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The scientific rationale and clinical application of short-chain fatty acids and medium-chain triacylglycerols

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Bases scientifiques et applications cliniques des lipides à chaîne courte et des triacylglycérols à chaîne moyenne

RÉSUMÉ

Cette revue discute les bases scientifiques et les applications cliniques des lipides à chaîne courte (LCC) et des triacylglycérols à chaîne moyenne (TCM).

Les LCC sont produits dans le côlon des mammifères à la suite de la fermentation microbienne des fibres, de l'amidon non digéré, et des protéines de l'alimentation. Leur concentration dans les segments différents du côlon est affectée par l'apport alimentaire; ceci a des effets métaboliques importants sur le colonocyte. Le butyrate, un des trois LCC les plus importants, a été impliqué comme agent protecteur dans la pathogénèse du cancer du côlon. Les LCC stimulent l'absorption du chlorure de Na et de l'eau dans la lumière du côlon. Le butyrate a des effets trophiques sur la muqueuse normale du côlon *in vivo* et stimule la prolifération physiologique des colonocytes normaux *in vitro*. En revanche, il bloque la croissance des colonocytes néoplastiques et inhibe l'hyperprolifération prénéoplastique induite par certains carcinogènes *in vitro*. Le butyrate induit une différenciation histologique des lignées cellulaires du cancer du côlon en parallèle avec des augmentations dans l'expression des marqueurs de la différenciation des colonocytes. Il inhibe également l'expression de plusieurs proto-oncogènes importants dans la carcinogénèse colorectale. Des études plus poussées s'imposent pour évaluer les effets thérapeutiques potentiels du butyrate *in vivo*, et pour comprendre son ou ses mécanismes d'action.

Les émulsions de lipides disponibles se composent de triacylglycérols à chaîne longues (TCL) qui fournissent de l'énergie aussi bien que des acides gras essentiels tels que l'acide linoléique. Cependant, le métabolisme des TCL est plus lent que celui des TCM, et plus de 50% des lipides dérivés des TCL sont stockés sous forme de graisses, au lieu d'être oxydées pour fournir de l'énergie. Une oxydation rapide, un transport indépendant de la carnitine vers les mitochondries (site de cette oxydation), et la capacité de fournir un apport rapide d'énergie par deux mécanismes (β-oxydation et formation de corps cétoniques), font des TCM une source d'énergie théoriquement avantageuse (en particulier dans le cas d'états cliniques déficients en carnitine tels que les traumatismes, les états septiques ou les chocs graves).

Short-chain fatty acids (SCFA) are the C_{2-5} organic fatty acids. These compounds are formed in the gastrointestinal tract of mammals as a result of anaerobic bacterial fermentation of undigested dietary components (Wolin, 1981; Wrong, 1981; Cummings & Branch, 1990). Although dietary fibre is the principal substrate for fermentation to SCFA in human subjects (Rombeau *et al.* 1990), undigested starch and protein also contribute to their production (Macfarlane *et al.* 1994). In the mammalian hindgut acetate, propionate and butyrate account for 83% of SCFA, with a total concentration of approximately 100 mmol/l (Nyman & Asp, 1982; Demigné & Rémésy, 1985; Rechkemmer *et al.* 1988) and are present in a nearly constant molar ratio of 60:25:15 respectively (Cummings & Branch, 1990). The highest concentrations of SCFA (120 mmol/l) in humans are usually found in the caecum (Cummings *et al.* 1987).

The total concentration, as well as the relative molar concentrations of individual SCFA, are greatly influenced by dietary carbohydrates and proteins (Wolin, 1981; Wrong, 1981; Cummings *et al.* 1987; Mortensen *et al.* 1990; Macfarlane *et al.* 1992). Proteins are preferentially fermented to *n*-valerate, *iso*-valerate, and 2-methylbutyrate (Macfarlane *et al.* 1992). The fact that different dietary substrates are preferentially fermented to different SCFA may be important in the relationship between diet and colonic pathology.

SCFA maintain the integrity of the intestinal mucosa (Roediger, 1980*a*), and stimulate proliferation of normal colonocytes (Jacobs, 1983). They are avidly absorbed by the normal intestinal epithelium (Cummings *et al.* 1987) and stimulate water and electrolyte absorption from the colonic lumen in animals and human subjects both *in vitro* and *in vivo* (Roediger, 1980*a*; Ruppin *et al.* 1980; Binder & Mehta, 1989; Bowling *et al.* 1993; Rajendran & Binder, 1994). Butyrate is the preferred oxidative fuel of colonocytes over glucose, glutamine or ketone bodies (Roediger, 1982).

Epidemiological studies suggest that dietary fat and protein may promote carcinogenesis in the colon, whereas increased fibre and complex carbohydrates in the diet may protect against colon cancer (Jacobs, 1986, 1988, 1990; Lee *et al.* 1993; McIntyre *et al.* 1993; Van Munster & Nagengast, 1993). Human case–control studies support this association (Howe *et al.* 1992). Intake of fibre-rich foods is inversely related to risk of both colon and rectal cancer (Howe *et al.* 1992). Colonic lumen butyrate concentrations are postulated to be a key protective component of high-fibre diets against colon cancer (McIntyre *et al.* 1993; Van Munster & Nagengast, 1993).

Butyrate modulates normal and neoplastic colonocyte growth and differentiation in animals (Otaka *et al.* 1989; Young, 1990; McIntyre *et al.* 1993), and in human cells *in vitro* (Kim *et al.* 1980; Tsao *et al.* 1982; Dexter *et al.* 1984; Whitehead *et al.* 1986; Czerniak *et al.* 1987; Gibson & Pavli, 1992; Scheppach *et al.* 1992*a*; Souleimani & Asselin, 1992; Bartram *et al.* 1993, 1995; Scheppach, 1994). The effects of propionate on colonocyte growth and differentiation are similar to those of butyrate, but less marked; acetate does not exert these effects.

The present article reviews the production, absorption and metabolism of SCFA as well as their effects on normal and neoplastic colonocyte growth and differentiation. The known and hypothetical 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 colonic pathology such as diversion colitis, ulcerative colitis, enteral-feeding-induced diarrhoea, and colon cancer. Finally, the scientific rationale and clinical applications for the use of medium-chain triacylglycerols (MCT) are reviewed.

SHORT-CHAIN FATTY ACID PRODUCTION

Although there are considerable variations in hindgut volume between carnivores, omnivores and herbivores, all mammals extensively ferment polysaccharides via anaerobic bacteria in the hindgut.

Production in animals

Studies of different dietary fibre intakes in rats indicate that high-fibre diets significantly increase microbial growth in the colon and influence the molar ratios of SCFA produced (Maczulak *et al.* 1983). Daily faecal output of total viable anaerobes is seventy-one times higher in high-fibre-fed rats. The excretion of total fermentation products is 2·4 *v.* 0·1 mmol for high-fibre diets and fibre-free diets respectively. Molar ratios for acetate: propionate:butyrate are 69:21:10 *v.* 92:7:1 in the high-fibre and fibre-free groups respectively (Maczulak *et al.* 1983). *In vivo* rat colonic lumen concentrations of butyrate may reach as high as 30 mmol/l (McIntyre *et al.* 1991) with dietary fibre supplementation.

A study using newborn piglets demonstrates limited production of SCFA as early as the first day of life. SCFA levels are low and stable between days 5 and 14, and then abruptly accumulate in the lumen after 14 d of life when colonic anaerobic flora achieve near adult-like population. Acetate is predominantly formed early, while propionate and butyrate are seen as late peaks. The major sites of production and absorption of SCFA in this model are proximal to the left colon (Murray *et al.* 1987).

Production in human subjects

Studies with human subjects have shown that neonates produce acetate, propionate, butyrate and other acids in the ratio 89:5:5:1 respectively by the end of 4 d (Rasmussen *et al.* 1988). The corresponding adult fermentation ratio is 55:18:11:6, which is consistent with postnatal microbial colonization of the human neonatal colon (Rasmussen *et al.* 1988).

The occurrence of microbial breakdown of carbohydrate and protein to SCFA in the human adult colon has been evaluated by obtaining large-intestine stool samples and portal, hepatic and peripheral venous blood samples from sudden-death victims, within 4 h of death (Cummings *et al.* 1987). Total SCFA concentrations (mmol/l) are low in the terminal ileum (13 (1SE 6)) and high in the colon, ranging from 131 (1SE 1) in the caecum to 80 (1SE 11) in the descending colon. These findings demonstrate a significant decreasing trend from caecum to descending colon, with an associated trend of increasing pH from the caecum (5·6 (1SE 0·2)) to the descending colon (6·6 (1SE 0·1)). Acetate, propionate and butyrate production, as well as branched-chain fatty acid production, is found. Total SCFA concentrations measured in the blood (mmol/l) are portal (375 (1SE 70)), hepatic (148 (1SE 42)) and peripheral (79 (1SE 22)). In this study, the molar ratios for the three principal SCFA recovered at the different sites tested indicate a preferential and greater uptake of butyrate (over other SCFA) by the colonic epithelium (Cummings *et al.* 1987).

