Metabolism of short-chain fatty acids by rat colonic mucosa in vivo

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Fitch, Mark D., and Sharon E. Fleming. Metabolism of short-chain fatty acids by rat colonic mucosa in vivo. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G31–G40, 1999.—To determine the influence of substrate concentration and substrate interactions on short-chain fatty acid metabolism in vivo, a surgical procedure was established. Rats were surgically operated to cannulate a 5-cm segment of proximal colon, isolate the vasculature, and cannulate the right colic vein draining this segment. Thus metabolism was restricted to the defined colonic segment. The appearance of total $^{14}$C and $^{14}$CO$_2$ in the mesenteric blood stabilized after 30 min of perfusion. Increasing luminal concentrations of butyrate from 2 to 40 mmol/l resulted in linear increases in total $^{14}$C, but $^{14}$CO$_2$ production from $[^{14}C]$butyrate increased as a function of concentration only up to 10 mmol/l and was stable at higher butyrate concentrations. In addition to CO$_2$, 3-hydroxybutyrate and lactate were major metabolites of acetate and butyrate in vivo. The presence of a mixture of alternative substrates in the lumen had no influence on the metabolism of butyrate to CO$_2$ but significantly reduced the metabolism of acetate to CO$_2$. When compared with young (4 mo old) animals, transport of butyrate was significantly lower for aged (48 mo old) animals, as evidenced by the rate of appearance in blood of total $^{14}$C (P = 0.04) and $^{14}$C in butyrate (P = 0.03), but metabolism was similar, since differences were not significant for $^{14}$C in the major metabolites 3-hydroxybutyrate (P = 0.06) and CO$_2$ (P = 0.17). These results show that important aspects of short-chain fatty acid transport and metabolism are not predicted from data using isolated colonocytes but require study using an in vivo model.

acetae; butyrate; intestine; oxidation; ketone bodies

SHORT-CHAIN FATTY ACIDS (SCFA), including acetate, propionate, and butyrate, are produced in the cecum and colon of nonruminant animals and humans via the fermentation of unabsorbed carbohydrates and dietary fiber. The type of fermentation substrate and the rate of fermentation can influence the luminal concentrations of SCFA and the relative proportion of the individual acids (reviewed in Ref. 14). The rate at which SCFA are transported across the mucosa of the large bowel has been shown to be concentration dependent (15, 38), suggesting that increased production rates will result in greater availability of these compounds to the intestinal cells and other tissues.

Butyrate is known to influence the proliferation and differentiation of multiple cell types, including colonocytes and intestinally derived cell types (reviewed in Ref. 45). Also, butyrate withdrawal from guinea pig colonic mucosa has been shown to cause time-dependent hypoplasia and rapid triggering of massive apoptosis (27), providing evidence that butyrate has the capacity to modulate survival and death of colonocytes. Recently, experimental colitis was shown to enhance carcinogenesis using a rat model, and butyrate was reported to reduce the incidence and size of tumors in this study (10), suggesting that butyrate may be useful in long-term therapy to reduce colon cancer risk in ulcerative colitis.

From in vitro studies, it appears also that butyrate is an important fuel for colonic epithelial cells. Butyrate has been shown to be oxidized more readily to CO$_2$ than other potential substrates such as acetate, propionate, glucose, glutamine, long-chain fatty acids, and keto bodies (15, 17, 18). Also, butyrate oxidation is not suppressed by the presence of other energy-providing substrates (3, 6, 15), whereas the presence of butyrate reportedly suppressed the oxidation of other SCFA including acetate (6, 18). Recently, colonocytes from aged rats were reported to oxidize fatty acids at an abnormally high rate, and in both the young and aged animals butyrate was found to provide 50% of the energy for isolated colonocytes (18). These and earlier observations have caused butyrate to be recognized as the preferred energy-providing substrate for colonic epithelial cells, leading to the speculation that butyrate deprivation jeopardizes colonocyte and mucosal health. The work in aged animals suggested abnormal fatty acid oxidation by colonocytes of these senescent animals.

With the use of in vitro techniques, SCFA have been shown to be metabolized by the colonic epithelial cells to CO$_2$ and other metabolites. The large bowel of nonruminants appears to resemble the rumen epithelium in its ketogenic capability (31). Specifically, instilling butyrate into the rat cecum increased acetoacetate and 3-hydroxybutyrate concentrations in the aorta blood (32), and butyrate increased net production of ketone bodies by isolated human (34) and rat colonocytes (2, 6, 35). In rat colonocytes, labeled butyrate was shown to be incorporated into ketone bodies (13).

