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REVIEW ARTICLE

Butyrate and the Colonocyte
Implications for Neoplasia

OMAIDA C. VELÁZQUEZ, MD, HOWARD M. LEDERER, MD, and JOHN L. ROMBEAU, MD

Butyrate is produced in the colon of mammals as a result of microbial fermentation of dietary fiber, undigested starch, and proteins. Butyrate may be an important protective agent in colonic carcinogenesis. Trophic effects on normal colonocytes *in vitro* and *in vivo* are induced by butyrate. In contrast, butyrate arrests the growth of neoplastic colonocytes and inhibits the preneoplastic hyperproliferation induced by some tumor promoters *in vitro*. We speculate that selective effects on G-protein activation may explain this paradox of butyrate's effects in normal versus neoplastic colonocytes. Butyrate induces differentiation of colon cancer cell lines. It also regulates the expression of molecules involved in colonocyte growth and adhesion and inhibits the expression of several protooncogenes relevant to colorectal carcinogenesis. Additional studies are needed to evaluate butyrate's antineoplastic effects *in vivo* and to understand its mechanism(s) of action.

KEY WORDS: butyrate; colonocytes; cancer; proliferation; differentiation; oncogenes.

Butyrate is a metabolic by-product of dietary fiber. Numerous studies have shown an inverse correlation between ingestion of a high fiber diet and the risk of colon cancer, thus leading many investigators to hypothesize an antineoplastic role for butyrate. This report reviews the effects of butyrate on normal and neoplastic colonocytes at the level of proliferation, differentiation, and gene expression. The known and hypothesized molecular events behind the mechanism of action of butyrate are detailed. The available data are examined in relation to their implications for understanding, preventing, and treating colon cancer.

Epidemiologic and animal studies suggest that dietary fat and protein may promote carcinogenesis in the colon, whereas increased fiber and complex carbohydrates in the diet may protect against colon cancer (1–6). Human case–control studies support this association (7). Intake of fiber-rich foods is inversely related to risk of cancers of both colon and rectum (7). Colonic luminal butyrate concentrations are postulated to be a key protective component of high-fiber diets against colon cancer (5, 6).

Butyrate is one of the short-chain fatty acids (SCFA) that are the C2-5 organic fatty acids. These compounds are formed in the gastrointestinal tract of mammals as a result of anaerobic bacterial fermentation of undigested dietary components (8–10) and are avidly absorbed by the colonic epithelium (11), with butyrate being a preferred oxidative fuel (12). Dietary fiber is the principal substrate for the fermentation of SCFA in humans (13); however, undigested starch and protein also contribute to their production (14). In the mammalian hindgut, acetate, propionate, and butyrate account for 83% of SCFA with a total concentration of approximately 100 mmol/liter (15–17) present in a nearly constant molar ratio of 60:25:15 (10). Of the three major SCFA, butyrate has the most profound effect on colonocyte growth and differentiation. Propionate has similar (sometimes less marked) effects as butyrate in colonocyte growth and

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From the Department Harrison Department of Surgical Research, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania; and Department of Surgery, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania.

Address for reprint requests: Dr. John L. Rombeau, Department of Surgery, 4 Silverstein Pavilion, 3400 Spruce Street, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania 19104.

TABLE 1. PARADOXICAL EFFECTS OF BUTYRATE ON CELL PROLIFERATION AND DIFFERENTIATION OF NORMAL AND NEOPLASTIC COLONIC EPITHELIAL CELLS*

	Effect of butyrate			
	Normal cells		*Cancer cells*	
	In vitro	In vivo	In vitro	In vivo
Cell proliferation	No change/?increased	Increased	Reduced	Probably reduced
Differentiation	Suppressed	No change	Induced	Unknown

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differentiation, but acetate does not exert these effects.

The total concentration and relative molar concentrations of individual SCFA are greatly influenced by dietary carbohydrates, protein, and types of fiber fermented (8–10, 18–21). Therefore, it may be possible to manipulate different dietary substrates to achieve desired amounts and ratios of SCFA, particularly with respect to butyrate, in order to influence the incidence of colonic disease.

BUTYRATE EFFECTS ON PROLIFERATION: NORMAL VERSUS NEOPLASTIC COLONOCYTES

The dividing cells within the normal colonic mucosa are located in the mid- to lower portion of the crypt (22). The products of these dividing cells then migrate towards the surface of the crypt and mature or fully differentiate. The process of carcinogenesis disrupts this orderly migration such that cells continue to proliferate as they migrate towards the crypt surface, failing to show characteristics of normal differentiation and eventually invading the basement membrane (23–25).

While butyrate decreases proliferation of neoplastic colonocytes *in vitro* (26–31) and *in vivo* (32, 33), it increases proliferation of normal colonic epithelium *in vitro* (34) and *in vivo* (35–37) (Table 1).

Trophic Effects on Normal Colonic Epithelium

Butyrate plays an important role in colonic mucosal growth and epithelial proliferation. A reduction in concentration of luminal butyrate by decreased delivery of fermentable substrate to the large intestine induces colonic mucosal atrophy (36, 38). Instillation of SCFA into the colonic lumen induces mucosal regeneration as shown by increased weight, DNA content, and crypt length (37). Of the three major SCFA, these effects on colonic mucosal proliferation are thought to be mostly due to butyrate (36, 37). In an *in vivo* rat model, both intravenous and intracolonic infusions of SCFA significantly reduced the mucosal atrophy associated with long-term total parenteral nutrition (TPN) (39). Another *in vivo* study using rats maintained on TPN showed that infusion of a SCFA mixture (60:25:15; acetate–propionate–butyrate) into the proximal colon significantly increased colonic mucosal height and DNA (40). A diet rich in wheat bran fed to rats significantly increased the concentration of butyrate throughout the colon (41). Of the SCFA examined, the colonic luminal concentration of butyrate had the strongest positive correlation to indices of cell proliferation. An *in vitro* study using human colonic mucosa demonstrated that propionate and more significantly, butyrate (10 mmol/liter), are trophic factors for the human cecal epithelium (42).

The mechanisms by which butyrate enhances normal colonic mucosal proliferation are not well understood, but they appear to be indirectly and directly mediated. Butyrate exerts indirect systemic trophic effects as noted when colonic instillation of SCFA stimulates proliferation not only in colonic mucosa, but also in unexposed, adjacent colonic epithelium, ileum and jejunum (36, 37, 43, 44). Moreover, infusion of SCFA into the colonic lumen stimulates growth in isolated, denervated loops of jejunum in rats (45). However, butyrate also directly affects proliferation because it stimulates epithelial proliferation in short-term organ culture of human colonic mucosa in the absence of circulating or neural factors (42).

Inhibitory Effects on Neoplastic Colonocyte Growth

Butyrate inhibits DNA synthesis and arrests the growth of neoplastic colonocytes in the G_1 phase of the cell cycle (46). These effects occur in multiple cultured tumor cell lines (47). The concentrations used to produce these effects are not toxic to the cells. RNA and protein synthesis inhibition is minimal and cells remain viable and functional. Butyrate inhibits the proliferation of LIM-1215 cultured colon cancer cells at concentrations of 1–10 mmol/liter (28). Bu-