Influence of dietary substrate on fermentation patterns

When attempting to manipulate colonic levels of butyrate it should be noted that

butyrate administered orally is absorbed before reaching the colon (Young & Gibson, 1994). Tributyrin and butyrate esters taken orally increase the butyrate concentration in the proximal colon of animals, but these compounds are expensive (Young & Gibson, 1994). A more physiological approach to increasing the butyrate concentration in the lumen of both the proximal and distal colon is by dietary manipulation which relies on bacterial fermentation of fibre (Topping *et al.* 1993). Butyrate is known to inhibit histone deacetylase leading to histone hyperacetylation *in vitro* (Boffa *et al.* 1978). In a study which modulated the concentration of butyrate in rat distal colon *in vivo*, a significant correlation between lumen butyrate levels and histone hyperacetylation was demonstrated in colonocytes (Boffa *et al.* 1992). These investigators were able to modulate colon butyrate levels by adding wheat bran to the diet. It appears, therefore, that dietary chemo-prevention (the ability to manipulate colonic butyrate levels with diet) may be a feasible goal (Jacobs, 1988).

Fermentation of some types of fibre consistently leads to increased proportions of butyrate production. Fermentation of soyabean fibre leads to higher proportions of butyrate and propionate than do citrus pectin, sugarbeet fibre, pea (*Pisum sativum*) fibre, and oat fibre (Titgemeyer *et al.* 1991). Fermentation of gums produces more butyrate and propionate than apple pectin (Titgemeyer *et al.* 1991). Oat bran, but not a β-glucan-enriched oat fraction, enhances butyrate production in the pig large intestine (Knudsen *et al.* 1993). This study indicated that arabinoxylan, and not β-glucan, in the cell walls of oat bran is responsible for the enhanced butyrate production of oat bran. Maize-starch fermentation by human colonic faecal suspensions yields more butyrate than cabbage fibre fermentation in samples obtained from twenty human subjects (Weaver *et al.* 1992). Slowly-fermentable fibre such as wheat bran is more effective than completely-fermentable fibre such as oat bran in producing high levels of butyrate throughout the proximal and distal colon in rats (McIntyre *et al.* 1991) and human subjects (Kashtan *et al.* 1992). Therefore, it may be possible to manipulate fibre sources in the diet to achieve desired amounts of total and individual SCFA production in the colon. Moreover, most of the studies indicate that variations in SCFA production are more likely to be related to the type of substrate fermented than to the individual tested.

SHORT-CHAIN FATTY ACID ABSORPTION AND EFFECTS ON WATER AND ELECTROLYTES

SCFA are weak acids with pK_a of 4·75, 4·87, and 4·81 for acetate, propionate, and butyrate respectively. More than 90% of the SCFA produced in the mammalian hindgut exist in anionic (or dissociated) form. These compounds constitute the major anions in the fluid phase of the colonic lumen (Engelhardt & Rechkemmer, 1983). SCFA are rapidly absorbed by the colonic mucosa *in vivo*, and enhance colonic Na, Cl^-, Ca and fluid absorption, and K secretion (Argenzio *et al.* 1977; McNeil *et al.* 1978; Argenzio & Whipp, 1979; Umesaki *et al.* 1979; Ruppin *et al.* 1980; Hoverstad, 1986; Binder & Mehta, 1989, 1990; Longo *et al.* 1991; Lutz & Sharrer, 1991; Sellin *et al.* 1993). This SCFA absorption in association with fluid and electrolyte fluxes are noted in horse (Argenzio *et al.* 1977), pig (Argenzio & Whipp, 1979), goat (Argenzio *et al.* 1975), sheep (Rübsamen *et al.* 1982), dog (Herschel *et al.* 1981), rabbit (Marty & Vernay, 1984), rat (Umesaki *et al.* 1979) and human subjects (Ruppin *et al.* 1980; Hoverstad, 1986; Bowling *et al.* 1993).

Absorption of short-chain fatty acids: proximal v. *distal colon*

Regional differences in the absorption of SCFA are noted in several experimental models (Ruppin *et al.* 1980; Engelhardt & Rechkemmer, 1983; Jacobs, 1983; Luciano *et al.* 1984; Stevens, 1988; Binder & Mehta, 1989; Sellin *et al.* 1993) including pig (Argenzio & Whipp, 1979), rabbit (Vernay, 1987), and guinea-pig (Rechkemmer *et al.* 1988). SCFA absorbed from the gut are transported via the portal vein and become important metabolic fuels for the liver (Topping *et al.* 1993).

Animal studies suggest that absorption of SCFA in the proximal colon takes place via three mechanisms: (1) the passive absorption of the lipid-soluble undissociated form, probably coupled to a Na^+–H^+ exchange; (2) a $SCFA^-$–HCO_3^- exchange; (3) the diffusion of the anionic form through paracellular 'leaky spots' (when transepithelial conductance is markedly increased by some secretory stimuli; Argenzio & Whipp, 1979; Luciano *et al.* 1984; Rechkemmer *et al.* 1988; Engelhardt, 1995). In the proximal colon (see Fig. 1(a)), 50% of SCFA are transported in the dissociated form and 50% in the undissociated form, according to measurements of diffusion potentials across isolated epithelium of guinea-pigs (Rönnau *et al.* 1989). In the distal colon, the absorption of SCFA was found to be primarily based on diffusion of the free acids in the lipid-soluble undissociated form (Ruppin *et al.* 1980; Engelhardt, 1995; see Fig. 1(b)). Non-ionic diffusion probably takes place after protonation of the SCFA provided by the Na^+–H^+ and H^+–K^+ exchange mechanisms on the apical membrane (Jacobs, 1983). The source of the H^+ needed for the absorption of the SCFA in the undissociated form is believed to be supplied by the Na^+–H^+ exchange and the HCO_3^- gain system (Engelhardt & Rechkemmer, 1983), from the K^+–H^+ pump in the apical membrane of the distal colon (Rechkemmer *et al.* 1988), and a small proportion provided by the bulk lumen solution.

Effect of lumen pH in short-chain fatty acid absorption

In the pH partition hypothesis (Jacobs, 1940; Shore *et al.* 1957; Hogben *et al.* 1959) the absorption of SCFA increases with decreasing pH because of increased concentrations of the lipid-soluble undissociated form of the SCFA. Simultaneously-perfused proximal and distal segments of colon used in anaesthetized guinea-pigs (Rechkemmer & Engelhardt, 1988) demonstrated that the clearance of SCFA was independent of bulk lumen pH, between pH 6·2 and 8·1 in both the proximal and distal colon. There was slightly higher clearance of SCFA at pH below 6. These unexpected findings were attributed to the existence of a presumed constant pH micro-climate at the surface of the colonic epithelium (Rechkemmer *et al.* 1986; Rechkemmer & Engelhardt, 1988). The pH of the micro-climate was found to be independent of the bulk lumen pH and close to neutral in both the proximal and distal colon, pH 7·08 and 6·91 respectively (Rechkemmer *et al.* 1986).