Whether the in vitro data accurately reflect the metabolism of SCFA by the colonic epithelia in vivo is unknown, since in vivo data are not available. Because the metabolic characteristics of cells in culture are often found to differ quite considerably from their metabolism in situ, in vivo studies of colonic SCFA metabolism were needed. To do these studies, it was necessary to establish appropriate methodologies. These techniques were used to 1) determine major metabolites of luminal butyrate in vivo, 2) determine how luminal butyrate concentration influences butyrate transport and metabolite formation by the proximal
colon, 3) evaluate whether butyrate metabolism is influenced by the presence of other oxidizable substrates, 4) compare metabolite formation from butyrate vs. acetate in a mixture of substrates, and 5) compare butyrate oxidation in the proximal colon of young adult vs. aged animals in vivo.

MATERIALS AND METHODS

Chemicals. All chemicals were obtained from commercial suppliers and were reagent grade. Radioisotopes ([1,2-\(^{14}\)C]acetate and [1-\(^{14}\)C]butyrate) were obtained from American Radiolabeled Chemicals (St. Louis, MO) and DuPont NEN Research Products (Boston, MA). Unlabeled substrates, acetylcysteine, and antibiotics were obtained from Sigma Chemical (St. Louis, MO). Pentobarbital sodium (50 mg/ml) was obtained from Abbott Laboratories (North Chicago, IL). Sodium heparin, injectable, was obtained from Elkins-Sinn (Cherry Hill, NJ). All silicone tubing was medical grade (Baxter-Scientific Products, McGaw Park, IL). Siliconized glass wool was obtained from Alltech Associates (Deerfield, IL).

Animals. For experiment 1, male Sprague-Dawley rats weighing 350–450 g (6–9 mo of age) were obtained from Simonsen Laboratories (Gilroy, CA). They were allowed free access to commercial rat diet (chow no. 5012, Ralston Purina, St. Louis, MO). For experiment 2, young (4 mo) and aged (24–25 mo) male Fischer 344 (F344) rats were purchased from the National Institute on Aging breeding colony maintained under barrier-reared conditions (Harlan Industries, Indianapolis, IN). They were allowed free access to National Institutes of Health 31 stock diet (Western Research Products, Hayward, CA) because they had been fed this diet throughout their lifetimes. All procedures involving animals were reviewed and approved by the Animal Care and Use Committee at the University of California (Berkeley, CA).

Substrates and solutions. All substrate solutions were prepared in Krebs-Henseleit buffer (26) but without Ca\(^{2+}\) and with antibiotics (2.5 µl/ml amphotericin B, 100 µg/ml kanamycin monosulfate, 250 U/ml penicillin G, and 250 µg/ml streptomycin sulfate) and acetylcysteine (10 mmol/l). Unlabeled sodium butyrate was dissolved in this solution to produce final concentrations of 2, 5, 10, or 40 mmol/l butyrate. Ethanolic solutions of [1-\(^{14}\)C]butyrate were evaporated to near dryness and then added to the butyrate solutions to produce a final specific activity of 0.1 µCi/µmol for the 2, 5, and 10 mmol/l substrate concentrations and of 0.06 µCi/µmol for the 40 mmol/l substrate concentration. Two substrates were also tested in a mixture of 10 mmol/l each of acetate, propionate, butyrate, glucose, and glutamine. The first contained [1-\(^{14}\)C]butyrate at a specific activity of 0.1 µCi/µmol, and the second contained [1,2-\(^{14}\)C]acetate at a specific activity of 0.3 µCi/µmol.

Dulbecco’s PBS (12) was used during the surgical preparation to keep abdominal tissues moistened. PBS containing 10 mmol/l acetylcysteine was used as a flush solution for the cannulated colon lumen before the infusion of labeled substrate solution.

Surgical procedures and experimental protocols. The surgical techniques were based on the protocols developed for studies in the rat small intestine (46–49). Details of the experimental procedures have been fully described (50). Modifications needed to adapt these procedures to the proximal colon are described below. A schematic of the overall surgical setup is provided (Fig. 1).