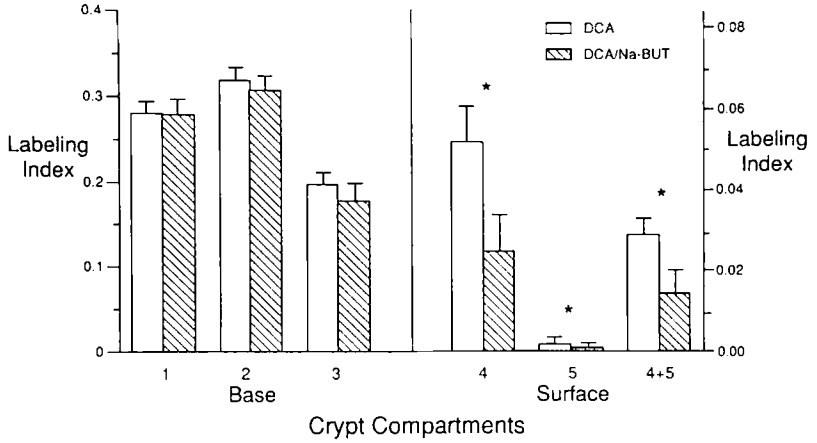

Fig 1. Labeling index by crypt compartment in human colon incubation experiments with 5 μM deoxycholate (DCA) and 5 μM DCA/10 mM sodium butyrate (Na-But); $N = 12$ subjects. Columns (bars), means (±SEM); $^{*}P < 0.05$ by Mann-Whitney U test. Adapted and reproduced with permission (49).

tyrate, in a concentration of 1 mmol/liter, increases cell doubling time from 26 to 72 hr and decreases the cloning efficiency from 1.1% to 0.05%. In this study, proliferation was only minimally inhibited by acetate and propionate. Similar inhibition of proliferation is noted in several other *in vitro* studies of many different colorectal cancer cell lines (22, 26, 27, 29, 31, 48). Anchor-dependent growth is inhibited by butyrate at a concentration of 2 mmol/liter in HRT-18 cells, under conditions that do not affect cell viability (27). Removal of butyrate from the culture medium rapidly reverses the effects on proliferation.

In vivo evidence that butyrate selectively inhibits malignant colonocyte growth is limited (32). We have recently shown that butyrate inhibits colorectal carcinoma cell growth *in vivo* in a model of liver metastases in mice when administered as a continuous intravenous infusion (2 g/kg/day) for 7 days (33).

Induction of Crypt Base Proliferation and Inhibition of Premalignant Crypt Surface Hyperproliferation

Patients at increased risk for colon cancer (eg, familial polyposis coli and Gardner's syndrome) have a "premalignant" type of proliferation of the colonic epithelium with a shift of the proliferative zone from the crypt base to the upper 40% of the crypt (24, 25). In endoscopically obtained biopsies from normal human mucosa, incubation with butyrate, at a concentration of 10 mmol/liter (which is an *in vivo* physiologic concentration), significantly increased colonic crypt cell proliferation. The butyrate-induced proliferation was observed only in the basal 60% of the crypt, which is considered to be the zone of physiologic crypt cell proliferation and not a premalignant response (42). In follow-up *in vitro* studies also using normal human colonic mucosa, deoxycholate-induced crypt surface premalignant hyperproliferation was inhibited by coincubation with butyrate (49, 50) (Figures 1 and 2).

In vivo evidence that butyrate inhibits colonic crypt surface hyperproliferation is limited. We have recently observed that crypt surface proliferation is decreased by butyrate (10 mmol/liter) and increased by deoxycholate (10 mmol/liter) *in vivo* in normal rat colon (51).

BUTYRATE EFFECTS ON DIFFERENTIATION: NORMAL VERSUS NEOPLASTIC COLONOCYTES

While butyrate induces differentiation of neoplastic colonocytes *in vitro* (26, 28, 30, 31) and *in vivo* (32), it either decreases or does not significantly affect the expression of differentiation markers in normal colonocytes *in vitro* (34) and *in vivo* (52) (Table 1).

Induction of Differentiation Markers in Neoplastic Colonocytes

In Vitro Regulation of Expression of Brush-Border Hydrolases. Brush-border membrane enzymes such as alkaline phosphatase are markers of differentiation, and their expression decreases with neoplastic transformation (53, 54). Evidence obtained from a study of brush-border hydrolases in biopsies of normal and neoplastic human colonic epithelium dem-

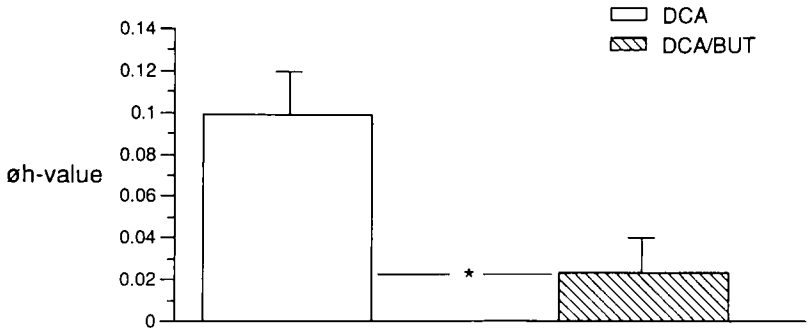

Fig 2. The ϕh-value obtained in biopsies of the sigmoid colon after incubation with 5 μM DCA and a combination of 5 μM plus 10 mM BUT (DCA/BUT). The ϕh value is an index of premalignant proliferation and represents the proliferation at the surface of the crypt (calculated by labeled cells in the upper 40% compartments divided by total labeled cells in the crypt) (N = 12 patients). Values are expressed as mean ± SEM. $^*P < 0.01$ by Wilcoxon's signed rank test. Adapted and reproduced with permission (50).

onstrate a low expression of brush-border hydrolases in adenomas, suggesting an impairment in the differentiation process early in colorectal carcinogenesis (53). A specific form of alkaline phosphatase [human placental-like alkaline phosphatase (PLAP)] is induced by sodium butyrate in several colorectal adenocarcinoma cell lines in culture (26, 27, 55–58). Butyrate increased the activity of alkaline phosphatase in 10 of 14 colon cancer cell lines, in magnitudes ranging from 2- to 123-fold increases (58). A significant correlation between the induction of alkaline phosphatase activity and the degree of morphologic differentiation was noted.

Studies with LIM-1215 cultured colon cancer cells incubated with butyrate at 1 mmol/liter concentrations demonstrate increased activity of alkaline phosphatase by 600%. Both the proliferative effects and the effects on alkaline phosphatase activity occurred at the same time in culture, therefore suggesting a possible link between butyrate's differentiating and antiproliferative effects (28). Associated with these effects on proliferation and differentiation were phenotypic changes such as higher cytoplasmic to nuclear ratios. In HT-29 cells, incubation with butyrate rapidly induced enterocytic-like differentiation and growth inhibition while concomitantly inducing alkaline phosphatase mRNA (31, 48).

***In Vitro* Regulation of Expression of SCLC Cluster-1 Antigen.** The expression of small cell lung cancer (SCLC) cluster-1 antigens are associated with increased differentiation in some adenocarcinoma cell lines. The effects of sodium butyrate on SCLC cluster-1 antigen expression has been studied in three poorly differentiated colorectal adenocarcinoma cell lines (59). After four days of culture with sodium butyrate, the expression of SCLC cluster-1 antigen was induced in these cell lines and enhanced induction correlated with increased levels of alkaline phosphatase.

Regulation of *in Vitro* Expression of Molecules Involved in Chemotherapy Resistance

The effects of butyrate on the level of expression of the multidrug resistance gene (*mdr-1*), which are believed to correlate with cellular differentiation, are variable (60, 61). Butyrate-treated SW60 human colon carcinoma cells have increased efflux of vinblastin, implying increased expression of the *mdr-1* gene; however, another study using DHD-K 12/TRV human colon cancer cells showed no effect on *mdr-1* gene product activity (62).

Contrasting Effects on Normal Colonocytes

The expression of differentiation markers decreases in normal human colonic cells after a 24-hr incubation with butyrate. This suggests that butyrate does not enhance, and in fact may suppress, differentiation of normal colonocytes (34). When large bowel luminal butyrate was increased in rats by feeding a wheat bran-rich diet, no significant *in vivo* effects were documented on the expression of the colonic brush-border hydrolases, alkaline phosphatase, and dipeptidyl peptidase IV (52).