Effect of short-chain fatty acid chain length on absorption

According to data compiled on the partition coefficient of weak electrolytes, the permeability of SCFA should increase by 3- –5-fold with each additional CH_2 group if absorption is primarily in the lipid-soluble undissociated form (Walter & Gutknecht, 1984). However, the absorption of SCFA in the proximal colon shows little relationship to chain length, indicating that lipid solubility at physiological pH plays only a partial

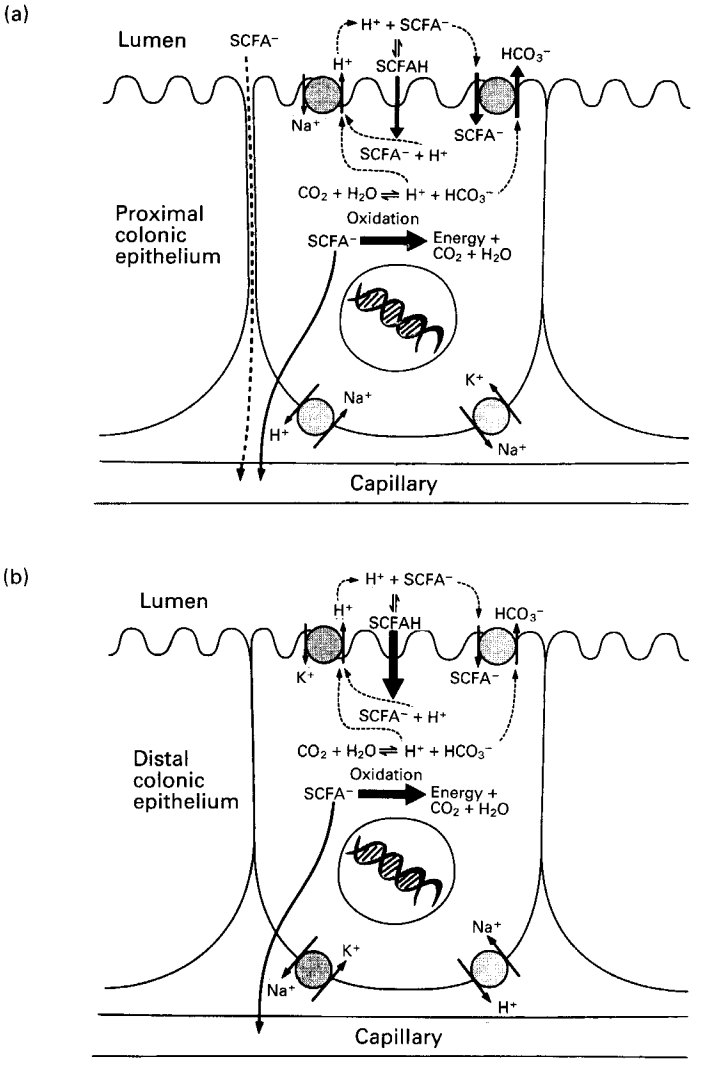

Fig. 1. Simplified, composite model of absorption and metabolism of short-chain fatty acids (SCFA) in (a) the proximal colonic epithelium and (b) the distal colonic epithelium. $SCFA^-$, dissociated form; SCFAH, undissociated form. In the proximal colonic epithelium the two main mechanisms for absorption are (1) passive diffusion of the lipid-soluble SCFAH coupled to Na^+–H^+ exchange and (2) the transport of the ionic form via an $SCFA^-$–HCO_3^- exchange. A third, less-important mechanism seen is the paracellular transport of the $SCFA^-$ in special conditions where transepithelial conductance is increased by secretory stimuli. In the distal colonic epithelium the main mechanism of absorption is passive diffusion of the lipid-soluble SCFAH coupled to Na^+–H^+ exchange. (Adapted and reproduced with permission from Engelhardt, 1995.)

role. The permeability of the proximal colon is higher for acetate, equal for propionate, and lower for butyrate when compared with the permeability in the distal colon. It is believed that these permeability differences can be accounted for by the partial absorption of SCFA in the dissociated form in the proximal colon (Engelhardt, 1995).

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Effects of short-chain fatty acids on water and electrolyte absorption

In vitro studies using isolated pig mucosa from the proximal and distal colon show that Na absorption is enhanced in the proximal colon with the addition of SCFA. This stimulation is concentration-dependent and follows Michaelis-Menten kinetics (Holtug *et al.* 1992).

Compared with the distal colon the proximal colon has twice the capacity to absorb Na and twice the capacity to secrete H^+ in the presence of SCFA (Roediger *et al.* 1986). These observations support the hypothesis that enhancement of Na absorption by free fatty acids is coupled to a Na^+–H^+ exchange mechanism in the proximal colon (Wrong, 1981; Engelhardt, 1995). The activity of carbonic anhydrase (*EC* 4.2.1.1) is important in supplying H^+ for the coupled absorption of Na and SCFA (Rübsamen *et al.* 1982; Engelhardt, 1995) (see Figs. 1 (a and b)).

An *in vivo* lumen perfusion technique in rat colon demonstrates that in the proximal colon butyrate significantly enhances Na and water absorption, while not influencing Ca absorption (Lutz & Sharrer, 1991). In the distal colon, however, butyrate and acetate significantly increase Ca absorption, while Na and water absorption is not affected. It is postulated that a Ca–H exchanger mechanism exists at the apical membrane of the distal colon which mediates Ca uptake into the epithelial cell.

Short-chain fatty acids and enteral-feeding-related diarrhoea

Evidence suggests that colonic secretion of water and electrolytes plays an important role in the pathogenesis of enteral-feeding-related diarrhoea (Bowling *et al.* 1993). Whether or not fibre should be added to artificial enteral nutrition is an issue that continues to be studied (Silk, 1989; Scheppach *et al.* 1990; Scheppach & Bartram, 1993). Initial work on SCFA and colonic fluid and electrolyte movement indicated that SCFA are involved in causing diarrhoea and producing fluid and electrolyte secretion (Bustos-Fernandez *et al.* 1971). Subsequent work, however, showed that SCFA are not involved in the pathogenesis of diarrhoea, and are responsible for compensatory and conservation mechanisms in some diarrhoea disorders as the result of their stimulation of Na, Cl^-, and colonic fluid absorption (Ruppin *et al.* 1980; Bowling *et al.* 1993). In fact, prolonged lack of SCFA probably plays an important role in Na malabsorption in the defunctionalized colon (Roediger *et al.* 1986). The removal of anions for more than 48 h converts the colonic mucosa from an absorptive to a secretory state (Roediger, 1992). The colonic secretion of water and electrolytes induced by the osmotic load of enteral feeding may be inhibited in healthy human volunteers by caecal infusion of SCFA mixtures in physiological concentrations (mmol/l; acetate 50, propionate 20, butyrate 20; Bowling *et al.* 1993).

Possible mechanisms for the effects of short-chain fatty acids on fluid and electrolytes

The mechanisms by which SCFA exert the aforementioned effects on fluid and electrolyte fluxes are not fully understood, but are thought to be dependent, at least in part, on CO_2 generation from the oxidation of butyrate (Roediger, 1982). Of significance are the findings that butyrate is an important energy source and the preferred fuel for the colonic epithelium (Roediger, 1982; Butler *et al.* 1990). Other mechanisms probably include the butyrate-induced up-regulation of Na^+–H^+ transport by inducing the

transcription of mRNA for the Na^+–H^+ exchanger (Bishop *et al.* 1992). Also, the induction of other key genes encoding for membrane ATPase ion exchangers may be involved. For example, induction of normal colonocytes by sodium butyrate resulted in a 2-fold increase in the amount of mRNA expression for the gene encoding the Na^+/K^+-ATPase (*EC* 3.6.1.37) α-subunit (Chehab *et al.* 1987). Hormonal, paracrine and autocrine factors may also play a role. In isolated rabbit distal colon, *n*-butyrate (10 mM) causes a stepwise concentration-dependent increase in peptide YY (PYY). PYY inhibits gastric acid and pepsin secretion, and increases gastric emptying and the mouth-to-caecum transit time in human subjects (Adrian *et al.* 1985).

METABOLISM

Several studies have shown that SCFA are the preferred fuel for colonocytes (Cummings, 1981; Roediger, 1982; Engelhardt & Rechkemmer, 1983; McNeil, 1984; Marty & Vernay, 1984; Awad *et al.* 1990; Topping *et al.* 1993). The preference of colonocytes for butyrate as an energy substrate appears to be a tissue-specific phenomenon that is greatest in the rectum and distal colon and less pronounced in the caecum (Windmueller & Spaeth, 1978; Roediger, 1982). This is interesting, in the light of the predominant anatomical distribution of colon cancer in the distal colon and rectum.

Normal colonocytes rapidly metabolize *n*-butyrate to CO_2 and ketones (Roediger, 1980*a*). *In vitro*, normal rat colonoctyes utilize SCFA in the following order of preference: butyrate > acetate > propionate (Awad *et al.* 1990). It has been postulated that the shift from aerobic to anaerobic metabolism that takes place with neoplastic transformation might lead to the inability to oxidize *n*-butyrate (Jass, 1985). The resulting accumulation of butyrate in the cytoplasm of the neoplastic colonocyte plus altered affinity of certain mutated proteins may be the cause of increased sensitivity to butyrate and the paradoxical effect of butyrate on neoplastic *v.* normal cells.

EFFECTS ON COLONIC MOTILITY

An *in vitro* study using isolated rat colon examined the contractile effect of SCFA on proximal, middle, and distal segments of the colon. Butyrate effectively stimulated colon contraction at 0·1 mM. The data suggest that butyrate may stimulate colonic contractions via an enteric reflex involving local sensory and cholinergic nerves (Yajima, 1985). The middle and distal colon showed a stimulatory effect of butyrate on contractile response. However, the proximal colon lacked any contractile response to either butyrate or other SCFA. The data suggest that butyrate is probably one of the important physiological lumen stimuli regulating colonic motility.