On the evening before the experiment, four or five animals were anesthetized by intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt), and blood was collected using cardiac puncture. During surgery, donor blood was reinfused into the operated experimental animal using a peristaltic pump equipped with an in-line blood filter and...
bubble trap. The silicone tubing terminated in a 25 gauge ×
11-mm needle that was inserted into the left saphenous vein.
On the day of the experiment, one rat was anesthetized by
intraperitoneal injection of pentobarbital sodium and pre-
pared for surgery as described (50). To prepare the colon for
cannulation, the lower and middle portions of the colon were
detached from the back body wall by selectively severing the
mesentry at the point of attachment. Fatty tissue was moved
to expose the underlying surface of the proximal colon and
associated vasculature. The middle colic artery and vein were
tied off and then cut to allow repositioning of the middle
region of the colon within the body cavity and facilitate
installation of the lumen cannula. With this one exception,
care was taken not to interfere with colonic innervation. The
dorsal aorta was exposed and cleared of fat and connective
tissue from the left renal artery to the iliac bifurcation, at
which point a blunt trocar was introduced into the abdomen
and tunneled toward the base of the tail. An incision through
the skin and muscle at this location allowed a saline-filled
cannula to be introduced and then positioned alongside the
aorta, as the aorta would be cannulated at a later time. This
system was connected to an electronic monitor (VT-15, Wins-
ton Electronics, Millbrae, CA) for measuring blood pressure.
The right colic vein was then exposed, taking care to avoid
trauma. Three 6-0 silk sutures were looped under the vessel
between the superior mesenteric vein and the branch point of
the vessel on the colon proper. These sutures were left loosely
draped while the lumen cannulas were installed, giving the
right colic vein time to relax and return to normal blood flow.
The stainless steel tips of the cannulas were inserted into the
lumen of the proximal colon at the position of the middle colic
vein and at the cecocolonic junction. The segment was then
flushed with PBS containing 10 mmol/l acetylcysteine to
destroy digesta and excess mucosa.
In preparation for the installation of three vascular cannu-
las, the animal was injected with 200 units of sodium heparin
into the right saphenous vein. The cannula supplying donor
blood was installed into the left saphenous vein, and the
previously positioned aorta cannula was installed into the
aorta between the left renal artery and the iliac bifurcation as
previously described (50). The right colic vein was then
 cannulated, and the 6-0 silk sutures were tightened to hold
the cannula tightly in place. The cannula consisted of an
11-mm portion of a 23-gauge stainless steel needle with a 90°
angle that was inserted into the left saphenous vein. The
donated blood was collected on ice at 10-min intervals under
300 µl mineral oil in 12 × 75-mm tubes. Luminal perfusion continued for 60 min.
Whole blood was analyzed immediately after collection for

total $^{14}C$ and for $^{14}CO_2$. Whole blood total $^{14}C$ was determined by
dissolving 50-µl aliquots in alkaline tissue solubilizer
(TS-2, RPI, Mt. Prospect, IL). They were decolorized with 300
µl of 15% benzoyl peroxide in toluene and counted with 5 ml
Hionic-Fluor scintillation cocktail (Packard Instruments, Me-
ridon, CT). Whole blood $^{14}CO_2$ was determined in 200-µl
aliquots using 25-ml Erlenmeyer flasks fitted with stoppers
and disposable center wells. Proteins were precipitated with
0.8 ml methanol, and the flask contents were acidified with
1.0 ml of 1 M NaH$_2$PO$_4$. Carbon dioxide was trapped in 0.45
ml of 10 N NaOH in the center well during a 2-h room
temperature incubation and counted with 1.0 ml of water and
15 ml of Hionic-Fluor cocktail.
Metabolites for HPLC analysis were extracted from frozen
whole blood aliquots using two volumes of 8% HClO$_4$ followed
by neutralization with 5.0 N and 1.0 N KOH. The superna-
tant was lyophilized, and the dried residue was extracted
three times with 93% ethanol. The ethanol supernatant was
 evaporated to dryness and derivatized using bromoacetophen-
one (BAP, 50 mg/ml) and 18-crown-6 ether (25 mg/ml) in
acetone (4). The BAP derivatives were dried and redis-
solved in 30% acetonitrile, and acid compounds were ana-
lized by reverse-phase HPLC using a 25 × 0.46-cm, 5-µm
particle size octadeysilsilane column (Ultrasound, Beckman,
Fullerton, CA). The elution rate (1.0 ml/min) and gradient (5
min at 30% acetonitrile, then a linear increase in acetonitrile
concentration of 1.5% per min, ending at 68% acetonitrile for
30 min) conditions were sufficient to resolve lactic, acetic,
propionic, butyric, acetoacetic, and 3-hydroxybutyric acids.
The HPLC column effluent fractions were collected and
 counted in 15 ml of 3a70b scintillation cocktail (RPI). All
scintillation counting was done in a 1600TR liquid scintilla-
tion counter (Packard). Metabolism was quantified as the
rate at which substrate carbon appeared in a specific metabo-
lite in mesenteric blood, and data are reported as nanomoles
of substrate carbon atoms per gram wet weight per minute,
where wet weight refers to the weight of the cannulated
intestinal segment.
Expired breath from the animal was continually collected
through a nose cone and bubbled under slight vacuum
through a liquid trap (800 ml of 0.5 N NaOH). At the
conclusion of the experiment, $^{14}CO_2$ was quantitated by
counting 1-ml aliquots of the trap solution using Hionic-Fluor
scintillation cocktail.
Sections of liver, lung, and abdominal fat weighing 1–2 g
were excised and minced by hand. Aliquots (200–250 mg)
were digested in a tissue solubilizer (TS-2) at 50°C for 16 h,
decolorized with 250 µl of 15% benzoyl peroxide in toluene,
and counted using Hionic-Fluor scintillation cocktail.
Experimental design and statistical analyses. Data from
two experiments are reported. In experiment 1, 21 Sprague-
Dawley rats were randomly assigned to seven treatments
(substrates) so that a total of 3 animals were exposed to each
treatment. In experiment 2, six young F344 rats and six aged
F344 rats were randomly assigned to two treatments (sub-
strates) so that three young and three aged animals were
exposed to each treatment. (The substrates evaluated in
experiments 1 and 2 are listed in Tables 2 and 3, respectively.)
Because of unequal variance among treatments in experi-
ment 1, differences among group means for experiment 1 were
determined on log-transformed data using one-way ANOVA
and the Tukey-HSD procedure as follow-up. Differences among
substrate groups for experiment 2 were determined on nontrans-
formed data using ANOVA with two main effects (age and
substrate) and their interaction as sources of variation. The
one-way and ANOVA procedures in SPSS (42) were used to
perform these statistical analyses. Differences were consid-
ered to be statistically significant at α = 0.05.
RESULTS
Assessing whether the perfusion technique limits metab-
olism to the cannulated segment. To assess the
validity of this technique, it was necessary to determine
whether the surgical procedure effectively prevented

