homologous to cytoskeletal-associated proteins. Int J Cancer **55:**947–951, 1993.
11. Heruth DP, Zirnstein GW, Bradley JF, Rothberg PG. Sodium butyrate causes an increase in the block to transcriptional elongation in the c-*myc* gene in SW837 rectal carcinoma cells. J Biol Chem **268:**20466–20472, 1993.
12. Barnard JA, Warwick G. Butyrate rapidly induces growth inhibition and differentiation in HT-29 cells. Cell Growth Differ **4:**495–501, 1993.
13. Souleiimani A, Asselin C. Regulation of c-*myc* expression by sodium butyrate in the colon carcinoma cell line Caco-2. FEBS Lett **326**(1–3): 45–50, 1993.
14. Craven RJ, Xu LH, Weiner TM, Fridell YW, Dent GA, Srivastava S, Varnum B, Liu ET, Cance WG. Receptor tyrosine kinases expressed in metastatic colon cancer. Int J Cancer **60:**791–797, 1995.
15. Park J, Meisler AI, Cartwright CA. c-*Yes* tyrosine kinase activity in human colon carcinoma. Oncogene **8:**2627–2635, 1993.
16. Pena SV, Melhem MF, Meisler AI, Cartwright CA. Elevated c-*yes* tyrosine kinase activity in premalignant lesions of the colon. Gastroenterology **108:**117–124, 1995.
17. Talamonti MS, Roh MS, Curley SA, Gallick GE. Increase in activity and level of pp60c-src in progressive stages of human colorectal cancer. J Clin Invest **91:**53–60, 1993.
18. Schwartz B, Lamprecht SA, Polak-Charcon S, Niv Y, Kim YS. Induction of the differentiated phenotype in human colon cancer cells is associated with the attenuation of subcellular tyrosine phosphorylation. Oncol Res **7:**277–287, 1995.
19. Heruth DP, Wetmore LA, Leyva A, Rothberg PG. Influence of protein tyrosine phosphorylation on the expression of the c-*myc* oncogene in cancer of the large bowel. J Cell Biochem **58:**83–94, 1995.
20. Basson MD, Modlin IM, Turowski G, Madri JA. Enterocyte-matrix interactions in the healing of mucosal injury. Eur J Gastroent **93**(5 Suppl 3):F21–F28, 1993.
21. Dahqlvist A. Assay of intestinal disaccharidases. Analyt Biochem **22:**99–107, 1968.
22. Nagatsu T, Hino M, Fuyamada H, Hayakawa T, Sakakibara S. New chromogenic substrates for *N*-propyl dipeptidyl-aminopeptidase. Anal Biochem **74:**466–476, 1976.
23. Turowski GA, Rashid Z, Hong F, Madri JA, Basson MD. Glutamine modulates phenotype and stimulates proliferation in human colon cancer cell lines. Cancer Res **54:**5974–5980, 1994.
24. Yannariello-Brown J, Wewer U, Liotta L, Madri JA. Distribution of a 69-kD laminin binding protein in aortic and microvascular endothelial cells: Modulation during cell attachment, spreading, and migration. J Cell Biol **106:**1773–1786, 1988.
25. Basson MD, Modlin IM, Madri JA. Human enterocyte (Caco-2) migration is modulated *in vitro* by extracellular matrix composition and epidermal growth factor. J Clin Invest **90:**15–23, 1992.
26. Hann SR, Abrams HD, Rohrschneider LR, Eisenman RN. Proteins encoded by v-*myc* and c-*myc* oncogenes: Identification and localization in acute leukemia virus transformants and bursal lymphoma cell lines. Cell **34:**789–798, 1983.
27. Binder HJ, Cummings J, Soergel KH, Eds. Short-chain fatty acids. Falk Symposium 73. Dordrecht: Kluwer Academic Press, 1993.
28. Dexter DL, Lev R, McKendall GR, Mitchell P, Calabresi P. Sodium butyrate-induced alteration of growth properties and glycogen levels in cultured human colon carcinoma cells. Histochem J **16:**137–149, 1984.
29. Young GP, Gibson PR. Butyrate and the colorectal cancer cell. In: Binder HJ, Cummings J, Soergel KH, Eds. Short Chain Fatty Acids,

Falk Symposium 73. Dordrecht: Kluwer Academic Press, pp 148–160, 1993.
30. Kim YS, Tsao D, Siddiqui B, Whitehead JS, Arnstein P, Bennett J, Hicks J. Effects of sodium butyrate and dimethylsulfoxide on biomedical properties of human colon cancer cells. Cancer **45**(5 Suppl):1185–1192, 1980.
31. Morita A, Tsao D, Kim YS. Effect of sodium butyrate on alkaline phospatase in HRT-18, a human rectal cancer cell line. Cancer Res **42:**4540–4545, 1982.
32. Gum JR, Kam WK, Byrd JC, Hicks JW, Sleisenger MH, Kim YS. Effects of sodium butyrate on human colonic adenocarcinoma cells. Induction of placental-like akaline phosphatase. J Biol Chem **262:**1092–1097, 1987.
33. Ho SB, Yan PS, Dahiya R, Neuschwander-Tetri BA, Basbaum C, Kim YS. Stable differentiation of a human colon adenocarcinoma cell line by sodium butyrate is associated with multidrug resistance. J Cell Physiol **160:**213–226, 1994.