THE PARADOX

The paradoxical effects of butyrate on normal and neoplastic colonocyte proliferation and differentiation is unlikely to be artifactual. We have recently observed that butyrate (10 mmol/liter) induces prolif-

eration of the colonic crypt base (undifferentiated rapidly proliferating normal cells) while inhibiting proliferation at the crypt surface (differentiated normal cells with low proliferation rates) in the surgically isolated normal rat colon *in vivo* (51).

Different metabolic profiles of neoplastic and normal colonocytes may account for this paradox. Colon cancer cells *in vivo* switch from principally aerobic to anaerobic metabolism (63, 64). Failure to completely oxidize butyrate may lead to higher intracellular concentrations or accumulation of metabolic intermediates as well as pH changes. Decreases in intracellular pH may enhance or inhibit gene expression when compared to normal cells (65). Alternatively, mutated proteins in the neoplastic colonocyte may have an altered specific affinity for butyrate. Similarly, normal colonocytes of different stages in the proliferation–differentiation continuum may express different isotypes of regulatory proteins that have varying intrinsic affinities for butyrate. Thus, butyrate may act as a cofactor to proteins in the transduction pathway or to the transcription regulatory proteins, thereby specifically stimulating or inhibiting certain cellular processes.

REGULATORY EFFECTS ON MOLECULES INVOLVED IN CELLULAR GROWTH, ADHESION AND MIGRATION

Increase in EGF Receptor Expression

In human HCT-116 colon tumor cells, butyrate increases the cell surface epidermal growth factor (EGF) receptor expression in association with the induction of a more differentiated phenotype in some of the clones studied (2 mmol/liter butyrate for 96 hr) (66). While all clones had a butyrate-induced inhibition of growth, the effect on alkaline phosphatase varied widely. These effects on EGF receptor expression may be important in controlling cell growth in neoplastic colonocytes.

Decrease in S19 and LBP and Increase in HLA-1

The differential expression of S19 ribosomal protein, laminin-binding protein (LBP), and human lymphocyte antigen class 1 (HLA-1) mRNA associated with human colon carcinoma correlates *in vitro* to tumor progression and differentiation (67). Increases of S19 and LBP expression combined with decreases in HLA-I expression are associated with increased malignant potential in human colon carcinoma cells. Incubation of human colorectal carcinoma cells with sodium butyrate decreases levels of S19 and LBP mRNA and increases levels of HLA-1 mRNA expression compared with untreated cells. These effects may be relevant in modulating cell adhesion and migration and thus malignant potential.

Decrease in Urokinase

Colon cancer cells secrete urokinase and express its receptors (68). The urokinase activity of these cells is greatest at the invading surface of the tumor (69). Butyrate is a potent inhibitor of urokinase secretion in LIM-1215 colon cancer cells as well as normal colon cells (70). This inhibition may affect the ability of carcinoma cells to invade and metastasize.

Increase in G_{M3} Ganglioside

Sialyltransferases are glycosyltransferases that add sialic acid to the nonreducing termini of oligosaccharides, forming molecules that are implicated in cellular recognition and adhesion as well as neoplastic transformation and metastasis (71). One such type of molecule are the sialylated glycolipids, such as G_{M3} ganglioside. Butyrate treatment of two colon cancer cell lines *in vitro* increased the synthesis of G_{M3} ganglioside while concomitantly inducing differentiation (26).

Decrease in Adhesion to Laminin

Laminin, a glycoprotein localized in the basement membrane, is involved in cell growth, differentiation, morphogenesis, migration, attachment, and cancer metastasis (72–78). Increased levels of laminin receptor correlate with tumor undifferentiation and metastatic potential (79, 80). Induction of differentiation of HT-29 human colon cancer cells with butyrate reduces the adhesion to laminin, possibly mediated by factors that modify galactosyltransferase (81). Undifferentiated human colon cancer cell lines bind preferentially to laminin substrate over fibronectin or collagen IV (81). Induction of differentiation with sodium butyrate decreases cellular adherence to laminin. Because laminin binding is partially inhibited by α-lactalbumin [a "modifier of galactosyltransferase specificity" (81)], galactosyltransferase has been implicated in the binding of cells to laminin. These findings suggest an interaction between increased differentiation and decreased neoplastic cell binding to laminin. Increased cellular differentiation might decrease accessibility to laminin binding protein on the basement membrane.

TABLE 2. SUMMARY OF EFFECTS OF BUTYRATE ON MOLECULAR EVENTS RELEVANT TO LARGE BOWEL NEOPLASIA*

Gene	*Chromosome*	*Effect of butyrate*
FAP/MCC	5q	Unknown
p53	17p	Unknown
DCC	18q	Unknown
c-*ras*	12p	p21 inhibited (HT29 cells)
c-*src*		pp60, p56 inhibited (SW620 cells)
c-*myc*		Inhibited
L/B/K alkaline phosphatase†	1p	Activated/induced (many cell lines)

* Adapted and reproduced with permission (84).
† Liver/bone/kidney alkaline phosphatase, the form expressed in colon.

BUTYRATE-INDUCED REGULATION OF GENE EXPRESSION

The multistage hypothesis of colon carcinogenesis is predicated on a steplike progression from normal epithelium, to hyperproliferative epithelium, to aberrant crypts, to various stages of dysplasia (present in adenomas), to preinvasive, invasive, and metastatic carcinoma (82, 83). Current data suggest that this multistep process results, in part, from an accumulation of genetic alterations including the familial adenomatous polyposis coli–mutated in colon cancer (FAP/MCC) locus on chromosome 5, the deleted colon cancer (DCC) gene on chromosome 18, the p53 gene on chromosome 17, and the *ras* protooncogene (82). Aberrations in the expression of many other protooncogenes have also been implicated, probably reflecting late events in the neoplastic transformation.

The effects of butyrate on the expression of FAP/MCC, DCC, and p53 protooncogenes in colonocytes are not known, but inhibition in the expression of c-*ras*, N-*ras*, c-*src*, c-*myc*, and c-*myb* genes have been reported in several colorectal cell lines *in vitro*. Table 2 summarizes the major effects of butyrate on important molecular events relevant to colon cancer (84).

Inhibition of the *ras* and *src* Protooncogenes and Inhibition of p21 and pp60

Butyrate suppresses the malignant transforming activity of a human N-*ras* oncogene in the human colon carcinoma cell line MIP 101 (85). This effect is associated with a more differentiated phenotype that includes decreased growth rate, eliminated anchorage-independent growth, and decreased tumorgenicity. Under these study conditions butyrate treatment did not have a detectable effect on the overall structure, methylation, and level of expression of the human N-*ras* gene from MIP 101 cells. It is possible that butyrate may activate a second gene that negates the action of the transforming N-*ras* gene.

Butyrate may exert its antineoplastic effects via p21, a GTP-binding protein product of the *ras* family. In the fibroblast cell line NIH 3T3, p21 is needed for cells to move from the G_1 to the S phase in the cell cycle, thereby initiating cell division (84). Butyrate exhibits a dose-dependent inhibition of the production of Ha-p21 in the HT-29 colon cancer cell line as well (29).

$PP60^{c\text{-}src}$ and $p56^{lck}$ are tyrosine kinases that are enzymatic products of the *src* family of protooncogenes. $PP60^{c\text{-}src}$ is expressed in both neoplastic and normal cells, whereas $p56^{lck}$ is expressed only in neoplastic cells. In SW620 colon cancer cells, butyrate decreases the expression of protein kinase activity as well as the expression of $pp60^{c\text{-}src}$ and $p56^{lck}$. In addition to these effects, butyrate decreases proliferation, decreases contact independent growth, and increases alkaline phosphatase expression (86).