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IN VIVO COLONIC METABOLISM OF SHORT-CHAIN FATTY ACIDS

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transport of the luminal perfusate into tissues other than the cannulated intestinal segment. To do this, breath gases were quantitatively collected throughout experiments, and tissue specimens were taken after a 1-h perfusion with 14C-labeled substrate. Breath gases taken from animals throughout the 1-h perfusion contained a small amount of radioactivity that comprised ~0.05% of radioactivity in perfusates or 3% of the radioactivity transported into the mesenteric blood (Table 1). Minute quantities of radioactivity were also detected in the liver and in blood taken from the aorta, and radioactivity in these tissues comprised <1% of the radioactivity transported into the mesenteric blood. Radioactivity in the visceral fat pad of experimental animals was equivalent to radioactivity in control animals that had never been exposed to radioactivity (data not shown). The sum of radioactivity in breath, fat, blood, and liver was calculated to be <5% of the radioactivity transported into the mesenteric blood during the 60-min perfusion period.

Steady-state metabolic conditions. Sufficient substrate was perfused through the cannulated colonic segment to prevent substantial changes in the availability of substrate to the epithelia during experimentation. This is evidenced by observing that <2% of substrate in the perfusate was transported into mesenteric blood during the 60-min perfusion period (Table 1). Also, direct measurements of the perfusate at 0 vs. 60 min indicated that substrate concentration changed on average by 2.6 ± 1.5% for data from experiment 1 (n = 21).

Total 14C and 14CO2 are presented as a function of perfusion time for selected treatments in which the lumen was perfused with 10 mmol/l butyrate. Total 14C and 14CO2 increased during the first 30 min of perfusion and then stabilized for at least 20–30 min (Fig. 2). In subsequent experiments, metabolite analyses were conducted on blood samples taken from the 40- to 60-min time points.

Influence of butyrate concentration on 14C in mesenteric blood. Four of the seven treatments evaluated in experiment 1 were used to determine the influence of luminal butyrate concentration on the rate of appearance in mesenteric blood of total 14C and its constituent metabolites. In these treatments, 95–99% of total 14C in mesenteric blood was accounted for as acetate, propionate, butyrate, CO2, 3-hydroxybutyrate, or lactate. Differences among the seven treatments in experiment 1 were statistically significant for total 14C and for 14C in constituents, including acetate, butyrate, CO2, 3-hydroxybutyrate, and lactate (Table 2). Follow-up analysis of differences among the mean values for the four treatments (butyrate at 2, 5, 10, and 40 mmol/l) relevant to this objective indicated that total 14C in mesenteric blood increased linearly (r = 0.998) with luminal butyrate concentration (Fig. 3A). Increasing luminal butyrate concentration also significantly in-

Table 1. Recovery of 14C after luminal perfusion for 1 h

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>% of 14C in Mesenteric Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>Breath CO2</td>
<td>3.41 ± 0.61</td>
</tr>
<tr>
<td>Aorta blood</td>
<td>0.74 ± 0.11</td>
</tr>
</tbody>
</table>

Values are means ± SE; data were taken from the 21 animals included in experiment 1. For this experiment, 1.43 ± 0.07% of 14C perfused through the lumen was collected in mesenteric blood in 60 min. Of the total 14C perfused through the lumen, 98.7 ± 0.31% was recovered. Aorta blood volume was assumed to be 5.8% of body weight (1).
creased the rate of appearance in mesenteric blood of 14C in butyrate (Fig. 3B) and in metabolites including acetate (Fig. 3C) and 3-hydroxybutyrate (Fig. 3E).