34. Tsao D, Shi ZR, Wong A, Kim YS. Effect of sodium butyrate on carcinoembryonic antigen production by human colonic adenocarcinoma cells in culture. Cancer Res **43:**1217–1222, 1983.
35. Boland CR, Montgomery CK, Kim YS. Alterations in human colonic mucin occurring with cellular differentiation and malignant transformation. Proc Natl Acad Sci USA **79:**2051–2055, 1992.
36. Gamet L, Daviaud D, Denis-Pouxviel C, Remesy C, Murat JC. Effects of short-chain fatty acids on growth and differentiation of the human colon-cancer cell line HT-29. Int J Cancer **52:**286–289, 1992.
37. Basson MD, Modlin IM, Flynn SD, Jena BP, Madri JA. Independent modulation of enterocyte migration and proliferation by growth factors, matrix proteins, and pharmacologic agents in an *in vitro* model of mucosal healing. Surgery **112:**299–308, 1992.
38. Basson MD, Beidler DR, Turowski G, Modlin IM, Jena BP, Madri JA. The effects of tyrosine kinase inhibition on basal and EGF-stimulated Caco-2 enterocyte sheet migration and proliferation. J Cell Phys **160:**491–501, 1994.
39. Basson MD, Turowski G, Emenaker NJ. Regulation of human (Caco-2) differentiation by extracellular matrix proteins. Exp Cell Res **225:**301–305, 1996.
40. Sgambati S, Zarif A, Basson MD. Octreotide differentially modulates human Caco-2 intestinal epithelial cell proliferation and differentiation by decreasing intracellular cAMP. Regul Pept **61:**219–227, 1996.
41. Basson MD, Rashid Z, Turowski GA, West AB, Emenaker NJ, Sgambati SA, Hong F, Perdikis DM, Datta S, Madri JA. Restitution at the cellular level: Regulation of the migrating phenotype. Yale J Biol Med **69:**119–129, 1996.
42. Lawrence JP, Brevetti L, Obiso RJ, Wilkins TD, Kimura K, Soper R. Effects of epidermal growth factor and *Clostridium difficile* toxin B in a model of mucosal injury. J Pediatr Surg **32:**430–433, 1997.
43. Mathur SN, Born E, Murthy S, Field FJ. Microsomal triglyceride transfer protein in Caco-2 cells: Characterization and regulation. J Lipid Res **38:**61–67, 1997.
44. Werner U, Kissel T, Stuber W. Effects of peptide structure on transport properties of seven thyrotropin releasing hormone (TRH) analogues in a human intestinal cell line (Caco-2). Pharm Res **14:**246–250, 1997.
45. Kagedahl M, Swaan PW, Redemann CT, Tang M, Craik CS, Szoka FC Jr., Oie S. Use of the intestinal bile acid transporter for the uptake of cholic acid conjugates with HIV-1 protease inhibitory activity. Pharm Res **14:**176–180, 1997.
46. Okumu FW, Pauletti GM, Vander Velde DG, Siahaan TJ, Borchardt RT. Effect of restricted conformational flexibility on the permeation of model hexapeptides across Caco-2 cell monolayers. Pharm Res **14:**169–175, 1997.
47. Pauletti GM, Okumu FW, Borchardt RT. Effect of size and charge on the passive diffusion of peptides across Caco-2 cell monolayers *via* the paracellular pathway. Pharm Res **14:**164–168, 1997.
48. Mackay K, Starr JR, Lawn RM, Ellsworth JL. Phosphatidylcholine hydrolysis is required for pancreatic cholesterol esterase- and phospholipase A2-facilitated cholesterol uptake into intestinal Caco-2 cells. J Biol Chem **272:**13380–13389, 1997.
49. Whitehead RH, Young GP, Bhathal PS. Effects of short-chain fatty acids on a new human colon carcinoma cell line (LIM1215). Gut **27:**1457–1463, 1986.
50. Hague A, Elder DJ, Hicks DJ, Paraskeva C. Apoptosis in colorectal tumor cells: Induction by the short-chain fatty acids butyrate, propionate, and acetate and by the bile salt deoxycholate. Int J Cancer **60:**400–406, 1995.
51. McRoberts JA, Riley NE. Modulation of growth factor and cytoskine-induced increases in T84 cell monolayer permeability by media components. Am J Physiol **267**(2 Pt 1):C537–543, 1994.
52. Chu S, Montrose MH. Extracellular pH regulation in microdomains of colonic crypts: Effects of short-chain fatty acids. Proc Nat Acad Sci **92:**3303–3307, 1995.
53. Huang N, Katz JP, Martin DR, Wu GD. Inhibition of IL-8 gene expression in Caco-2 cells by compounds which induce histone hyperacetylation. Cytokine **9:**27–36, 1997.
54. Staecker JL, Sawada N, Pitot HC. Stimulation of DNA synthesis in primary cultures of adult rat hepatocytes by sodium butyrate. Biochem Biophys Res Comm **147:**78–85, 1987.
55. Stanley F, Samuels HH. *n*-Butyrate affects thyroid hormone stimulation of prolactin production and mRNA levels in GH1 cells. J Biol Chem **259:**9768–9775, 1984.
56. McKnight GS, Hager L, Palmiter RD. Butyrate and related inhibitors of histone deacetylation block the induction of egg white genes by steroid hormones. Cell **22**(2 Pt 2):469–477, 1980.
57. Cousens LS, Gallwitz D, Alberts BM. Different accessibilities in chromatin to histone acetylase. J Biol Chem **254:**1716–1723, 1979.