EFFECTS ON COLONIC BLOOD FLOW

SCFA infused into autoperfused dog colon in physiological concentrations induce a 24% increase in colonic blood flow (Kvietys & Granger, 1981). Dietary manipulations that increase caecal SCFA concentrations lead to an increase in caecal blood flow in rats (Demigné & Rémésy, 1985). Acetate is the primary cause of these blood flow effects in the dog model. It is postulated that a direct vasodilatory effect of SCFA may mediate this stimulation of blood flow (Rombeau *et al.* 1990).

EFFECTS ON COLONIC ANASTOMOTIC HEALING

Intralumen infusion of SCFA mixture (mmol/l; acetate 75, propionate 35, butyrate 20) in rats significantly increases colonic anastomotic strength (Rolandelli *et al.* 1986). This SCFA-mediated enhancement of colonic anastomosis healing is thought to be the result of increased mucosal proliferation (Sakata & Engelhardt, 1983) and stimulation of colonic blood flow with enhanced uptake of O_2 (Kvietys & Granger, 1981).

IMPLICATIONS FOR ULCERATIVE COLITIS

Studies suggest that the inhibition of fatty acid oxidation in ulcerative colitis may be of pathogenic importance (Butzer *et al.* 1995). Details of the proposed pathogenic model for ulcerative colitis and the relevance for butyrate have been reviewed by Gibson & Pavli (1992). Lack of SCFA in the colonic lumen leads to a form of 'nutritional colitis' (Roediger, 1980*b*). In fact, decreased faecal concentrations of SCFA are noted in patients with ulcerative colitis, but not in those with Crohn's colitis (Vernay, 1987). The distal colonic epithelium in patients with ulcerative colitis exhibits an impaired ability to oxidize butyrate *in vitro* (Roediger, 1980*b*) and *in vivo* (Roediger *et al.* 1984). This evidence suggests that colonic epithelium in ulcerative colitis does not fully compensate for its energy deficiency through use of alternative substrates (Kameyama *et al.* 1984). Histopathologically, the lesions of diversion colitis and ulcerative colitis are indistinguishable (Glotzer *et al.* 1981). The chemically-induced inhibition of fatty acid oxidation in experimental animals produces mucosal inflammation very similar to that of ulcerative colitis (Glotzer *et al.* 1981). The predominant dependence of the distal colon on butyrate as its source of energy may be the explanation for the characteristic distribution of ulcerative colitis (Roediger, 1980*b*). Butyrate enemas (100 mmol/l) were used to treat ten patients with ulcerative colitis in a placebo-controlled single-lined randomized trial (Scheppach *et al.* 1992*b*). Findings from this study support the view that butyrate deficiency may play a role in the pathogenesis of distal ulcerative colitis and that butyrate irrigation is beneficial in the treatment of this condition. Study variables in this 2-week study included stool frequency, discharge of blood per rectum, endoscopic score, histological degree of inflammation and upper crypt labelling index, all of which were significantly reduced in patients treated with the butyrate enemas. The control of fatty acid oxidation probably plays an essential role in the development and therapy of active ulcerative colitis (Roediger *et al.* 1986). In an *in vitro* study of human colonic epithelial cells from patients with ulcerative colitis incubated with butyrate, there was a significant increase in protein synthesis (Frankel *et al.* 1994). The authors postulated that the therapeutic effects of butyrate in patients with ulcerative colitis may be due to its use as a metabolic fuel to increase protein production and, thus, promote healing (Frankel *et al.* 1994).

IMPLICATIONS FOR DIVERSION COLITIS

SCFA deficiency induced by diverting the faecal stream usually results in the development of diversion colitis. This colitis may be reversed by the instillation of butyrate and other SCFA into the diverted colon (Harig *et al.* 1989). Therefore, it seems that SCFA are essential in maintaining the integrity of the colonic mucosa. Four patients with diversion colitis were treated with colonic irrigations of SCFA mixtures (mmol/l; acetate

60, propionate 30, butyrate 40) for 2–3 weeks. This therapy resulted in macroscopic and histological resolution of inflammation (Harig *et al.* 1989). However, in a prospective 2-week double-blind study using rectal enemas of SCFA (mmol/l; acetate 60, propionate 30, butyrate 40) twice daily for 14 d in fourteen patients, there was no significant endoscopic and histological improvement (Guillmot *et al.* 1991). The authors concluded that the heterogeneity of the bacterial population in different types of excluded colon or inadequate total amount of SCFA infusate may have accounted for these negative findings. The aetiology of diversion may be another reason for conflicting results.

IMPLICATIONS FOR COLON CANCER

Increased ingestion of fibre-rich diets is associated with a low risk of colon cancer (Jacobs, 1986; McIntyre *et al.* 1993). Human epidemiological studies indicate an inverse correlation between fibre consumption and colon-cancer rates (Jacobs, 1986). Statistical analysis of thirteen case–control studies summarizing data records for 5287 subjects with colon cancer and 10 470 control subjects were pooled for analysis of the relationship between dietary fibre intake and colo-rectal cancer risk (Howe *et al.* 1992). An inverse association was seen in twelve of the thirteen studies, thus providing substantive evidence that intake of fibre-rich foods is associated with a decrease in colo-rectal cancer risk. If causality is assumed, 31% of all colo-rectal cancers could be prevented by an average increase in fibre intake of about 13 g/d (Howe *et al.* 1992).

Important factors that have been emphasized in the literature include: the carcinogenic effects of bile acids, high colon lumen pH, and the protective role of SCFA, particularly butyrate (Jacobs, 1988; Van Munster & Nagengast, 1993). *In vivo* rat studies demonstrate that dietary fibre and fat mediate cell proliferation of the colon in an interactive, site-specific manner (Lee *et al.* 1993). *In vivo* human studies indicate that a short-term increase in dietary fat and decrease in dietary fibre does not increase colonocyte proliferation rate, thus suggesting that long-term rather than acute exposure might be of significance in human subjects (Gregoire *et al.* 1991).

The products of fibre fermentation within the colonic lumen are probably key factors in protection against colonic carcinogenesis (Van Munster & Nagengast, 1993), although there may be many other factors. Butyrate is one of the most significant products of fermentation since it exhibits anti-neoplastic properties both *in vitro* and *in vivo* (Kim *et al.* 1980; Tsao *et al.* 1982; Dexter *et al.* 1984; Whitehead *et al.* 1986; Czerniak *et al.* 1987; Otaka *et al.* 1989; Young, 1990; Gibson & Pavli, 1992; Scheppach *et al.* 1992*a*; Souleimani & Asselin, 1992; Bartram *et al.* 1993, 1995; McIntyre *et al.* 1993; Van Munster & Nagengast, 1993; Scheppach, 1994). Patients with adenomatous polyps or colon cancer have lower rates of butyrate fermentation than normal subjects (Weaver *et al.* 1988), but whether altered levels of butyrate are merely an associated or causative factor in colon cancer remains in question (Bradburn *et al.* 1993).

The experimental data using butyrate as a direct dietary supplement either to treat or prevent colonic neoplasia has been discouraging. An *in vivo* study using 10–20 g sodium butyrate/l in the drinking water of rats failed to demonstrate any anti-neoplastic effect in 1,2-dimethylhydrazine-induced rat intestinal neoplasia (Freeman, 1986). In another *in vivo* study, butyrate was introduced in the diet of rats treated with the carcinogen azoxymethane. In this work, butyrate also failed to protect against the development of aberrant crypts, which was the anti-neoplastic end-point examined (Wargovich *et al.*

1992). These results should be interpreted with caution since orally-ingested butyrate is almost completely absorbed in the small intestine before reaching the colon (Young & Gibson, 1994).

However, the *in vivo* effects of diets rich in insoluble fibre as a substrate precursor to increase colonic butyrate levels are promising. Wheat-bran-rich diets were studied in rats throughout periods of tumour initiation and promotion (Young, 1990). This study demonstrated that wheat-bran-rich diets protected against tumour growth. Other studies which examined diets rich in soluble fibre such as guar gum or pectin (which are known not to increase the colonic butyrate concentrations), failed to demonstrate a protective effect against tumourigenesis (McIntyre *et al.* 1991).

In a rat colon-cancer model, increased faecal levels of butyrate (an index of distal colon butyrate levels) are induced by dietary manipulations (wheat-bran-rich diets) and are associated with a significant decrease in tumour size *in vivo* (McIntyre *et al.* 1993). In human subjects, the ingestion of processed wheat bran in patients with familial adenomatous polyposis decreases the incidence of adenoma formation in the remaining rectum within 9 months of follow up (DeCosse *et al.* 1989).