Differences among these four treatments were not statistically significant for CO2 (Fig. 3D) or lactate (Fig. 3F).

Luminal butyrate concentration influenced the relative proportions of 14C in the main metabolites. When the lumen was perfused with 2 mmol/l butyrate, 14CO2 accounted for more than 50% of all 14C in metabolites, but this proportion decreased with increasing butyrate concentration until, at 10 and 40 mmol/l, CO2 represented only 37 and 35%, respectively, of all metabolites (Fig. 4). Increasing butyrate concentration also decreased the proportion of butyrate metabolized to lactate. In contrast, 3-hydroxybutyrate represented only 28% of metabolites at 2 mmol/l butyrate but 51 and 50%, respectively, at 10 and 40 mmol/l butyrate.

Colonic metabolism of acetate vs. butyrate. As part of experiment 1 (Table 2), colonic metabolism of acetate and butyrate was compared. To do this, data from four treatments were considered: [14C]acetate in acetate, [14C]butyrate in butyrate, [14C]acetate in a mixture of five substrates (acetate, propionate, butyrate, glucose and glutamine), and [14C]butyrate in the mixture of five substrates.

Fig. 3. Influence of luminal butyrate concentration on the rate of appearance (nmol carbon atoms·g wet wt intestine\(^{-1}\)·min\(^{-1}\)) in mesenteric blood of total carbon atoms from butyrate (A) and on the rate of appearance of butyrate carbon in metabolites (B–F). Data were taken from experiment 1. Proximal colon was continuously perfused with [1-14C]butyrate in butyrate at 2, 5, 10, or 40 mmol/l. Aliquots of mesenteric blood collected during the 40- to 60-min perfusion periods were analyzed for total 14C and for 14C in metabolites. Values are means ± SE; n = 3 for each concentration. These data are part of experiment 1 as presented in Table 2. Values (●) with different superscripts are significantly different at P < 0.05.

Fig. 4. Influence of butyrate concentration on the relative proportion of substrate carbon in metabolites (%). Data were taken from experiment 1. Proximal colon was continuously perfused with [1-14C]butyrate in butyrate at 2, 5, 10, or 40 mmol/l. Aliquots of mesenteric blood collected during the 40- to 60-min perfusion periods were analyzed for total 14C and for 14C in metabolites, and these values were used to calculate percentage of total substrate carbon in each metabolite (substrate carbon identified as butyrate was excluded from this calculation). Values are percent total substrate carbon in nonsubstrate metabolites; n = 3 for each concentration.
The rate of appearance in mesenteric blood of total \(^{14}\text{C}\) and of \(^{14}\text{CO}_2\) was not significantly lower for acetate than for butyrate when only acetate or butyrate was present in the lumen (Fig. 5A). When a mixture of substrates was present, however, the rate of appearance of total butyrate carbon was 3.5-fold higher than the rate of appearance of total acetate carbon, and the rate of appearance in \(^{14}\text{CO}_2\) was 8.25-fold higher for butyrate than for acetate carbon. The rate of appearance of \(^{[3-^{14}C]}\)hydroxybutyrate was significantly higher for butyrate than for acetate when the lumen was perfused with a single substrate or with the mixture (Fig. 5E), and values for butyrate were more than 12-fold higher than the values for acetate. Similarly, \(^{[14}C\)lactate appearance rate was nearly fourfold higher for butyrate than for acetate when the lumen was perfused with the mixture of substrates (Fig. 5F).

Of the \(^{14}\text{C}\) present in mesenteric blood, metabolites accounted for a greater proportion of total \(^{14}\text{C}\) for butyrate than for acetate (~45% for the two butyrate treatments vs. 20 and 34% for the two acetate treatments). This difference was particularly evident when the lumen was perfused with the mixture of substrates: only 54% of total butyrate carbon was found in butyrate, whereas 80% of total acetate carbon was found in acetate. The relative proportion of carbon in the main metabolites also differed for acetate and butyrate (Fig. 6). When these SCFA represented the sole luminal substrate, \(^{14}\text{CO}_2\) was the most abundant metabolite of acetate, whereas both \(^{14}\text{CO}_2\) and 3-hydroxybutyrate were main metabolites of butyrate. Also, more than 20% of metabolized acetate was recovered in lactate vs. 8% for butyrate.

Influence of alternative fuels on metabolism of acetate and butyrate. To determine the influence of alternative fuels on the metabolism of acetate and butyrate, data for the single-substrate treatments were compared with data for the corresponding treatments in the mixture of substrates (\(^{14}C\)acetate in acetate vs. \(^{14}C\)acetate in a mixture of five substrates; \(^{14}C\)butyrate in butyrate vs. \(^{14}C\)butyrate in a mixture of five substrates).