Inhibition of c-*myc* Protooncogene

The protooncogene c-*myc* encodes a nuclear protein that functions as a transcription factor as well as being involved in DNA replication (87). The c-*myc* gene expression is linked to cell proliferation, differentiation, and apoptosis and is down-regulated with induced differentiation. Colonic carcinogenesis is associated with increased expression of c-*myc* mRNA and protein in the absence of gene amplification or rearrangement (88–92). Increases of c-*myc* protein correlate with growth rates in six human colon tumor cell lines examined *in vitro*. Butyrate significantly reduced the levels of c-*myc* in all colon cancer cell phenotypes treated (93). In HT-29 cells butyrate arrests cell growth early in the G_1 phase of the cell cycle, in association with the emergence of markers of differentiation and down-regulation of c-*myc* mRNA expression (94). Sodium butyrate increases the block to transcriptional elongation in the c-*myc* gene in *in vitro* studies of SW837 rectal cancer cells. Therefore, butyrate might regulate c-*myc* expression by regulating transcriptional elongation in these cell types (95). All of the cells examined in this study had a normal c-*myc* DNA sequence, indicating that the deregulation of c-*myc* expression in colon cancer is not due to a *cis* mutation of this region. *In vitro* studies on the regulation of c-*myc* expression by butyrate in the colon carcinoma cell line CaCo-2 show that butyrate induces a factor involved in c-*myc* mRNA degradation (96). Therefore, it is also possible that posttran-

scriptional modification of gene expression could be one of the major targets for the antiproliferative effects of butyrate. In this study, butyrate reduced c-*myc* mRNA levels after a 30-min delay. In contrast to the findings in SW837 cells, butyrate appeared to affect c-*myc* expression at the posttranscriptional level in CaCo-2 cells, but not at the level of transcriptional initiation or elongation.

The c-*fos* and c-*jun* Protooncogenes

The *jun* and *fos* gene families encode for DNA binding proteins that dimerize to form the AP-1 transcription factor, which regulates multiple genes involved in cellular growth and differentiation (97). Butyrate induces c-*fos* very rapidly at a posttranscriptional level and more slowly at a transcriptional level in human colon carcinoma CaCo-2 cells (98). This transcriptional induction does not result in increases of steady-state mRNA levels, indicating that butyrate can affect specific transcription factors important for cell growth and differentiation.

Whether butyrate can affect gene expression *in vivo* remains to be thoroughly investigated. We have recently shown that butyrate increases c-*jun* but not c-*fos* in normal rat colon *in vivo*, under the same conditions where the tumor promoter deoxycholate induces c-*fos* but not c-*jun* (51).

Regulation of CEA Antigen Expression

Incubation of several human colon cancer cell lines with sodium butyrate at 2 mmol/liter concentration shows a considerable heterogeneity in the induction and expression of carcinoembryonic antigen (CEA) (99). Each of the cell lines studied show a distinct pattern of CEA antigen expression from a limited number of mRNA transfers. The induction of these CEA-related antigens began as early as 24 hr after exposure and occurred primarily at the transcriptional level.

Induction of Cytochrome Oxidase

Expression levels of COXIII, a mitochondrial gene encoding for one of the 13 subunits of cytochrome *c* oxidase, are abnormally low in colon tumors, and in colonic tissue genetically at risk for neoplasia (100). *In vitro* studies using the HT-29 human colon carcinoma cells showed that incubation with butyrate increased cytochrome oxidase activity and the expression of genes encoding for subunits of cytochrome oxidase (100).

Induction of Apoptosis

In vitro studies using cell lines that originated from six colorectal adenomas and seven colorectal carcinomas examined the ability of butyrate to induce apoptosis (programmed cell death) (101). Sodium butyrate, at physiologic concentrations, induced apoptosis in two adenoma cell lines (RG-C2, AA-Ci) and in one carcinoma cell line (PC/JW/FI). Because escape from the induction of programmed cell death is important in colorectal carcinogenesis, butyrate may have an important regulatory effect on this process and thus explain, in part, why high-fiber diets appear to be protective in colon cancer.

POSSIBLE MOLECULAR MECHANISMS OF ACTION FOR BUTYRATE EFFECTS ON COLONOCYTE GROWTH/DIFFERENTIATION

Butyrate regulates the transcriptional expression of multiple genes (102) and has several effects on nuclear proteins that could modify gene expression. These effects include: (1) hyperacetylation of core histones through the inhibition of histone deacetylase (103), (2) selective inhibition of phosphorylation of histones H1 and H2 (104, 105), (3) selective increase in the phosphorylation of H3 histone (106), and (4) hypermethylation of cytosine residues in DNA (102, 104, 107). Butyrate also enhances the acetylation and phosphorylation of nonhistone proteins and increases ADP ribosylation (102, 105, 108–110). The significance of these varied molecular effects is not well understood and differs among the multiple *in vitro* cell lines studied. In general, histone hyperacetylation is associated with increased gene expression (111) and an increase in DNase I sensitivity (112). Hypermethylation of cytosine residues in DNA is associated with decreased gene expression. H1 histone phosphorylation is associated with progression of the cells in the cell cycle (104). The induction or inhibition of gene expression produced by histone hyperacetylation, regulation of phosphorylation in specific histones, and DNA hypermethylation are thought to be nonspecific. However, butyrate can also specifically modulate gene expression. For example, in CaCo-2 cells, butyrate directly activates the PLAP promoter (113). Therefore, some investigators hypothesize that butyrate acts as a cofactor for regulatory transcription proteins in the nucleus that are directly involved in gene expression (102) (see Figure 4 below).

Alternatively, butyrate may act at the membrane–cytoplasm interface by interacting with the G-proteins, which are key components of the signal

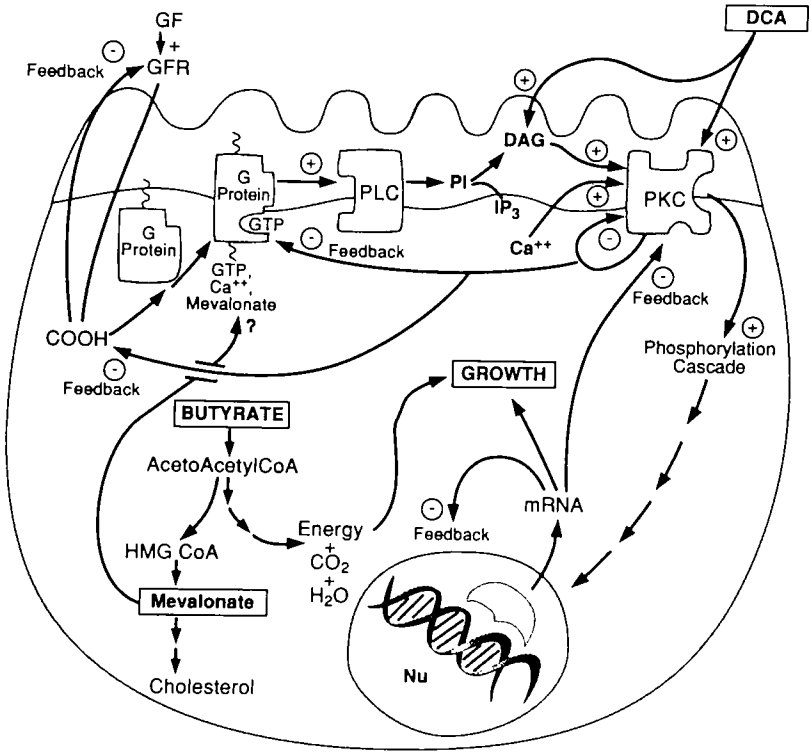

Fig 3. Speculative mechanism of action for the proliferation effects of butyrate and DCA on normal colonocytes. In the normal colonocyte, intact feedback inhibitory mechanisms regulate the growth process. Butyrate is the preferred oxidative fuel for normal colonocytes. Butyrate-induced proliferation may represent, in part, a fuel-mediated effect. It is postulated that butyrate may also enhance G-protein activation indirectly via mevalonate. The secondary bile acid, deoxycholate (DCA), in high concentrations, may promote tumor growth. DCA may induce a hyperproliferative effect by directly stimulating protein kinase C (PKC) or by increasing membrane phospholipid turnover, thereby increasing diacylglycerol (DAG) levels. GF, growth factor; GFR, growth factor receptor; HMGCoA, 3-hydroxy-3-methylglutaryl-CoA; IP3, inositol triphosphate; PI, phosphatidylinositol; PLC, phospholipase C.

transduction pathway and play an important role in cellular proliferation. Binding of the G-protein to the SCFA mevalonate (or one of its metabolic intermediates), is required for membrane translocation and subsequent *ras* protein activation in yeast and humans (114, 115). Altered mevalonate-binding affinity in mutated G-proteins (G*) may explain the paradox of butyrate's different effects on proliferation in normal versus neoplastic colonocytes (Table 1, Figures 3 and 4).