It is not possible to draw definitive conclusions regarding butyrate effects from experiments using diets enriched with wheat fibre since other aspects of the diet may be confounding variables; however, the data are encouraging and certainly lend validity to the *in vitro* findings on the anti-neoplastic effects of butyrate.

BUTYRATE EFFECTS ON PROLIFERATION: NORMAL *v.* NEOPLASTIC COLONOCYTES

Although butyrate decreases proliferation of neoplastic colonoctyes *in vitro* (Kim *et al.* 1980; Tsao *et al.* 1982; Whitehead *et al.* 1986; Czerniak *et al.* 1987; Colony, 1989; Awad *et al.* 1991; Barnard & Warwick, 1992; Souleimani & Asselin, 1992), and *in vivo* (DeCosse *et al.* 1989; McIntyre *et al.* 1993), it increases proliferation of normal colonic epithelium *in vitro* (Gibson & Pavli, 1992) and *in vivo* (Sakata, 1984, 1987; Kripke *et al.* 1989; see Table 1).

Trophic effects on normal colonic epithelium

Ample evidence exists to show that butyrate in the colonic lumen plays an important role in mucosal growth and epithelial proliferation. A reduction in lumen butyrate by

Table 1. *The paradoxical effects of butyrate on cell proliferation and differentiation of normal and neoplastic colonic epithelial cells* (Reproduced with permission from Young & Gibson, 1995)

	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

decreased delivery of fermentable substrate to the large intestine induces colonic mucosal atrophy (Janne *et al.* 1977; Goodlad & Write, 1983; Saka, 1988). Subsequent instillation of SCFA into the colonic lumen induces mucosal regeneration as shown by increased mucosal weight and DNA content, increased crypt length, and increased mitotic index (Sakata, 1984; Kripke *et al.* 1989). Of the three major SCFA, these effects on colonic mucosal proliferation are thought to be mostly due to butyrate (Sakata, 1987; Kripke *et al.* 1989). In an *in vivo* rat model, both intravenous and intracolonic infusions of SCFA significantly reduce the mucosal atrophy associated with long-term total parenteral nutrition (TPN; Koruda *et al.* 1990). Another *in vivo* study using rats maintained on TPN shows that infusion of a SCFA mixture (acetate–propionate–butyrate; 60:25:15 by vol.) into the proximal colon significantly increases colonic mucosal height and colonic mucosal DNA (Freidel & Levine, 1992). A diet rich in wheat bran fed to rats significantly increases the concentration of butyrate throughout the colon (Lupton & Kurtz, 1993). The colonic lumen 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/l) are lumen trophic factors for the human caecal epithelium (Scheppach *et al.* 1992*a*).

The mechanisms by which butyrate enhances normal colonic mucosal proliferation are not well understood. There is evidence that butyrate exerts an indirect systemic effect since colonic lumen SCFA mixtures stimulate proliferation not only in colonic mucosa, but also in unexposed adjacent colonic epithelium, ileum and jejunum (Sakata & Engelhardt, 1983; Sakata, 1987; Kripke *et al.* 1989; Frankel *et al.* 1994). Infusion of SCFA into the colonic lumen stimulates growth in unperfused isolated denervated loops of jejunum in rats (Sakata, 1987). However, butyrate probably also has a direct effect on proliferation, since it stimulates epithelial proliferation in short-term organ culture of human colonic mucosa in the absence of circulating or neural factors (Scheppach *et al.* 1992*a*). A more detailed discussion of the possible mechanisms of action for butyrate on proliferation will be discussed later (see p. 64).

Inhibitory effect on neoplastic colonocyte growth

Butyrate inhibits DNA synthesis and arrests the growth of neoplastic colonocytes in the G1 phase of the cell cycle (Toscani *et al.* 1988). These effects are shown in multiple cultured tumour cell lines (Young & Gibson, 1994). The concentrations used to produce these effects are not toxic to cells. RNA and protein synthesis 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/l (Whitehead *et al.* 1986). Butyrate at a concentration of 1 mmol/l increases cell doubling time from 26 to 72 h and decreases the cloning efficiency from 1·1 to 0·05% (Whitehead *et al.* 1986). In this study, acetate and propionate showed only minimal effects of proliferation. Similar inhibition of proliferation is noted in several other *in vitro* studies using colo-rectal cancer cell lines such as SW480, SW620, HRT-18, HT-29, CaCo-2 and LS180 (Kim *et al.* 1980; Tsao *et al.* 1982; Whitehead *et al.* 1986; Czerniak *et al.* 1987; Colony, 1989; Barnard & Warwick, 1992; Souleimani & Asselin, 1992). Anchor-dependent growth is inhibited by butyrate at a concentration of 2 mmol/l in the HRT-18 cell line, under conditions which do not affect cell viability (Tsao *et al.* 1982). Withdrawal of butyrate from the culture medium rapidly

reverses the effects on proliferation. Higher concentrations (5 mmol/l) lead to increases in cell toxicity and decreases in cell viability (Tsao *et al.* 1982).

Inhibition of pre-malignant crypt surface hyperproliferation

Patients at risk for increased colon cancer (e.g. familial polyposis coli and Gardner's Syndrome) have pre-malignant colonocyte proliferation with a shift of the proliferative zone from the crypt base to the upper 40% of the crypt (Lipkin, 1988; Deschner *et al.* 1990). In endoscopically-obtained biopsies from normal human mucosa, incubation with butyrate significantly increases colonic crypt cell proliferation at the physiological concentration of 10 mmol/l. Butyrate-induced proliferation (observed only in the basal 60% of the crypt) is considered to be the zone of physiological crypt cell proliferation and not a pre-malignant event (Scheppach *et al.* 1992*a*). In follow-up *in vitro* studies also using normal human colonic mucosa, deoxycholate-induced crypt surface (pre-malignant) hyperproliferation was inhibited by co-incubation with butyrate (Bartram *et al.* 1993).

BUTYRATE EFFECTS ON DIFFERENTIATION: NORMAL *v.* NEOPLASTIC COLONOCYTES

While butyrate induces differentiation of neoplastic colonocytes *in vitro* (Kim *et al.* 1980; Dexter *et al.* 1984; Whitehead *et al.* 1986; Awad *et al.* 1991; Barnard & Warwick, 1992) and *in vivo* (Otaka *et al.* 1989), it decreases, or has no significant effect on, the expression of differentiation markers in normal colonocytes *in vitro* (Gibson & Pavli, 1992) and *in vivo* (Young, 1991; Young & Gibson, 1995; see Table 1).

It has been suggested that differentiation is a state in which a cell is locked in the G1 phase of the cell cycle where components necessary for mature cellular function are expressed in an orderly manner (Higgins, 1989). Neoplasia is thought to arise when a series of defects (usually at the genomic level) lead to the ability of the cell to escape the state of terminal differentiation (Kim *et al.* 1980). Butyrate has generated great interest in its ability to lock colonocytes in the G1 phase and induce a morphologically differentiated phenotype (Whitehead *et al.* 1986; Young & Gibson, 1994).

Induction of differentiation markers in neoplastic colonocytes

In vitro *modulation of the expression of brush-border hydrolases*. Brush-border-membrane enzymes such as alkaline phosphatase (*EC* 3.1.3.1) are considered markers of differentiation, and their expression decreases with neoplastic transformation (Bell & Williams, 1979; Young *et al.* 1992). Evidence obtained from a study of brush-border hydrolases in normal and neoplastic human colonic epithelium biopsies demonstrate a low expression of brush-border hydrolases in adenomas, which suggests a block in the differentiation process early in colo-rectal carcinogenesis (Young *et al.* 1992). Patchy re-expression of brush-border hydrolases as well as a loss of cellular polarity is noted in the carcinomas, suggesting a subsequent derepression of gene expression for these enzymes. A specific form of alkaline phosphatase (human placental-like alkaline phosphatase (PLAP)) is induced by sodium butyrate in several colo-rectal adenocarcinoma cell lines in culture (Kim *et al.* 1980; Herz *et al.* 1981; Morita *et al.* 1982; Tsao *et al.* 1982; Ito & Chou, 1984; Chung *et al.* 1985).