The rate of appearance in mesenteric blood of butyrate carbon was not significantly different when butyrate was the sole luminal substrate from when the mixture of five substrates was present in the lumen for total \(^{14}\text{C}\), \(^{14}\text{C}\) in butyrate, or for \(^{14}\text{C}\) in any of the metabolites including acetate, \(^{14}\text{CO}_2\), 3-hydroxybutyrate, or lactate (Fig. 5). For acetate carbon, however, the rate of \(^{14}\text{CO}_2\) appearance was significantly lower when the mixture of substrates was present than when only acetate was provided in the lumen (Fig. 5D). Differences were not statistically significant for other metabolites.

The presence of the mixture of substrates had little, if any, effect on the relative distribution of butyrate

![Fig. 5. Metabolite appearance from acetate vs. butyrate and influence of substrate composition on metabolite appearance rate (nmol carbon atoms·g wet wt intestine\(^{-1}\)·min\(^{-1}\)) from both substrates. Data were taken from experiment 1. Proximal colon was perfused with trace quantities of \(^{14}C\)butyrate in 10 mmol/l butyrate (butyr) or in a mixture of substrates (10 mmol/l each of acetate, propionate, butyrate, glucose, and glutamine) and trace quantities of \(^{14}C\)acetate in 10 mmol/l acetate (Ac) or in a mixture of substrates. Aliquots of mesenteric blood collected during the 40- to 60-min perfusion periods were analyzed for total \(^{14}\text{C}\) (A) and for \(^{14}\text{C}\) in metabolites (B–F). Values (bars) are means ± SE; n = 3 for each treatment. Values (bars) with different superscripts are significantly different at P < 0.05.](image-url)
carbon among the main metabolites (Fig. 6) but generally reduced the proportion of acetate carbon in CO₂ and increased the proportion transported as 3-hydroxybutyrate.

The metabolic advantage of having more than one SCFA available in the lumen was assessed by summing data for the two treatments in which the lumen was perfused with the mixture of substrates (10 mmol/l each of acetate, propionate, butyrate, glucose, and glutamine) and trace quantities of [14C]acetate in 10 mmol/l acetate or in a mixture of substrates. Aliquots of mesenteric blood collected during the 40- to 60-min perfusion periods were analyzed for total 14C and for 13C in metabolites, and these values were used to calculate percentage of total substrate carbon in each metabolite (substrate carbon identified as either acetate or butyrate was excluded from these calculations). Values are percent total substrate carbon in nonsubstrate metabolites; n = 3 for each concentration.

Fig. 6. Influence of perfusate composition on the relative proportion of acetate and butyrate carbon in metabolites (%). Data were taken from experiment 1. Proximal colon was perfused with trace quantities of [14C]butyrate in 10 mmol/l butyrate or in a mixture of substrates (10 mmol/l each of acetate, propionate, butyrate, glucose, and glutamine) and trace quantities of [14C]acetate in 10 mmol/l acetate or in a mixture of substrates. Aliquots of mesenteric blood collected during the 40- to 60-min perfusion periods were analyzed for total 14C and for 13C in metabolites, and these values were used to calculate percentage of total substrate carbon in each metabolite (substrate carbon identified as either acetate or butyrate was excluded from these calculations). Values are percent total substrate carbon in nonsubstrate metabolites; n = 3 for each concentration.

perfusion substrate (butyrate alone vs. butyrate in a mixture of five substrates) were not statistically significant, differences due to animal age could be determined using data pooled for the two perfusates. The pooled data showed a significant effect of age on the rate of appearance of total 14C carbon in blood, with younger animals having a higher rate than older animals.

Influence of animal age on transport and metabolism of butyrate. Animal age significantly influenced the rate of appearance in mesenteric blood of butyrate carbon in several metabolites (Table 3). Because interactions between animal age (young vs. aged) and perfusion substrate (butyrate alone vs. butyrate in a mixture of five substrates) were not statistically significant, differences due to animal age could be determined using data pooled for the two perfusates. The pooled data showed a significant effect of age on the rate of appearance of total 14C carbon in blood, with younger animals having a higher rate than older animals.