We hypothesize that in the normal colonocyte, mevalonate (derived from the metabolism of butyrate) mediates the proliferative effect of butyrate (Figure 3). If true, inhibitors of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase such as mevastatin, should block butyrate-induced growth in normal cells by inhibiting the synthesis of mevalonate. Additionally, exogenous mevalonate should restore the proliferative response. Studies are ongoing to test this hypothesis.

Mutated G-proteins are the products of activated *ras* oncogenes, which are thought to play a role in early colorectal carcinogenesis (116, 117). With a change to anaerobic metabolism in the neoplastic colonocyte, butyrate may accumulate in the cytoplasm. Mutated G-proteins may have altered mevalonate-binding affinity, and we speculate that butyrate competes with mevalonate binding to G* but results in an inactivated form of the G*-protein (Figure 4). If this hypothesis is true, then one would expect an additive or synergistic inhibitory effect of mevastatin and butyrate on proliferation in these transformed cells. Moreover, exogenous mevalonate should eliminate the antiproliferative effects of both butyrate and mevastatin. We have recently observed that in MC-26 murine colorectal carcinoma cells coincubation with

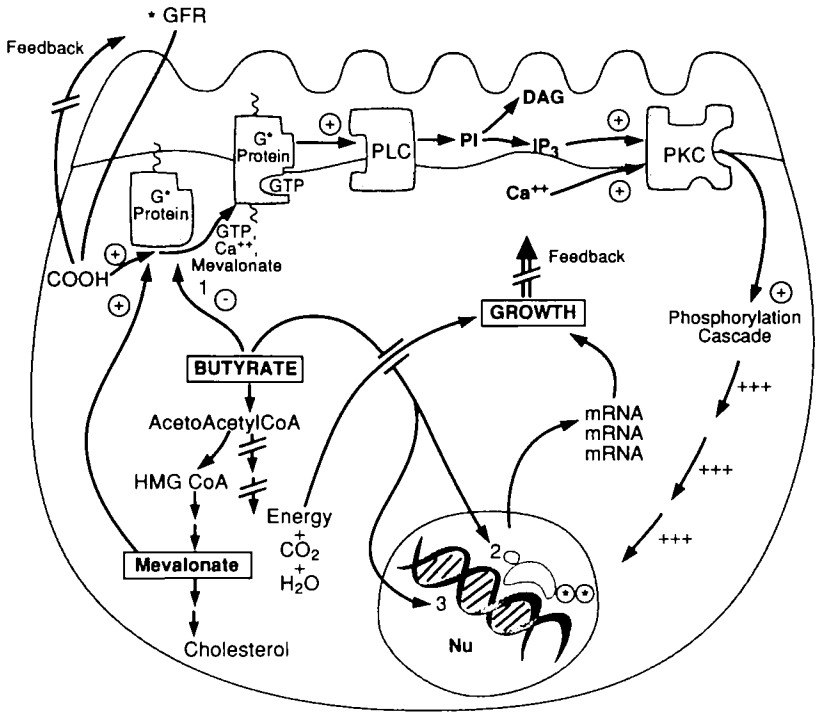

Fig 4. Speculative mechanisms for butyrate-induced growth inhibition in neoplastic colonocytes. Evidence suggests that G-proteins require binding to mevalonate or a mevalonate intermediate for translocation into the membrane and subsequent activation (114). The activated G-protein then stimulates the activity of protein kinase C (PKC) via the activation of phospholipase C (PLC). The cascade of enzymatic phosphorylations resulting from the activation of PKC leads to a signal for growth. Activated *ras* oncogenes encode for mutated G-proteins (G^*). With a switch to anaerobic metabolism in the neoplastic colonocyte, butyrate may accumulate in the cytoplasm. (1) We hypothesize that butyrate may then compete with mevalonate (or a mevalonate intermediate) in the binding to G^*, but the butyrate-G^* combination results in an inactive form of the G^*-protein. Alternative mechanisms have been proposed (102) as depicted: (2) butyrate may specifically modulate gene expression as a cofactor to the transcription proteins, and (3) butyrate may nonspecifically modulate gene expression via chromatin changes induced by DNA methylation and histone acetylation (102).

mevalonate reverses butyrate's antiproliferative effects and coincubation with mevastatin synergistically potentiates these antiproliferative effects (33).

CONCLUSIONS AND FUTURE RESEARCH

The many effects of butyrate on the colonocyte probably reflect its ability to both specifically and nonspecifically affect genomic expression. The study of butyrate's varied effects may help clarify the process of colorectal carcinogenesis at a molecular level. Future work is needed to evaluate the *in vivo* balance between colonic carcinogenic substances and potentially protective agents such as butyrate, which can be modulated by dietary interventions. Additionally, further investigations are needed to identify the mechanism(s) of action of butyrate and to identify its role in the mediation, prevention, and treatment of colon cancer.

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REFERENCES

1. Jacobs LR: Relationship between dietary fiber and cancer: metabolic, physiologic, and cellular mechanism. Proc Soc Exp Biol Med 182:299–310, 1986
2. Jacobs LR: Role of dietary factors in cell replication and colon cancer. Am J Clin Nutr 48:775–779, 1988
3. Jacobs LR. Influence of soluble fibers on experimental colon carcinogenesis. *In* Dietary Fiber: Basic and Clinical Aspects.