Studies with Lim 1215 cultured colon cancer cells and butyrate at concentrations of 1 mmol/l demonstrate a 600% increase in the activity of alkaline phosphatase. Acetate and propionate have similar but less marked effects. Both proliferative effects and effects on alkaline phosphatase activity occur at the same time in culture, suggesting a possible link between differentiating and anti-proliferative effects (Whitehead *et al.* 1986). Associated with these effects on proliferation and differentiation are phenotypic changes such as higher cytoplasm:nucleus values. In HT-29 cells, incubation with butyrate rapidly induces 'enterocytic-like' differentiation and growth inhibition while concomitantly inducing alkaline phosphastase mRNA (Barnard & Warwick, 1992).

The *in vitro* effects of butyrate on brush-border-membrane hydrolases were studied using fourteen different colon-cancer cell lines (Chung *et al.* 1985). Butyrate increased the activity of alkaline phosphatase in ten of the fourteen cell lines examined, in magnitudes ranging from 2- to 123-fold increases. This effect on alkaline phosphatase activity is inhibited by the addition of cycloheximide (in the HRT-18 cell line), thus providing evidence that this increase is secondary to new protein synthesis rather than gradual protein breakdown (Tsao *et al.* 1982). The investigators reported a significant correlation between induction of alkaline phosphatase activity and the degree of morphological differentiation.

In vivo *modulation of the expression of brush-border hydrolases*. Using encapsulated liposomes that are covalently linked to an anti-Le^X monoclonal antibody (Otaka *et al.* 1989), selective *in vivo* delivery of butyrate was accomplished in nude mice bearing human colon adenocarcinoma tumours. Increases in membrane-bound alkaline phosphatase and γ-glutamyl transpeptidase (*EC* 2.3.2.2) were noted, indicating the occurrence of *in vivo* butyrate-induced differentiation of neoplastic colonocytes. However, when large-bowel lumen butyrate was experimentally increased in rats by feeding a wheat-bran-rich diet, no significant *in vivo* effects were documented on the expression of the brush-border hydrolases, alkaline phosphatase and dipeptidyl peptidase IV (*EC* 3.4.14.5), by the normal colonic mucosa (Young, 1991).

Contrasting effects on normal colonocytes

When cells harvested from microscopically normal human colonic mucosa (obtained from cancer-bearing colon) are cultured *in vitro*, incubation with butyrate does not show a reduction in the rates of energy-consuming processes such as DNA, protein, and glycoprotein synthesis. Furthermore, total DNA content is not altered (Gibson *et al.* 1991). Moreover, the expression of differentiation markers significantly decreases in these cells after a 24 h incubation with butyrate, suggesting that it does not enhance, but may suppress, differentiation of normal colonocytes (Gibson *et al.* 1991). This paradoxical effect of butyrate on normal and neoplastic colonocytes and their differentiation is unlikely to be artifactual (Young & Gibson, 1994).

BUTYRATE-INDUCED MODULATION OF GENE EXPRESSION

Multi-stage colo-rectal carcinogenesis

The multi-stage process of carcinogenesis in the aetiology of colon cancer (Fearon & Vogelstein, 1990) is now well accepted. There seems to be a step-like progression from

normal epithelium, to hyperproliferative epithelium, to aberrant crypts, to various stages of dysplasia (seen in adenomas), to pre-invasive and finally, to invasive and metastatic carcinomas (Willson, 1989; Fearon & Vogelstein, 1990). Data at this time suggest that this multi-step process results from an accumulation of genetic alterations (Fearon & Vogelstein, 1990). The known series of genetic alterations in the development of human colo-rectal carcinoma include the familial adenomatous polyposis coli-mutated in colon cancer locus (FAP/MCC) on chromosome 5, the DCC (deleted in colo-rectal cancer) gene on chromosome 18, and the p53 gene on chromosome 17 (Young & Gibson, 1994). A mutation of the *ras* proto-oncogene is thought to be aetiologically important as well. Mutations in the FAP/MCC gene may be inherited, whereas the other previously mentioned genetic events are acquired in random order (Young & Gibson, 1994). These genetic changes, whether acquired or inherited, express themselves as disordered protein synthesis, and thus alter cellular growth and differentiation. The p53 gene is commonly involved in colo-rectal carcinoma, but not adenomas (Fearon & Vogelstein, 1990), and may be important in normal cells as a tumour suppressor gene. A deletion of the allelic pair, or a mutation that produces an oncogenic form, may provide a selective growth advantage or the ability to invade (Fearon & Vogelstein, 1990). In intermediate and late adenomas, a mutation of the *ras* proto-oncogene is a common finding. It is possible that this mutation may initiate events in some colo-rectal carcinomas, or may be responsible for increasing dysplasia in some adenomas (Young & Gibson, 1994). Other oncogenes are implicated in colo-rectal carcinogenesis such as the amplification and gene re-arrangements of c-*myc*, c-*myb*, *src*, and the *trk* (Foss *et al.* 1989; Fearon & Vogelstein, 1990).

The effects of butyrate on the expression of FAP/MCC, DCC, and p53 proto-oncogenes 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 colo-rectal cell lines *in vitro* (Young & Gibson, 1994). Table 2 summarizes the major effects of butyrate on key molecular events relevant to large-bowel neoplasia (Young & Gibson, 1995).

Table 2. *Summary of effects of butyrate on molecular events relevant to large-bowel neoplasia* (Reproduced with permission from Young & Gibson, 1995)

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 and B and K alkaline phosphatase (*EC* 3.1.3.1)	1p	Activated or induced (many cell lines)

L and B and K alkaline phosphatase, liver, bone and kidney alkaline phosphatase, the form expressed in colon.

POSSIBLE MECHANISM OF ACTION FOR BUTYRATE EFFECTS ON COLONOCYTE GROWTH AND DIFFERENTIATION

Butyrate modulates the transcriptional expression of multiple genes (Toribara *et al.* 1989; Kruh *et al.* 1995). Butyrate has several effects on nuclear proteins through which it could modify gene expression. These effects include: (1) hyperacetylation of core histones through the inhibition of histone deacetylase (Boffa *et al.* 1978), (2) selective inhibition of the phosphorylation of histones H1 and H2 (Boffa *et al.* 1981; Kruh, 1982), (3) selective increase in the phosphorylation of H3 histone (Parker *et al.* 1985), and (4) hypermethylation of cytosine residues in DNA (Boffa *et al.* 1981; Parker *et al.* 1985; Kruh *et al.* 1994). Butyrate also enhances the acetylation and phosphorylation of non-histone proteins and increases ADP ribosylation (Rastl & Swetly, 1978; Cousenes *et al.* 1979; Christman *et al.* 1980; Boffa *et al.* 1981; Kruh, 1982; Kruh *et al.* 1994). 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 (Reeves & Dserjesi, 1979) and increased DNAase I (*EC* 3.1.21.1) sensitivity (Vidali *et al.* 1978). 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 (Boffa *et al.* 1981). The induction and inhibition of gene expression produced by DNA methylation are thought to be non-specific. However, butyrate can also specifically modulate gene expression. For example, in the human colon adenocarcinoma cell line CaCo-2, butyrate causes a direct activation of the PLAP promoter (Deng *et al.* 1992). It is suggested that butyrate acts as a cofactor of regulatory transcription proteins in the nucleus which are directly involved in gene expression (Kruh *et al.* 1994; see Fig. 2).

Alternatively, butyrate may act at the membrane–cytoplasmic interface by interacting with the G-proteins which are key components of the signal transduction pathway in cellular growth and differentiation (see Fig. 2). Mutated G-proteins are the products of activated *ras* oncogenes which are thought to play a role in early colo-rectal carcinogenesis (Bos *et al.* 1987; Vogelstein *et al.* 1988). Butyrate suppresses the transforming activity of an activated N-*ras* oncogene in cultured human colon carcinoma cells (Stoddart *et al.* 1989). Binding of the G-protein to SCFA, mevalonate (or one of its metabolic intermediates), is required for membrane translocation and subsequent *ras* protein activation in yeast and humans (Willumsen *et al.* 1984; Schafer *et al.* 1989). Colon cancer cells *in vivo* have been shown to switch from principally aerobic to anaerobic metabolism (Jass, 1985). We postulate that in the neoplastic colonocyte, butyrate (which may accumulate as a result of impaired oxidation) may compete for mevalonate (or a mevalonate intermediate)-binding and, thus, interfere with the process of membrane translocation and activation of the mutated *ras* protein. Studies are ongoing to investigate this hypothesis (see Fig. 2).