Table 3. Experiment 2: significance of differences regarding influence of animal age and luminal substrate on production of metabolites from butyrate

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Animal age</th>
<th>Substrate</th>
<th>Age x Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>0.38</td>
<td>0.90</td>
<td>0.96</td>
</tr>
<tr>
<td>Butyrate</td>
<td>0.03</td>
<td>0.51</td>
<td>0.82</td>
</tr>
<tr>
<td>CO₂</td>
<td>0.17</td>
<td>0.26</td>
<td>0.91</td>
</tr>
<tr>
<td>3-Hydroxybutyrate</td>
<td>0.06</td>
<td>0.22</td>
<td>0.33</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.02</td>
<td>0.47</td>
<td>0.96</td>
</tr>
<tr>
<td>Propionate</td>
<td>0.14</td>
<td>0.049</td>
<td>0.23</td>
</tr>
<tr>
<td>Other</td>
<td>0.62</td>
<td>0.95</td>
<td>0.84</td>
</tr>
<tr>
<td>Total</td>
<td>0.04</td>
<td>0.39</td>
<td>0.71</td>
</tr>
</tbody>
</table>

P values of <0.05 indicate that there was a statistically significant effect of age or substrate, or a significant interaction between age and substrate, on the rate at which the identified metabolite was produced from butyrate. This analysis included data from the following 4 treatments: 1) young F344 rats perfused with [14C]butyrate in butyrate at 10 mmol/l; 2) young F344 rats perfused with [14C]butyrate in a mix of 5 substrates; 3) aged F344 rats perfused with [14C]butyrate in butyrate at 10 mmol/l; 4) aged F344 rats perfused with [14C]butyrate in a mix of 5 substrates. The mix of 5 substrates was comprised of 10 mmol/l each of acetate, propionate, butyrate, glucose, and glutamine. Metabolites were analyzed in blood collected after 40–60 min of perfusion. Other = [total 14C in blood – (sum of 14C in all identified metabolites)].
means for young animals were significantly higher than those for aged animals for total \(^{14}C,^{13}C\) in butyrate, and \(^{14}C\) in lactate (Fig. 8). Animal age, however, did not significantly influence the metabolism of butyrate to CO\(_2\) or 3-hydroxybutyrate.

**DISCUSSION**

To our knowledge, this is the first model that simultaneously restricts metabolism to a selected colonic segment and permits the metabolism to be studied in vivo. With the use of a perfusion model based on the jejunal model developed by Windmueller and Spaeth (47), metabolism in the proximal colon could be studied under steady-state conditions. Our results suggest that labeled metabolites did not escape from the lumen of the perfused segment. Thus it seems unlikely that metabolites originated from tissues other than from the perfused segment.

Influence of butyrate concentration on metabolite production. Absorption of butyrate carbon into the mesenteric blood increased linearly with luminal butyrate concentration. Similar observations have been made previously using the human rectum (38) and rat cecum (15). Our observations do not preclude carrier-mediated transport, as observed in the small intestine (39, 40) and colon (21, 29, 30, 33, 41), but demonstrate the important and direct effect of luminal butyrate concentration on the net rate of transport across the intact proximal colon of the rat in vivo.

The appearance in mesenteric blood of CO\(_2\) from butyrate was not significantly influenced by luminal butyrate concentration, presumably due to the high variability among the small number of replicates. Thus the current data suggest that luminal butyrate concentrations may limit butyrate oxidation, since the mean values for the 2 mmol/l treatment was only 50% of the value for the 10 mmol/l treatment. Butyrate concentrations in cecal fluid and colonic contents of rats, pigs, and monkeys have been reported to be as low 3–7 mmol/l when diets contained little or no fermentable dietary fiber (7, 11, 16, 24, 43) and as high as 40 mmol/l when the diets provided ample fermentable fiber (5, 43, 44). In studies that have used isolated colonocytes, the Michaelis-Menten constant for butyrate oxidation to CO\(_2\) has been reported to range from 0.06 to 0.3 mmol/l (6, 8, 25).

From in vitro data, one would predict that butyrate oxidation by colonocytes would always be maximal, and this contrasts sharply with observations in vivo. There are several possible explanations for these differences. First, a higher concentration of butyrate may be needed in vivo than in vitro to achieve a certain intercellular concentration of butyrate. This could be due to the presence of mucus in vivo or to differences in the absorptive surface per cell, since cells are exposed to butyrate at the luminal surface only in the in vivo model but on all surfaces in the in vitro model. Second, the butyrate concentration gradient between the lumen and intracellular space is likely to be greater in vivo than in isolated cells. In isolated cells, the transport of absorbed butyrate out of the cell would be inhibited by butyrate in the medium, thus maintaining a near equilibrium with the substrate in vitro. In vivo, a large percentage of absorbed butyrate (up to 80%) is continually secreted into the blood stream.

Third, the in vivo procedure is unable to eliminate metabolic inhibitors from unintentionally entering the cells via the basolateral membrane. Although further studies would be needed to determine the explanation for these differences, it appears that the luminal concentration of butyrate needed for maximal butyrate oxidation cannot be accurately assessed using isolated colonocytes.