GB Vahouny, D Kritchevesky (eds). New York, Plenum Press, 1990, pp 389–403
4. Lee DK, Chapkin RS, Lupton JR: Dietary fat and fiber modulate colonic cell proliferation in an interactive site-specific manner. Nutr Cancer 20:107–118, 1993
5. Van Munster IP, Nagengast FM: The role of carbohydrate fermentation in colon cancer prevention. Scand J Gastroenterol 28(S200):80–86, 1993
6. McIntyre A, Gibson PR, Young GP: Butyrate production from dietary fibre and protection against large bowel cancer in a rat model. Gut 34:386–391, 1993
7. Howe GR, Benito E, Castelleto R, Cornée J, Estève J, Gallagher RP, Iscovich JM, Deng-ao J, Kaaks R, Kune GA, Kune S, L'Abbé, Lee HP, Lee M, Miller AB, Peters RK, Potter JD, Riboli E, Slattery ML, Trichopoulos D, Tuyns A, Tzonou A, Whittemore AS, Wu-Williams AH, Shu Z: Dietary intake of fiber and decreased risk of cancers of the colon and rectum: Evidence from the combined analysis of 13 case-control studies. JNCI 84:1887–1896, 1992
8. Wolin MJ: Fermentation in the rumen and human large intestine. Science 213:1463–1468, 1981
9. Wrong OM: Carbohydrates. *In* The Large Intestine: Its Role in Mammalian Nutrition and Homeostasis. OM Wrong, CJ Edmonds, VS Chadwick (eds). New York, Halsted Press, 1981, pp 20–21, 113–114
10. Cummings JH, Branch WJ: Fermentation and production of short-chain fatty acids in human large intestine. *In* Dietary Fiber: Basic and Clinical Aspects. GB Vahouny and D Kritchevesky (eds). New York, Plenum Press, 1990, pp 131–152
11. Cummings JH, Pomare EW, Branch WJ, Naylor CPE, MacFarlane GT: Short chain fatty acids in human large intestine, portal, hepatic, and venous blood. Gut 28:1221–1227, 1987
12. Roediger WEW: Utilization of nutrients by isolated epithelial cells of the rat colon. Gastroenterology 83:424–429, 1982
13. Rombeau JL, Kripke SA, Settle RG: Short-chain fatty acids: Production, absorption, metabolism, and intestinal effects. *In* Dietary Fiber: Basic and Clinical Aspects. GB Vahouny and D Kritchevesky (eds). New York, Plenum Press, 1990, pp 317–339
14. Macfarlane GT, Gibson GR, Macfarlane S: Short-chain fatty acid and lactate production by human intestinal bacteria grown in batch and continuous culture. *In* Short-Chain Fatty Acids. J Cummings, HJ Binder, K Soergel (eds). Boston, Kluwer Academic Publishers, 1994, pp 44–60
15. Nyman M, Aso NG: Fermentation of dietary fiber components in rat intestinal tract. Br J Nutr 47:357–366, 1982
16. Demigné C, Remesy C: Stimulation of absorption of volatile fatty acids and minerals in cecum of rats adapted to a very high fiber diet. J Nutr 115:53–60, 1985
17. Rechkemmer G, Ronnau K, von Englehardt W: Fermentation of polysaccharides and absorption of short chain fatty acids in the mammalian hindgut. Comp Biochem Physiol 90A:563–568, 1988
18. Macfarlane GT, Gibson GR, Cummings JH: Comparison of fermentation reactions in different regions of the human colon. J Appl Bacteriol 72:54–64, 1992
19. Titegeyer EC, Bourquin LD, Fahey GC, Garleb KA: Fermentability of various fiber sources by human fecal bacteria *in vitro*. Am J Clin Nutr 53:418–424, 1991
20. Knudsen KE, Jensen BB, Hansen I: Oat bran but not a β-glucan enriched oat fraction enhances butyrate production in the large intestine of pigs. J Nutr 123:1235–1247, 1993
21. Weaver GA, Krause JA, Miller TL, Wolin MJ: Cornstarch fermentation by the colonic microbial community yields more butyrate than does cabbage fiber fermentation: cornstarch fermentation rates correlate negatively with methanogenesis. Am J Clin Nutr 55:70–77, 1992
22. Colony PC: The identification of cell types in the normal adult colon. *In* Cell and Molecular Biology of Colon Cancer. LH Augenlicht (ed). Boca Raton, CRC Press, 1989, pp 2–21
23. Higgins PJ: Antigenic and cytoarchitectural 'markers' of differentiation pathways in normal and malignant colonic epithelial cells. *In* Cell and Molecular Biology of Colon Cancer. LH Augenlicht (ed). Boca Raton, CRC Press, 1989, pp 112–132
24. Lipkin M, Enker WE, Winawer SJ: Tritiated-thymidine labeling of rectal epithelial cells in non-prep biopsies of individuals at increased risk for colonic neoplasia. Cancer Lett 37:153–161, 1987
25. Lipkin M: Biomarkers of increased susceptibility to gastrointestinal cancer: New application to studies of cancer prevention in human subjects. Cancer Res 48:235–245, 1988
26. Kim YS, Tsao D, Siddiqui B, Whitehead JS, Arnstein P, Bennette J, Hicks J: Effects of sodium butyrate and dimethylsulfoxide on biochemical properties of human colon cancer cells. Cancer 45:1185–1192, 1980
27. Tsao D, Morita A, Bella A, Luu P, Kim YS: Differential effects of sodium butyrate, dimethylfoxide, retinoic acid on membrane-associated antigen, enzymes and glycoproteins of human rectal adenocarcinoma cells. Cancer Res 42:1052–1058, 1982
28. Whitehead RH, Young GP, Bhathal PS: Effects of short chain fatty acids on a new human colon carcinoma cell line (LIM1215). Gut 27:1457–1436, 1986
29. Czerniak B, Herz F, Westo RP, Koss LG: Modification of H-*ras* oncogene p-21 expression and cell cycle progression in the human colon cancer cell line HT-29. Cancer Res 47:2826–2830, 1987
30. Awad AB, Horvath PJ, Andersen MS: Influence of butyrate on lipid metabolism, survival, and differentiating of colon cancer cells. Nutr Cancer 16:125–133, 1991
31. Barnard JA, Warwick G: Sodium butyrate rapidly induces 'enterocytic-like' differentiation and growth inhibition of HT-29 cells. Gastroenterology 102:A199, 1992
32. Otaka M, Singhal A, Hakomori S: Antibody-mediated targeting of differentiation inducers to tumor cells: Inhibition of colonic cancer cell growth *in vitro* and *in vivo*. Biochem Biophys Res Commun 158:202–208, 1989
33. Velázquez OC, Jabbar A, DeMatteo RP, Rombeau JL: Butyrate inhibits hepatic tumor growth in a murine model of metastatic colorectal cancer. Surgery (in press).
34. Gibson PR, Moeller I, Kagelari O, Folino M, Young GP: Contrasting effects of butyrate on differentiation of neoplastic and nonneoplastic colonic epithelial cells. J Gastroenterol Hepatol 7:165–172, 1992
35. Sakata T: Influence of short-chain fatty acids on epithelial cell division of digestive tract. J Exp Physiol 69:639–648, 1984
36. 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 58:95–101, 1987
37. Kripke SA, Fox AD, Berman JM, Settle RG, Rombeau JL:

Stimulation of intestinal mucosal growth with intracolonic infusion of short-chain fatty acids. J Parenter Enter Nutr 13:109–116, 1989
38. Janne P, Carpenter Y, Willems G: Colonic mucosal atrophy induced by a liquid elemental diet in rats. Am J Dig Dis 22:808–812, 1977
39. Koruda MJ, Rolandelli RH, Bliss DZ, Hastings J, Rombeau JL, Settle RG: Parenteral nutrition supplemented with short-chain fatty acids: Effect on the small-bowel mucosa in normal rats. Am J Clin Nutr 51:685–689, 1990
40. Friedel D, Levine GM: Effect of short-chain fatty acids on colonic function and structure. J Parenter Enter Nutr 16(1):1–4, 1992
41. Lupton JR, Kurtz PP: Relationship of colonic luminal short-chain fatty acids and pH to *in vivo* cell proliferation in rats. J Nutr 123:1522–1530, 1993
42. Scheppach W, Bartram P, Richter A, Richter F, Dusel G, Liepold H, Hofstetter G, Ruthlein J, Kasper H: Effect of short-chain fatty acids on the human colonic mucosa *in vitro*. J Parenter Enter Nutr 16:43–48, 1992
43. Sakata T, von Englehardt W: Stimulatory effects of short chain fatty acids on the epithelial cell proliferation in rat large intestine. Comp Biochem Physiol 74A:459–462, 1983
44. Frankel WL, Lew J, Su B, Bain A, Klurfeld D, Einhorn E, MacDermott RP, Rombeau JL: Mediation of the trophic effects of short-chain fatty acids on the rat jejunum and colon. Gastroenterology 106:375–380, 1994
45. Sakata T: Stimulatory effect of short-chain fatty acids on epithelial cell proliferation of isolated and denervated jejunal segment of the rat. Scand J Gastroenterol 24:886–890, 1989
46. Toscani A, Soprano DR, Soprano KJ: Molecular analysis of sodium butyrate-induced growth arrest. Oncogene Res 3:223–238, 1988
47. Young GP, Gibson PR: Butyrate and colorectal cancer cell. *In* Short-Chain Fatty Acids. J Cummings, HJ Binder, K Soergel (eds). Boston, Kluwer Academic Publishers, 1994, pp 148–160
48. Barnard JA, Warwick G: Butyrate rapidly induces growth inhibition and differentiation in HT-29 cells. Cell Growth Differ 4:495–501, 1993
49. Bartram HP, Scheppach W, Schmid H, Hofmann A, Dusel G, Richter F, Richter A, Kasper H: Proliferation of human colonic mucosa as an intermediate biomarker of carcinogenesis: Effects of butyrate, deoxycholate, calcium, ammonia, and pH. Cancer Res 53:3283–3288, 1993
50. Bartram HP, Scheppach W, Englert S, Dusel G, Richter A, Richter F, Kasper H: Effects of deoxycholic acid and butyrate on mucosal prostaglandin E_2 release and cell proliferation in the human sigmoid colon. J Parenter Enter Nutr 19:182–186 1994
51. Velázquez OC, Zhou D, Seto R, Jabbar A, Choi J, Lederer HM, Rombeau J: *In vivo* crypt surface proliferation is decreased by butyrate and increased by deoxycholate in normal rat colon: Associated *in vivo* effects on c-*fos* and c-*jun* expression. J Parenter Enter Nutr (in press)
52. Young GP, Gibson PR: Contrasting effects of butyrate on proliferation and differentiation of normal and neoplastic cells. *In* Short Chain Fatty Acids: Metabolism and Clinical Importance. Report of the Tenth Ross Conference on Medical Research. Columbus, Ohio, Ross Laboratories, 1991, pp 50–55
53. Young GP, Macrae FA, Gibson PR, Alexeyeff M, Whitehead R: Brush border hydrolases in normal and neoplastic colonic epithelium. J Gastroenterol Hepatol 7:347–357, 1992
54. Bell L, Williams L: Histochemical demonstration of alkaline phosphatase in human large intestine, normal and deceased. Histochemistry 60:84–90, 1979
55. Herz F, Schermer A, Halwer M, Bogart LH: Alkaline phosphatase in HT-29, a human colon cancer cell line: influence of sodium butyrate and hyperosmolality. Arch Biochem Biophys 210:581–591, 1981
56. Morita A, Tsao D, Kim YS: Effect of sodium butyrate on alkaline phosphatase in HRT-18, a human rectal cancer cell line. Cancer Res 42:4540–4545, 1982
57. Ito F, Chou JY: Induction of placental alkaline phosphatase biosynthesis by sodium butyrate. J Biol Chem 259:2526–2530, 1984
58. Chung YS, Song IS, Erickson RH, Sleisenger MH, Kim YS: Effect of growth and sodium butyrate on brush border membrane associated hydrolases in human colorectal cancer cell lines. Cancer Res 45:2976–2982, 1985
59. Hay FG, Duncan LW, Langdon SP, Leonard RCF: Modulation of the cluster 1 and mucin antigens in human small cell lung cancer and other epithelial tumour cell lines after treatment with the differentiation inducing agent, sodium butyrate. Br J Cancer 14:33–35, 1991
60. Frommel TO, Coon JS, Tsuro T, Roninson IB: Variable effects of sodium butyrate on the expression and function of the MDR-1 (P-glycoprotein) gene in colon carcinoma cell lines. Int J Cancer 55:297–302, 1993
61. Mickley LA, Batest SE, Richert ND, Currier S, Tanaka S, Foss F, Rosen N, Fojo AT: Modulation of the expression of a multidrug resistance gene (mdr-1/P-glycoprotein) by differentiating agents. J Biol Chem 264:18031–18040, 1989
62. Petit JM, Chauffert B, Dimanche-Boitrel MT, Genne P, Duchamp O, Martin Francois: mdr 1 gene-expression and villin synthesis in a colon cancer cell line differentiated by sodium butyrate. Anticancer Res 13:487–490, 1993
63. White MW: Metabolism of the malignant cell, *in vivo* is anaerobic and significantly plays a factor in the pathway to carcinogenesis. Med Hypotheses 39(4):323–333 1992
64. Fischer H: Malignancy may contribute to rapid evolution. Med Hypotheses 28(1):35–38 1989
65. Isfort RJ, Cody DB, Asquith TN, Ridder GM, Stuard SB, LeBoeuf RA: Induction of protein phosphorylation, protein synthesis, immediate early gene expression and cellular proliferation by intracellular pH modulation. Eur J Biochem 213(1):349–357, 1993
66. Nathan DF, Burkhart SR, Morin MJ: Increased cell surface EGF receptor expression during the butyrate-induced differentiation of human HCT-116 colon tumor cell clone. Exp Cell Res 190:76–84, 1990
67. Kondoh N, Schweinfest CW, Henderson KW, Papas TS: Differential expression of s19 ribosomal protein, laminin-binding protein, and human lymphocyte antigen class I messenger RNAs associated with colon carcinoma progression and differentiation. Cancer Res 52:791–796, 1992
68. Boyd D, Florent G, Kim P, Brattain M: Determination of the levels of urokinase and its receptors in human colon carcinoma cell lines. Cancer Res 48:3112–3116, 1988
69. Kohga S, Harvey Sr, Weaver Rm, Markus G: Localization of plasminogen activators in human colon cancer by immunoperoxidase staining. Cancer Res 45:1787–1796, 1985
70. Gibson PR, Rosella O, Rosella G, Young GP: Butyrate is a