MEDIUM-CHAIN TRIACYLGLYCEROLS

Medium-chain triacylglycerols and long-chain triacylglycerols (LCT)

Recently, the use of MCT as a parenteral high-density energy source has been investigated. MCT are obtained from hydrolysed coconut oil and consist of a mixture of (mol/100 mol): $C_{6:0}$ <1, $C_{8:0}$ 65–75, $C_{10:0}$ 25–35, $C_{12:0}$ <1; Wan *et al.* (1988). MCT were

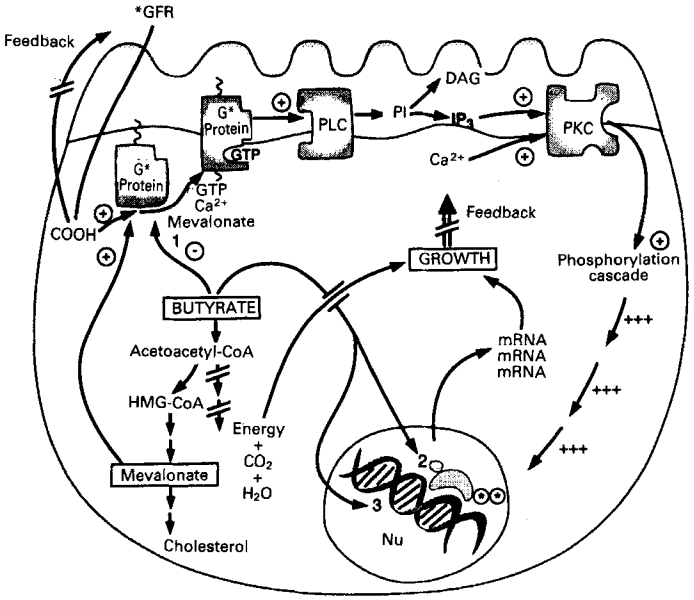

Fig. 2. Speculative mechanisms for butyrate-induced growth inhibition in neoplastic colonocytes. Evidence suggests that G proteins require binding to mevalonate or a mevalonate intermediate for translocation into the membrane and subsequent activation (Schafer *et al.* 1989). The activated G protein then stimulates the activity of protein kinase C (PKC) via the activation of phospholipase C (*EC* 3.1.4.3; PLC). The cascade of enzymic phosphorylations resulting from the activation of PKC leads to a signal for growth. Activated *ras* oncogenes encode for mutated G proteins (G*). Butyrate has been shown to inhibit the transforming action of an activated human *ras* oncogene (Stoddart *et al.* 1989). With a switch to anaerobic metabolism in the neoplastic colonocyte, butyrate may accumulate in the cytoplasm (Jass, 1985). (1) We speculate that butyrate may then compete with mevalonate (or a mevalonate intermediate) in the binding to G*, but the butyrate–G* combination results in an active form of the G* protein. Alternative mechanisms have been proposed (Kruh *et al.* 1994) that include: (2) butyrate may specifically modulate gene expression as a cofactor to the transcription proteins, and (3) non-specific modulation of gene expression via chromatin changes induced by DNA methylation and histone acetylation. GFR, growth factor receptor; HMG-CoA, hydroxymethylglutaryl-CoA; PI, phosphatidyl inositol; PI3, inositol triphosphate; DAG, diacylglycerol; Nu, nucleus.

postulated to be a more-efficient energy source than LCT because of their non-carnitine-dependent transport into the mitochondria, higher plasma clearance, and higher oxidation rate (Bohmer *et al.* 1974; Bach & Babayan, 1982). Also, animal studies demonstrated improved N-sparing effects with MCT (Maiz *et al.* 1984*b*; Mok *et al.* 1984; Sandberg *et al.* 1985; Dawes *et al.* 1986; Magnussen *et al.* 1986), and less accumulation in the reticulo-endothelial system during long-term treatment (Hamawy *et al.* 1985*b*; Sobrado *et al.* 1985). However, large doses of pure MCT emulsions are toxic, lack the important essential polyunsaturated fatty acids, and induce metabolic acidosis. In contrast, intravenous LCT emulsions are generally well-tolerated and safe.

LCT are made from soyabean or safflower vegetable oils, contain fatty acyl chains of C_{16}–C_{18} in length, and represent the standard of care in parenterally-administered fat emulsions. Lipid emulsions composed of LCT have been in use for many years in parenteral nutrition to provide a concentrated form of energy and essential fatty acids while reducing the requirement for high glucose loads.

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Metabolism of medium-chain triacylglycerols v. *long-chain triacylglycerols*

LCT-derived fatty acids must use the carnitine transport pathway to enter the mitochondria for oxidation (Mitchell, 1978). Researchers have suggested that in clinically-stressed situations, the carnitine transfer system eventually becomes saturated, leading to decreased efficiency of LCT metabolism (Bohmer *et al.* 1974; Wan *et al.* 1988). In these patients, LCT are not completely oxidized, and some long-chain fatty acids are re-esterified to triacylglycerols in the liver (Goodenough & Wolfe, 1984). In contrast, MCT undergo β-oxidation without requiring the presence of carnitine (Bremer, 1980; Bach & Babayan, 1982). Moreover, the physical properties of MCT favour normal hepatic and splenic reticulo-endothelial system function (Mascioli *et al.* 1988).

MCT have been studied in varied types of animal models. MCT infusion in burned animals was noted to reduce pulmonary sequestration of bacteria (Sobrado *et al.* 1985). In rats receiving TPN with MCT emulsions for cancer cachexia there is decreased tumour growth and lung metastases (Bartlet *et al.* 1992).

Human studies have shown that MCT are metabolized faster than LCT (Yeh *et al.* 1978; Wolfram *et al.* 1985; Bach *et al.* 1988; Deckelbaum *et al.* 1990), thereby providing a readily-available energy burst. The β-oxidation of MCT yields C_2 units and energy (Sailer & Berg, 1974). MCT can overwhelm the biochemical pathway which allows them to enter into the Krebs cycle and then be metabolized by an alternative pathway to produce ketones (acetoacetate and β-hydroxybutyrate; Sailer & Berg, 1974; Yeh & Zee, 1976; Bach *et al.* 1977). These ketone bodies are utilized as fuel by peripheral tissues (Guisard & Debry, 1972; Robinson & Williamson, 1980; Sailer & Muller, 1981). Patients stressed by surgery, burns, and shock form ketone bodies within skeletal muscle mass as an alternative form of fuel, thereby reducing proteolysis from branched-chain amino acids (Maiz *et al.* 1984*a*; Mok *et al.* 1984). For these reasons, MCT have been proposed as feasible non-carbohydrate sources of energy which may be useful in severely ill patients, especially those with glucose intolerance. However, in a study of nine healthy male subjects MCT administration led to pronounced ketogenesis without significant improvements in oral glucose tolerance (Weissman *et al.* 1988). Other human studies have reported more favourable results. In twelve adult patients requiring major abdominal surgery for gastric ulcer, gastric cancer, and colon cancer, TPN with MCT emulsions resulted in improved muscle energy utilization, improved N balance and less weight loss postoperatively compared with standard LCT emulsions (Jiang *et al.* 1993).

Absorption: long-chain triacylglycerols v. *medium-chain triacylglycerols*

Oral administration of MCT has been useful for many years in the treatment of disorders of lipid absorption (Hashim *et al.* 1960, 1962). MCT emulsions are hydrolysed efficiently in the lumen of the small intestine under conditions where LCT hydrolysis is impaired, such as in pancreatic insufficiency (see Fig. 3 (*a* and *b*)). While LCT are entirely absorbed through the lymphatic system, orally-administered MCT are hydrolysed by mucosal lipase (*EC* 3.1.1.3) and the resulting medium-chain fatty acids (MCFA) are absorbed through the intestinal capillaries. These MCFA bind to albumin and are transported to the liver via the portal venous system (Bach & Babayan, 1982; Babayan, 1987; Hashim & Tantibhedyangkul, 1987). Parenterally-administered MCT are hydrolysed by the lipoprotein lipase (*EC* 3.1.1.34) system within the capillaries and released MCFA bind to albumin and are transported to various tissues for utilization (Sailer &