In our studies, there was substantial incorporation of butyrate into 3-hydroxybutyrate but acetacetoacetate was undetectable. In studies with isolated colonocytes, the presence of butyrate was reported to cause a greater net production of acetacetoacetate than 3-hydroxybutyrate (2, 13, 35, 36) and low 3-hydroxybutyrate-to-acetacetoacetate ratios (8, 9). Results of studies using epithelial slices (19, 20) have been more consistent with the in vivo data presented here, however. Differences among these studies are likely due to differences in the redox status of the cells rather than due to differences in relative flux through specific metabolic pathways. The current study demonstrates that ketone bodies (predominantly 3-hydroxybutyrate) are major metabolites of butyrate in vivo, since more than 50% of the metabolized butyrate was incorporated into 3-hydroxybutyrate when the lumen was perfused with 40 mmol/l butyrate. This is the first report, to our knowledge, of ketone body production from butyrate by the rat colon in vivo, and these results suggest similarities in the metabolic fate of butyrate between rats and ruminant animals (22, 31).
Small amounts of butyrate were incorporated also into lactate, although incorporation was not significantly different for the 2 vs. 40 mmol/l butyrate treatments. Using isolated colonocytes, others have reported that butyrate did not stimulate lactate production when presented alone (2, 6, 35) or in the presence of other SCFA (28).

Influence of alternative fuels on butyrate metabolism and comparison to acetate metabolism. When the lumen was perfused with a mixture of five substrates, significantly more carbon atoms from butyrate than acetate were transported across the colonic mucosa, and significantly more butyrate carbon was incorporated into CO₂, 3-hydroxybutyrate, and lactate. If the 3.5-fold higher value for butyrate carbon vs. acetate carbon in mesenteric blood is converted to nanomoles of substrate transported, butyrate molecules were transported across the epithelium at a 1.7-fold higher rate than for acetate molecules. This observation is not consistent with previous data attained using the rat cecum, which showed that the three main SCFA were transported out of the lumen at equivalent molar rates (15). The transport kinetics may differ between the cecum and proximal colon, since others have reported that SCFA transport differs along the longitudinal axis of the colon (23, 37). Although the factor responsible for these differences is not known, the current data describe SCFA transport in the proximal colon for the first time, to our knowledge, and the differences between acetate and butyrate are noteworthy. The data show that acetate transport was not significantly inhibited by the presence of other SCFA, whereas acetate metabolism to CO₂ was suppressed by the mixture of substrates. This suppression effect may be due to the presence of butyrate in the mixture, since others have shown, using isolated colonocytes, that butyrate suppresses acetate oxidation (6, 18).

Influence of animal age on butyrate transport and metabolism. The transport of total butyrate carbon and unmetabolized butyrate across the proximal colon was significantly higher for young (4 mo) than for aged (48 mo) animals. These differences were significant also when transport was calculated in nanomoles per centimeter per minute (data not shown), indicating that the aging effect was not likely due to differences in the density (wt/length) of the epithelial cell layer and verifying previous observations (18). Metabolism of butyrate to the major metabolites, CO₂ and 3-hydroxybutyrate, was not significantly influenced by age in these in vivo studies, although there was a tendency for butyrate metabolism to change in parallel with butyrate carbon transport. Previously, butyrate oxidation to CO₂ was reported to be significantly lower for rat colonocytes isolated from young vs. aged F 344 rats (17, 18). Thus, for reasons that cannot be elucidated from the current studies, the effect of aging on colonocyte metabolism appears not to be accurately predicted using isolated colonocytes. It is conceivable that changes in the cellular membrane may occur as a consequence of the aging process, and these changes might reduce butyrate transport across the cellular and subcellular membranes in vivo, which, in turn, would reduce its metabolism. If colonocyte membranes are influenced by the aging process, the chemical and mechanical processes used to isolate colonocytes might have a different effect on colonocytes of aged than on those of young animals. The differences between in vitro and in vivo studies support this suggestion and raise doubts that isolated cells can be reliably used to evaluate the effects of aging on transport and metabolism.

In conclusion, a rat model was developed that permitted the metabolism of SCFA by the mucosa of the proximal colon to be studied in vivo. Transport of butyrate from the lumen into the mesenteric blood increased linearly with increasing butyrate concentrations, whereas butyrate metabolism followed saturation kinetics. The major metabolites of both acetate and butyrate were CO₂, 3-hydroxybutyrate, and lactate, and the proportions of these metabolites were dependent on substrate concentration. Neither the transport nor metabolism of butyrate was influenced by the presence of alternative substrates, including acetate or propionate. In contrast, the oxidation of acetate to CO₂ was suppressed by the presence of alternative substrates. When present with other substrates, transport and metabolism were significantly lower for acetate than for butyrate. The transport of butyrate and, to a lesser extent, its metabolism were lower in the proximal colon of aged than in that of young animals. These results show that aspects of SCFA transport and metabolism are not predicted from data using isolated colonocytes but require study using an in vivo model.

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REFERENCES