potent inhibitor of urokinase secretion by normal colonic epithelium *in vitro*. Gastroenterology 107(2):410–419, 1994
71. Bresalier RS, Rockwell RW, Dahiya R, Duh QY, Kim YS: Cell surface sialoprotein alterations in metastatic murine colon cancer cell lines selected in an animal model for colon cancer metastasis. Cancer Res 50:1299–1307, 1990
72. Bryant G, Haberern C, Rao CN, Liotta LA: Butyrate induced reduction of tumor cell laminin receptors. Cancer Res 46:807–811, 1986
73. Aumailley M, Nurcombe V, Edgar D, Paulsson M, Timpl R: The cellular interactions of laminin fragments. Cell adhesion correlates with two fragment-specific high affinity binding sites. J Biol Chem 262:11,532–11,538, 1987
74. Yannariello-Brown J, Weaver U, Liotta L, Madri JA: Distribution of a 69-kD laminin-binding protein in aortic and microvascular endothelial cell: Modulation during cell attachment, spreading, and migration. J Cell Biol 106:1773–1786, 1988
75. Herbst TJ, McCarthy JB, Tsilibary EC, Furcht LT: Differential effects of laminin, intact type IV collagen, and specific domains of type IV colagen on endothelial cell adhesion and migration. J Cell Biol 106:1365–1373, 1988
76. Ocalan M, Goodman SL, Kuhl U, Hanuschka SD, de Mark K: Laminin alters cell shape and stimulates motility and proliferation of marine skeletal myoblasts. Dev Biol 125:158–167, 1988
77. Kubota Y, Kleinman HK, Martin GR, Lawlwy TJ: Role of laminin and basement membrane in the morphological differentiation of human endothelial cells into capillary-like structures. J Cell Biol 107:1589–1598, 1988
78. Daneker GW Jr, Piazza AJ, Steele GS Jr, Mercurio AM: Relationship between extracellular matrix interactions and degree of differentiation in human colon carcinoma cell lines. Cancer Res 49:681–686, 1989
79. Terranova VP, Rao CN, Kalebic T, Margulies IM, Liotta LA: Laminin receptor on human breast carcinoma cells. Proc Natl Acad Sci USA 80:444–448, 1983
80. Wewer UM, Liotta LA, Jaye M, Ricca GA, Drohan WN, Claysmith AP, Rao CN, Wirth P, Coligan JE, Albrechtsen R, Mudryi M, Sobel ME: Altered levels of laminin receptor mRNA in various human carcinoma cells that have different abilities to bind laminin. Proc Natl Acad Sci USA 83:7137–7141, 1986
81. Wilson JR, Weiser M: Colonic cancer cell (HT29) adhesion to laminin is altered by differentiation: Adhesion may involve galactosyltransferase. Exp Cell Res 201:330–334, 1992
82. Fearon ER, Volgelstein B: A genetic model for colorectal tumorigenesis. Cell 61:759–767, 1990
83. Willson JKV: Biology of large bowel cancer. Hematol Oncol Clin North Am 3:19–34, 1989
84. Young GP, Gibson PR: Butyrate and the human cancer cell. *In* Physiologic and Clinical Aspects of Short-Chain Fatty Acids. JH Cummings, JL Rombeau, T Sakata (eds). Cambridge, Cambridge University Press, 1995, pp 319–336
85. Stoddart JH, Lane MA, Niles RM: Sodium butyrate suppresses the transforming activity of an activated N-*ras* oncogene in human colon carcinoma cells. Exp Cell Res 184:16–27, 1989
86. Foss FM, Veillette A, Sartor O, Rose N, Bolen JB: Alterations in the expression of $pp60^{c\text{-}src}$ and $p56^{lck}$ associated with butyrate-induced differentiation of human colon carcinoma cells. Oncogene Res 5:13–23, 1989
87. Marcu KB, Bossone SA, Patel AJ: *Myc* function and regulation. Annu Rev Biochem 61:809–860, 1992
88. Tsuboi K, Hirayoshi K, Takeuchi K, Sabe H, Shimada Y, Ohshio G, Tobe T, Hatanaka M: Expression of the c-*myc* gene in human gastrointestinal malignancies. Biochem Biophys Res Commun 146:669–704, 1987
89. Stewart J, Evan G, Watson J, Sikora K: Detection of the c-*myc* oncogene in colonic polyps and carcinomas. Br J Cancer 53:1–6, 1986
90. Finley GG, Schulz NT, Hill SA, Geiser JR, Pipas JM, Meisler AI: Expression of the *myc* gene family in different stages of human colorectal cancer. Oncogene 4:963–971, 1989
91. Untawale S, Blick M: Oncogene expression in adenocarcinomas of the colon and in colon tumour-derived cell lines. Anticancer Res 8:1–7, 1988
92. Dolcetti R, DeRe V, Viel A, Pistello M, Tavian M, Boicchi M, Meisler AI: Nuclear oncogene amplification or rearrangement is not involved in human colorectal malignancies. Eur J Cancer Clin Oncol 24:1321–1328, 1988
93. Taylor CW, Kim YS, Childress-Fields KE, Yeoman LC: Sensitivity of nuclear c-*myc* levels and induction to differentiation-inducing agents in human colon tumor cell lines. Cancer Lett 62:95–105, 1992
94. Barnard JA, Warwick G: Butyrate rapidly induces growth inhibition and differentiation in HT-29 cells. Cell Growth Differ 4:495–501, 1993
95. 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 Res 268:20466–20472, 1993
96. Souleimani A, Asselin C: Regulation of c-*myc* expression by sodium butyrate in the colon carcinoma cell line CaCo-2. FEBS Lett 326:45–50, 1993
97. Bohmann D, Bos TJ, Admon A, Nishimura T, Vogt PK, Tjian R: Human proto-oncogene c-*jun* encodes a DNA binding protein with structural and functional properties of transcription factor AP-1. Science 238:1386–1392, 1987
98. Souleimani A, Asselin C: Regulation of c-*fos* expression by sodium butyrate in the human colon carcinoma cell line CaCo-2. Biochem and Biophys Resch Commun 193(1):330–336, 1993
99. Toribara NW, Sack TL, Gum JR, Ho SB, Shively JE, Willson JKV, Kim YS: Heterogeneity in the induction and expression of carcinoembryonic antigen-related antigens in human colon cancer cell lines. Cancer Res 49:3321–3327, 1989
100. Heerdt BG, Augenlicht LH: Effects of fatty acid on expression of genes encoding subunits of cytochrome *c* oxidase and cytochrome *c* oxidase activity in HT29 human colonic adenocarcinoma cells. J Biol Chem 266:19120–19126, 1991
101. Hague A, Manning AM, Hanlon KA, Huschtscha LI, Hart D, Paraskeva C: Sodium butyrate induces apoptosis in the human colonic tumour cell line in a p-53 independent pathway: implications for the possible role of dietary fibre in the prevention of large bowel cancer. Int J Cancer 55:498–505, 1993
102. Kruh J, Tichonicky L, Defer N: Effect of butyrate on gene expression. *In* Short-Chain Fatty Acids. J Cummings, HJ Binder, K Soergel (eds). Boston, Kluwer Academic Publishers, 1994, pp 135–147
103. Boffa LC, Vidali G, Mann RS, Allfrey VG: Suppression of histone deacetylation *in vivo* and *in vitro* by sodium butyrate. J Biol Chem 253:3364–3366, 1978
104. Boffa L, Vidali G, Mann R, Allfrey V: Manifold effects of

sodium butyrate on nuclear function. Selective and reversible inhibition of phosphorylation of histones H1 and H2A and impaired methylation of lysine and arginine residues in nuclear protein fractions. J Biol Chem 256:9612–9621, 1981
105. Kruh J: Effect of sodium butyrate, a new pharmacological agent, on cells in culture. Mol Cell Biol 42:65–82, 1982
106. Whitlock JP, Galeazzi D, Schulman H: Acetylation and calcium dependent phosphorylation of histone H3 in nuclei from butyrate-treated HeLa cells. J Biol Chem 258:1299–1304, 1983
107. Parker MI, de Haan JB, Gevers W: DNA hypermethylation in sodium byturate-treated WI-38 fibroblasts. J Biol Chem 261:2786–2790, 1985
108. Rastl E, Swetly P: Expression of poly-(adenosine diphosphate-ribose) polymerase activity in erythroleukemic mouse cells during cell cycle and erythropoietic differentiation. J Biol Chem 253:4333–4340, 1978
109. Cousenes LS, Gallwitz D, Alberts BM: Different accessibilities in chromatin to histone acetylase. J Biol Chem 254:1716–1723, 1979
110. Christman JK, Weich N, Schoenbrun B, Schneidman N, Acs G: Hypomethylation of DNA during differentiation of Friend erythroleukemia cells. J Cell Biol 86:366–370, 1980
111. Reeves R, Dserjesi P: Sodium butyrate induces new gene expression in Friend erythroleukemic cells. J Biol Chem 254:4383–4290, 1979
112. Vidali G, Boffa LC, Bradbury EM, Allfrey VG: Butyrate suppression of histone deacetylation leads to accumulation of multiacetylated forms of histone H3 and H4 and increased DNase I sensitivity of the associated DNA sequences. Proc Natl Acad Sci USA 75:2239, 1978
113. Deng G, Liu G, Hu L, Gum JR, Kim YS: Transcriptional regulation of the human placental-like alkaline phosphatase gene and mechanisms involved in its induction by sodium butyrate. Cancer Res 52:3378–3383, 1992
114. Willumsen BM, Christensen A, Hubbert NL, Papageorge AG, Lowy DR: The p21 *ras* C-terminus is required for transformation and membrane association. Nature 310:583–586, 1984
115. Schafer WR, Kim R, Sterne R, Thorner J, Kim S, Rine J. Genetic and pharmacological suppression of oncogenic mutations in *ras* genes of yeast and humans. Science 245:379–385, 1989
116. Bos JL, Fearon ER, Hamilton SR, Verlaan-de Vries M, van Boom JH, van der Eb AJ, Vogelstein B: Prevalence of *ras* gene mutations in human colorectal cancers. Nature 327:293–297, 1987
117. Volgelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL: Genetic alterations during colorectal-tumor development. N Engl J Med 319:525, 1988