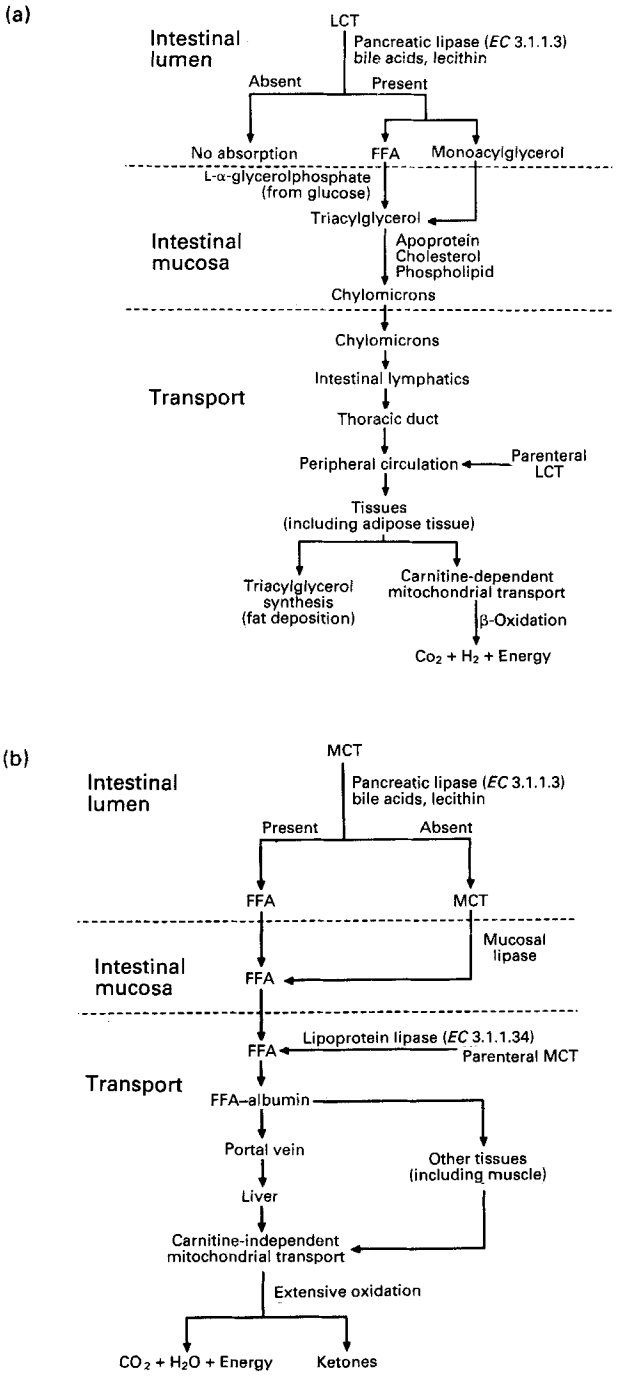

Fig. 3. Digestion, absorption, transport, and metabolism of (a) long-chain triacylglycerols (LCT) and (b) medium-chain triacylglycerols (MCT). FFA, free fatty acid. (Adapted and reproduced with permission from Hashim & Tantibhedyangkul, 1987.)

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Berg, 1974). Unlike LCT, MCT are rapidly cleared from circulation (Dawes *et al.* 1986) and their resulting MCFA enter cells at a higher rate than the long-chain fatty acids (Thomson, 1978).

Side effects of parenterally-administered lipids

Although LCT are generally well-tolerated, they are not free from side effects. Adverse reactions have been reported with rapid infusion rates in excess of 4·2 kJ/kg per h (Klein & Miles, 1994). Adverse effects include impaired immune, pulmonary, hepatic and platelet function (Miles, 1991). In addition, only 30% of the LCT administered are oxidized whereas the remainder are stored (Lanser & Saba, 1981; Bennegård *et al.* 1984). In traumatized animals, excessive administration of LCT may overwhelm the reticulo-endothelial system with resulting hepatomegaly and splenomegaly (Sobrado *et al.* 1985).

In general, pure MCT emulsions are less well-tolerated than LCT. Adverse effects with parenteral MCT emulsions reported in animals include poor growth and N balance and increased energy expenditure (Wiley & Leveille, 1973; Stein *et al.* 1984; Hill *et al.* 1989). Some reports have indicated that MCT may not spare protein catabolism as well as LCT (Rodriguez *et al.* 1986). More importantly, MCT are not well tolerated by patients, and moderate to severe side effects have been reported, such as neurotoxicity (Miles *et al.* 1991), emesis, somnolence, coma (Miles *et al.* 1991), narcosis, essential fatty acid deficiency, and ketosis in susceptible diabetic patients (Bach *et al.* 1974; Gordon & Duger, 1975). Early enthusiasm for parenteral MCT emulsions was tempered by reports of acute toxicity associated with excess administration. Doses as low as 1·5 g/kg body weight per h in dogs resulted in nausea, vomiting, somnolence, and in higher doses, coma and death (Cotter *et al.* 1987). Increased mortality in septic rats infused with MCT as a component of their TPN has been reported (Stein *et al.* 1986). Some of these side effects have been attributed to uncoupling of oxidative phosphorylation resulting from the uncontrolled entry of MCFA into the mitochondria when excess MCT are provided (Pressman & Lardy, 1956; Stein *et al.* 1984).

MCT–LCT mixtures and structured lipids (*SL*). It has been proposed that a combination of MCT and LCT may offer advantages over pure LCT or MCT emulsions. This combination could be obtained by either a physical mixture of LCT and MCT, or by re-esterification of LCT and MCT on the backbone of glycerol, otherwise known as SL, or structured triacylglycerols. Active research is currently targeting these areas.

Physical mixing of MCT and LCT emulsions does not alter their subsequent clearance kinetics such that corresponding triacylglycerols are cleared from the blood at rates unaffected by the presence of the other. Similarly, the oxidation rate of MCT and LCT emulsions is unaffected by the presence or absence of other triacylglycerols (Hamawy *et al.* 1985). Nonetheless, emulsions containing MCT–LCT (50:50, v/v) are relatively safe for use in parenteral nutrition, and may provide an alternative fuel that improves protein metabolism (Jiang *et al.* 1993).

Trans-esterification of MCT with LCT forms new triacylglycerols with intermediate clearance and oxidation rates between MCT and LCT (Babayan, 1987; Ekman *et al.* 1987). These SL have the benefits of MCT without some of the side effects. In the burned animal model, SL have superior protein-sparing effects over conventional triacylglycerols (Maiz *et al.* 1984*b*; DeMichele *et al.* 1988). These animals have improved

weight gain, N balance, liver and muscle protein content and serum albumin synthesis. SL do not increase energy expenditure as MCT emulsions often do (Mok *et al.* 1984). SL included in TPN of burned rats improves N balance and hepatic protein synthesis, and decreases leucine oxidation (Maiz *et al.* 1984*b*; Mok *et al.* 1984). SL stimulates muscle protein synthesis in rats after partial hepatectomy (Magnussen *et al.* 1986). Parenteral SL do not adversely affect the reticulo-endothelial system in burned guinea-pigs (Sobrado *et al.* 1985). SL improve N balance and protein loss, decrease hypertriacylglycerolaemia, and prevent fat accumulation in the liver (Bach *et al.* 1988). These animal studies have shown that SL are more beneficial than either LCT or a physical mixture of LCT and MCT (Maiz *et al.* 1984*b*; Mok *et al.* 1984; Bach *et al.* 1988; Teo *et al.* 1989), and lack the side effects of pure MCT emulsions (Moyer *et al.* 1989).

To date, few human studies with SL have been reported. In patients undergoing elective surgery infusion of SL demonstrated no significant difference in safety or tolerance compared with the clinical standard LCT emulsion, Intralipid (200 ml/l; Sandström *et al.* 1993). The advantages and clinical applications of SL parenteral emulsions in human subjects will probably be an active and fruitful area of future investigations.

CONCLUSIONS AND FUTURE RESEARCH

SCFA are essential for the normal structure and function of the colonic epithelium, and may have therapeutic potential in certain colonic pathologies such as diversion colitis, ulcerative colitis, and enteral-feeding-induced diarrhoea. Butyrate may be the key byproduct of fibre fermentation that has a role in the prevention and treatment of colon cancer. The many effects of butyrate on the colonocyte probably reflect its ability to both specifically and non-specifically affect genomic expression. Butyrate may also serve as a useful model to delineate the process of colo-rectal carcinogenesis at a molecular level. Future work is needed to evaluate the *in vivo* importance of maintaining a balance between possible colon carcinogenic substances such as bile acids and possible protective agents such as butyrate, both of which can be modulated by dietary interventions. Further work is needed to delineate the mechanism(s) of action of butyrate and to determine its role in the understanding, prevention, and treatment of colon cancer.

In general, parenteral LCT are effective and well-tolerated. However, adverse effects with rapid infusions of LCT emulsions and the requirement for the carnitine-dependent active transport into the mitochondria for oxidation have led to the search for alternate lipid fuels. MCT have attracted a great deal of attention for their theoretical benefit in conditions associated with carnitine depletion such as sepsis, burns, and multi-organ trauma. With the recognition that pure MCT have adverse side effects, the search continues for the ideal fat emulsion. The design of SL has offered marked advances over pure MCT emulsions. Initial studies with SL are promising. It is likely that SL will not replace, but instead supplement, the available choices in lipid parenteral nutrition.

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